Two new compounds from edible mushroom *Sarcomyxa edulis*

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ABSTRACT

Chemical investigation of the edible mushroom *Sarcomyxa edulis* led to the isolation of one new highly degraded sterol (**1**), and one new β-carboline alkaloid (**2**), along with nine known compounds (**3**-**11**) for the first time from this mushroom. The structures of new compounds were elucidated using HR-ESI-MS data and NMR spectroscopy. In addition, anti-inflammatory activity of new compounds was evaluated against lipopolysaccharide-induced NO production in RAW 264.7 macrophages. Compound **2** exhibited a good anti-inflammatory activity with IC_{50} value of 9.88 ± 0.48 μM, and compound **1** exhibited a weak inhibitory effect with IC_{50} value of 71.36 ± 5.11 μM.

3.1. General experimental procedures

NMR spectra were recorded on Bruker DRX-600 spectrometer. Ultraviolet (UV) spectra were recorded on a TU-1810DPC spectrophotometer. HR-ESI-MS data were measured on a Waters Xevo G2 Q-TOF spectrometer. Silica gel (200-300 mesh, Qingdao Marine Chemical Ltd., People′s Republic of China), Sephadex LH-20 (Amersham Biosciences, Sweden), ODS (Merck) were used for column chromatography (CC). HPLC grade solvents (acetonitrile and water) were purchased from Thermo Fisher Scientific Korea Ltd. High-performance liquid chromatography (HPLC) was carried out using a Waters 2535 chromatography system (Waters, Milford, MA, USA) equipped with a Waters 2489 UV/visible detector with a YMC-Pack ODS-A (250 × 10 mm, 5 μm) column (YMC Co., Ltd., Kyoto, Japan). Acetonitrile and water (HPLC grade) were purchased from Fisher Scientific Ltd.

3.2. Extraction and isolation

The dried fruiting bodies of *S. edulis* (2 kg) were powdered and then extracted with 95% ethyl alcohol (3 × 5 L, 2 days each) at room temperature to yield the ethanol extract (260 g). The ethanol extract was suspended in water (2 L), and extracted successively with petroleum ether (3 × 2.0 L) and ethyl acetate (3 × 2.0 L), respectively.

The ethyl acetate extract (SE, 32 g) was subjected to silica gel column chromatography (400 g, 200-300 mesh) and eluted using dichloromethane-methanol (100:1-1:1) to obtain 8 fractions (SE1-SE8). SE1 (1.4 g) was purified by semi-preparative RP-HPLC to give compounds **1** (12.1 mg, 100% CH_3CN, t_R 20.5 min). SE3 (5.9 g) was further fractionated on ODS column chromatography eluting with mixtures of methano-water (30:70-70:30) to give 6 subfraction (SE3A-SE3F). SE3E (690 mg) was purified by semi-preparative RP-HPLC to give compounds **2** (8.4 mg, 30% CH_3CN, t_R 26.4 min), **6** (6.8 mg, 25% CH_3CN, t_R 32.0 min), **7** (5.2 mg, 25% CH_3CN, t_R 27.5 min), and **8** (5.2 mg, 35% CH_3CN, t_R 18.9 min). SE4 (4.5 g) was separated on ODS column chromatography and eluted with mixtures of methano-water (20:80-70:30) to obtain 6 subfraction (SE4A-SE4F). SE4B (1.2 g) was

isolated by semi-preparative RP-HPLC to obtain compounds **11** (3.2 mg, 15% CH_3CN, t_R 10.8 min), **3** (7.4 mg, 15% CH_3CN, t_R 16.5 min), and **4** (8.5 mg, 15% CH_3CN, t_R 19.2 min). Compounds **5** (12.2 mg, 28% CH_3CN, t_R 26.9 min), **9** (10.5 mg, 28% CH_3CN, t_R 23.5 min), and **10** (3.6 mg, 30% CH_3CN, t_R 19.4 min) were obtained from SE4D (1.5 g) by semi-preparative RP-HPLC.

3.3. Cell viability assay

Cell viability was evaluated using the CCK8 assay kit. After pre-incubation of RAW264.7 cells (100 μL, 1×10^5 /mL, 96-well plate) at 37ºC under 5% CO_2 for 24 h, various concentrations of the compounds (20, 40, and 80 μM) were added into the cells and incubated for another 24 h. Then, 10 μL CCK-8 solution was added to each well, and the plate was protected from light and incubated at 37℃ for 2 h. Absorbance was measured at 450 nm using a microplate reader.

3.4. Determination of NO production

After pre-incubation of RAW 264.7 cells (100 μL, 1×10^5 /mL, 96-well plate) for 24 h, cells were treated with test compounds, and aminoguanidine was used as a positive control. Then the cells were stimulated with lipopolysaccharide (1 μg/mL) for 24 h. The supernatants were collected and tested for NO production by Griess' reagent method.

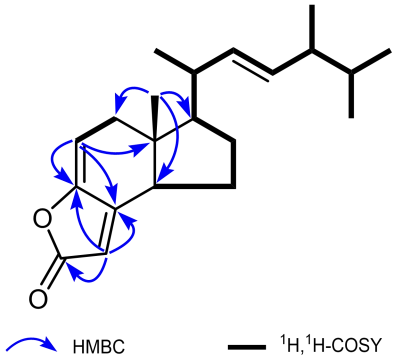


Figure S1. The HMBC and ^{1}H,^{1}H-COSY correlations of compound **1**

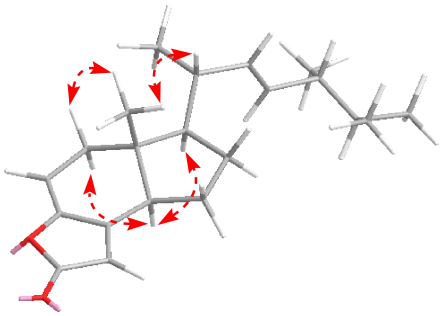


Figure S2. The significant NOESY correlations of compound **1**

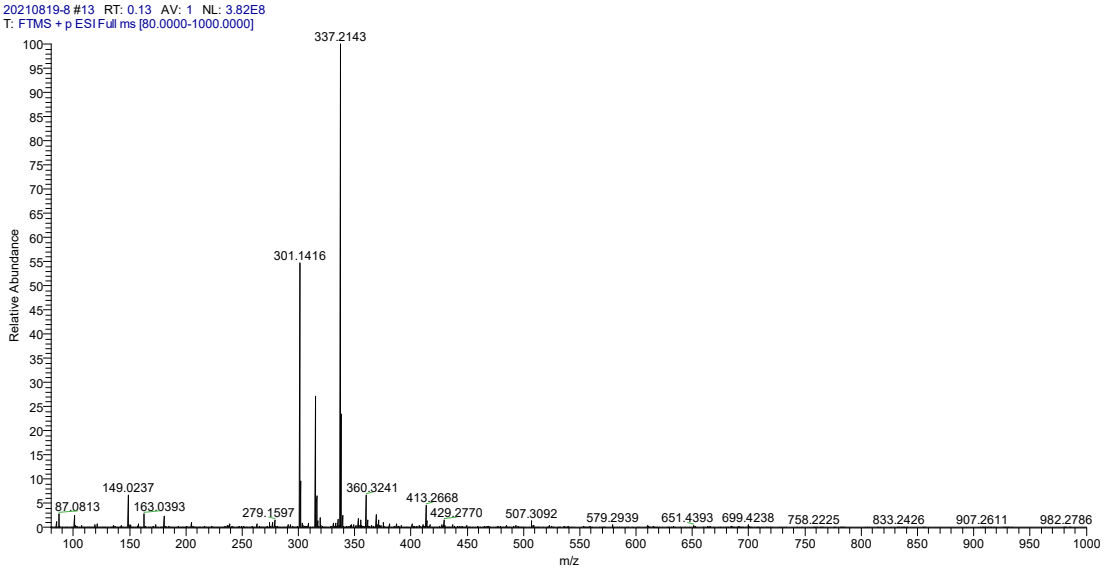


Figure S3. HR-ESI-MS spectrum of compound **1**

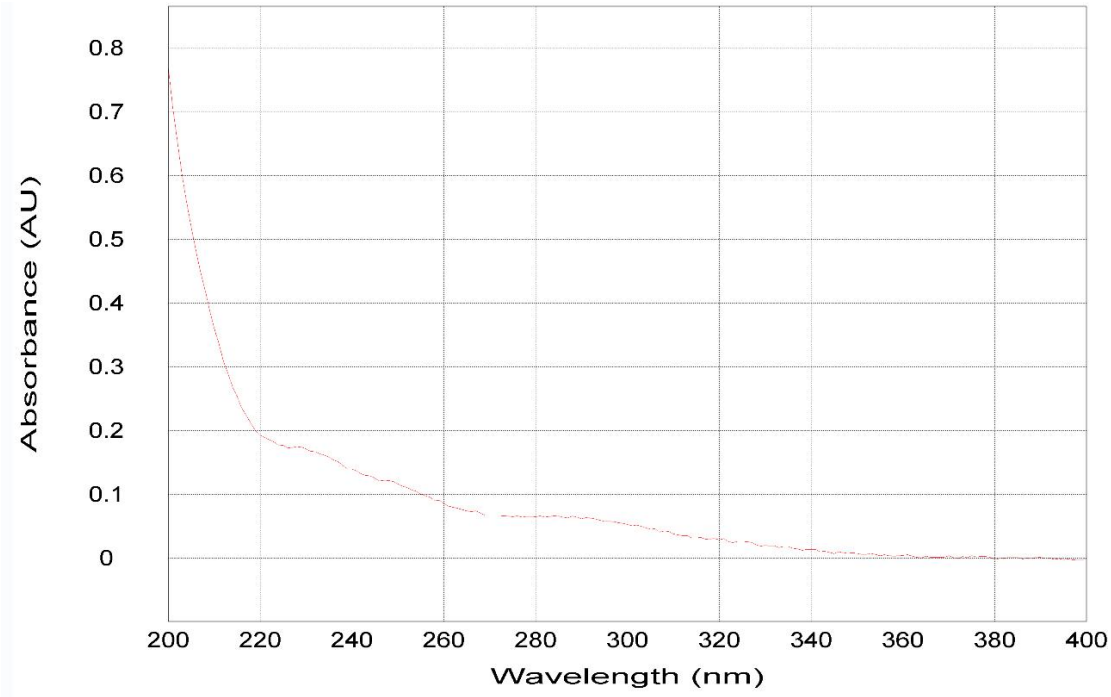


Figure S4. UV spectrum of compound **1**

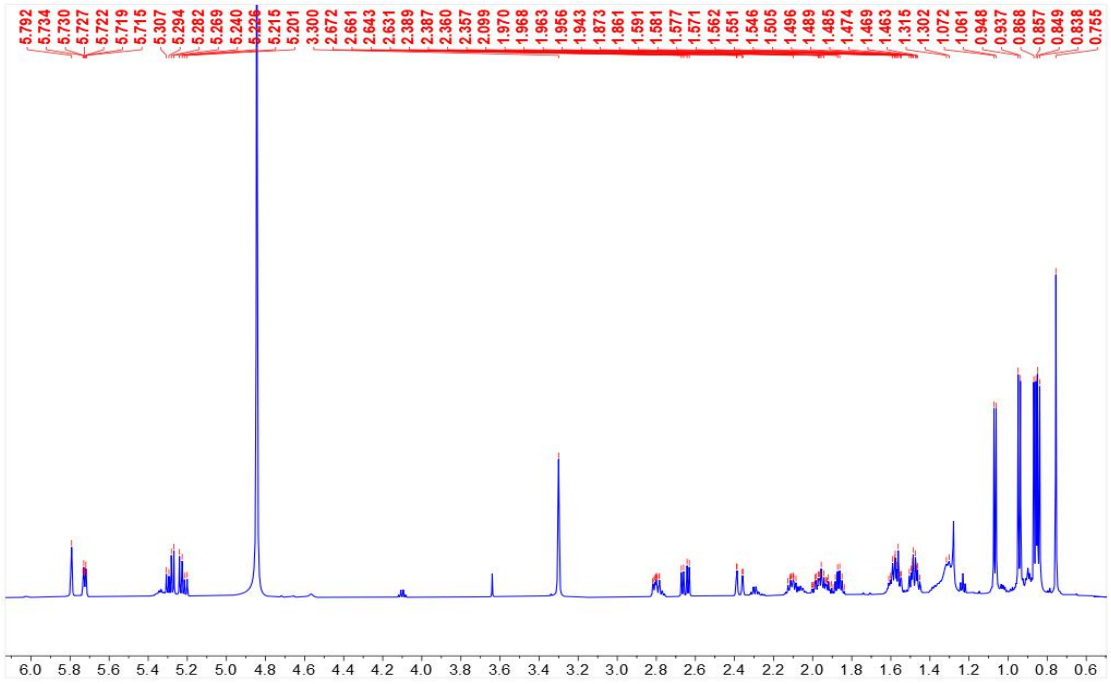


Figure S5. ^{1}H-NMR spectrum of compound **1**

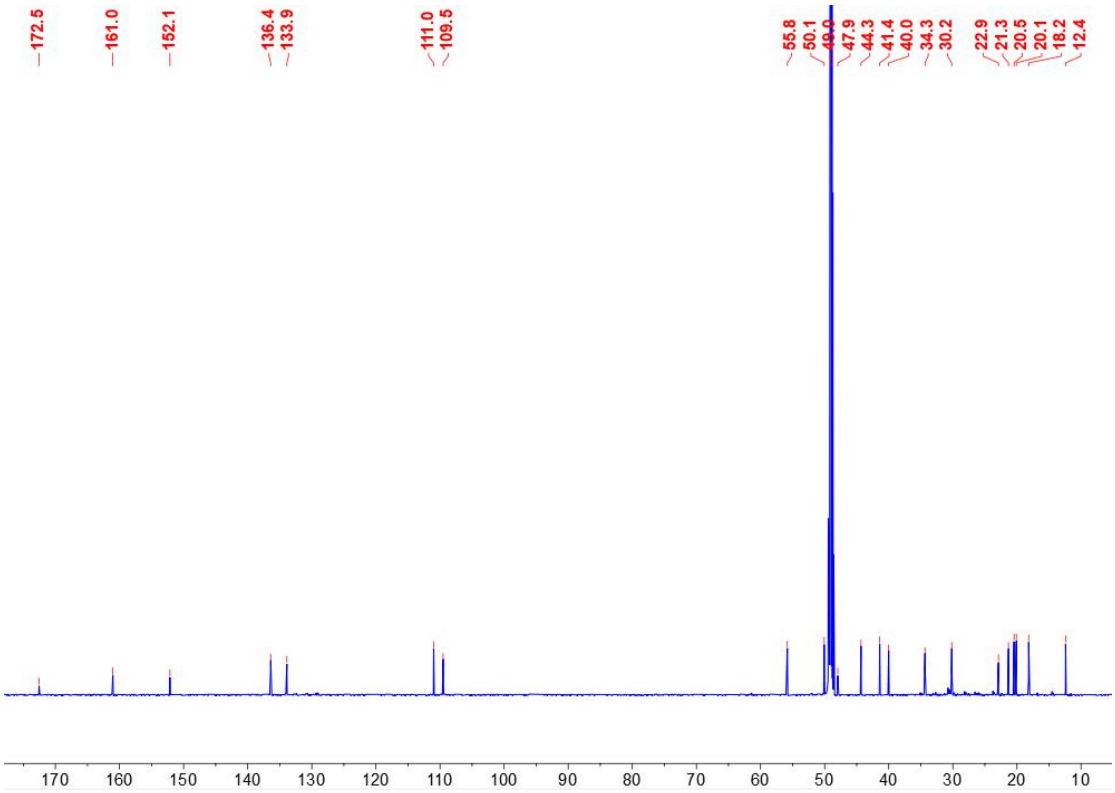


Figure S6. ^{13}C-NMR spectrum of compound **1**

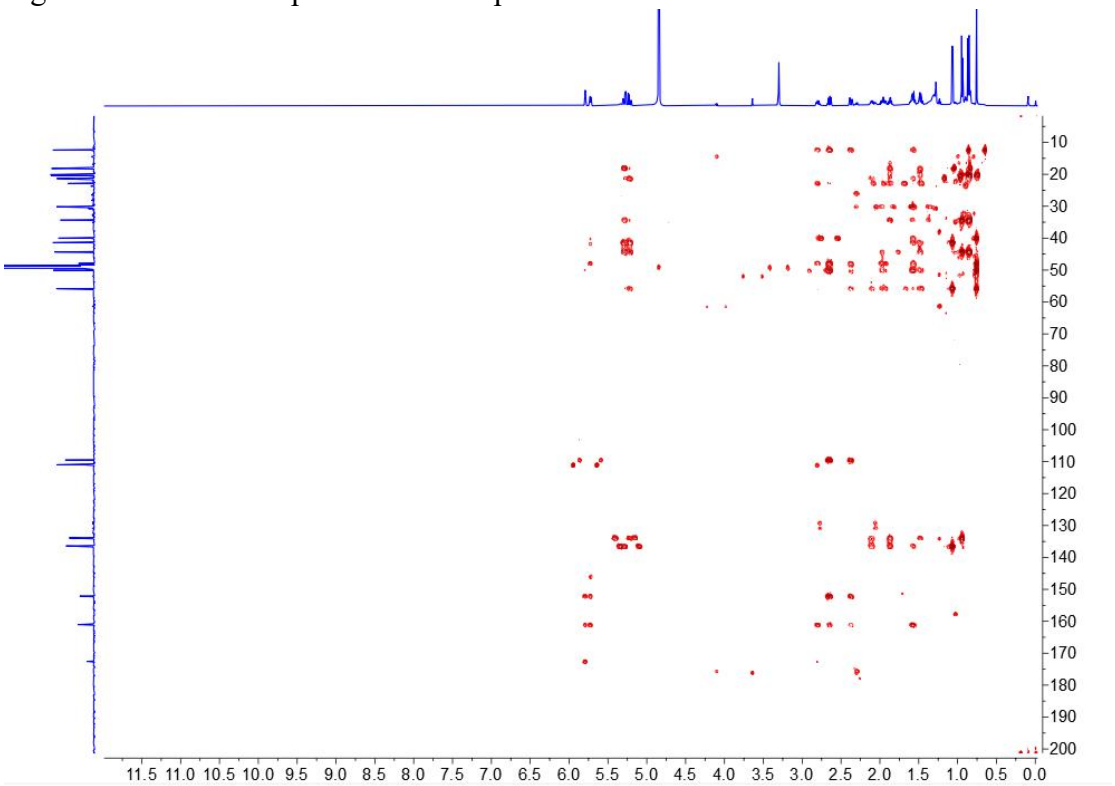


Figure S7. HMBC spectrum of compound **1**

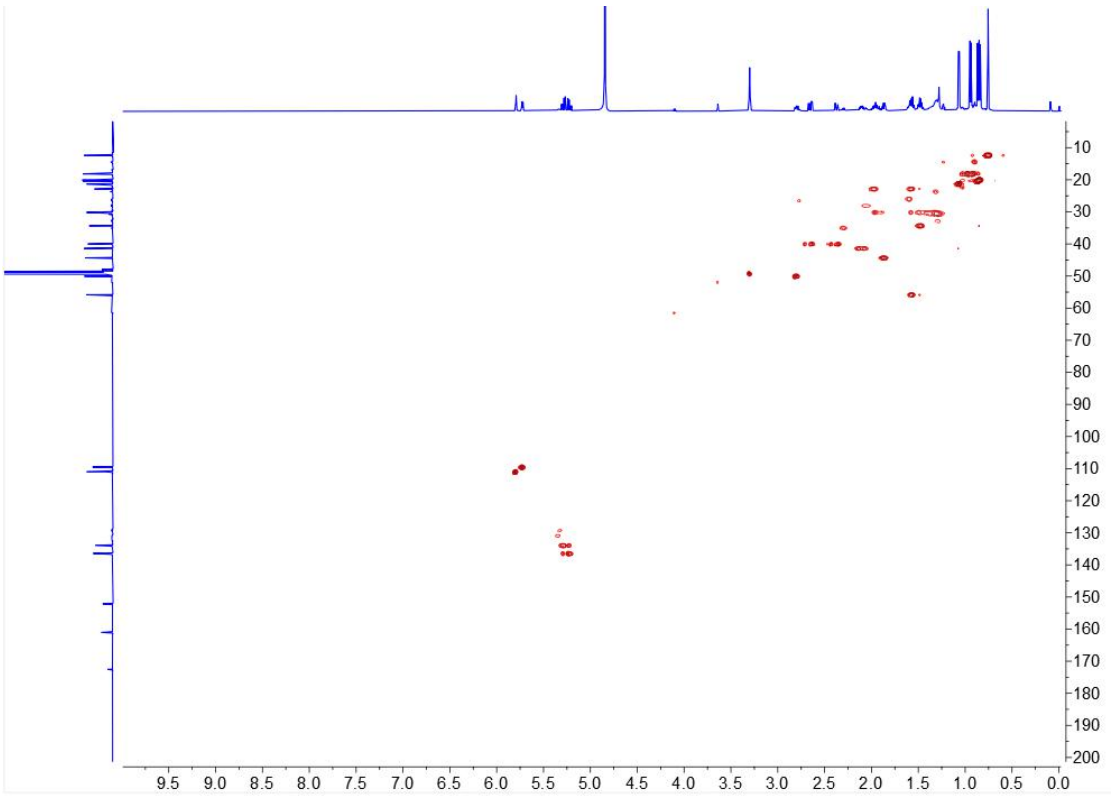


Figure S8. HSQC spectrum of compound **1**

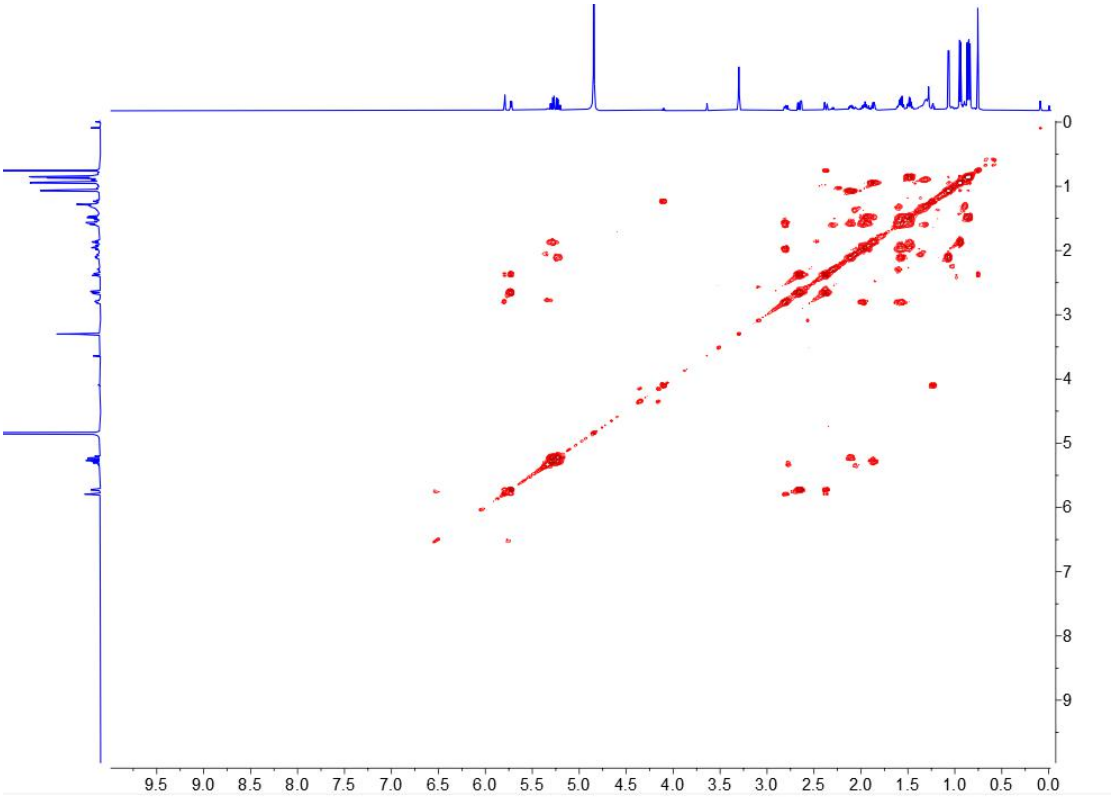


Figure S9. ^{1}H-^{1}H COSY spectrum of compound **1**

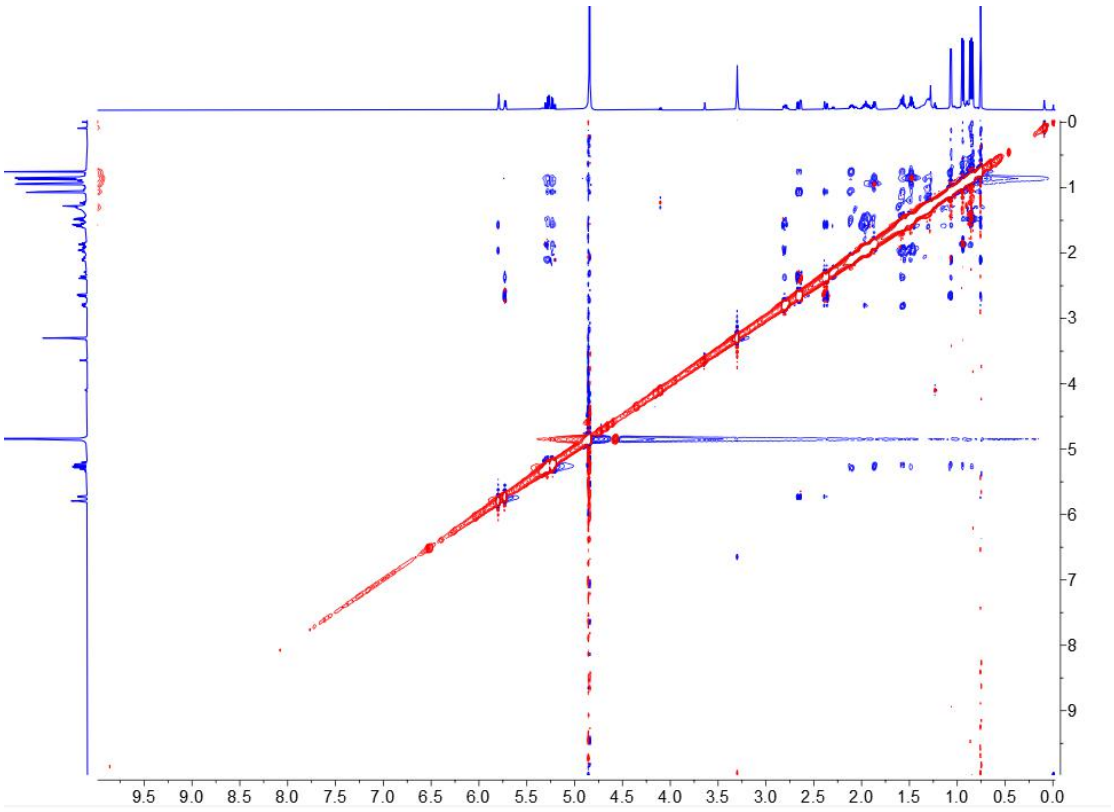


Figure S10. NOESY spectrum of compound **1**

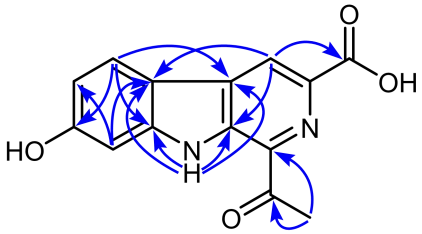
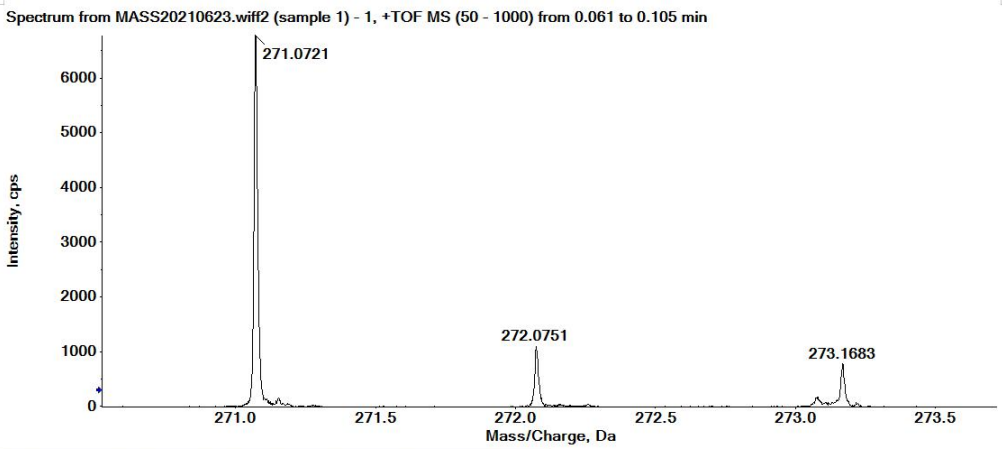


Figure S11. The HMBC correlations of compound **2**



Hit	Formula	m/z	RDB	ppm	MS Rank	MSMS ppm	MSMS Rank	Found
1	C14H10N2O4	271.0713	11.0	2.8	1			NA/NA

Figure S12. HR-ESI-MS spectrum of compound **2**

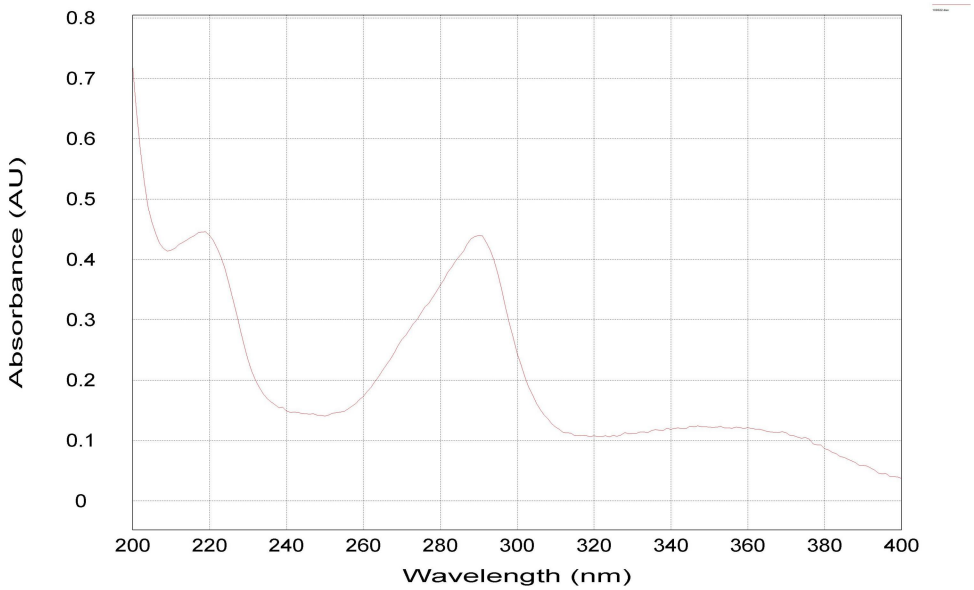


Figure S13. UV spectrum of compound **2**

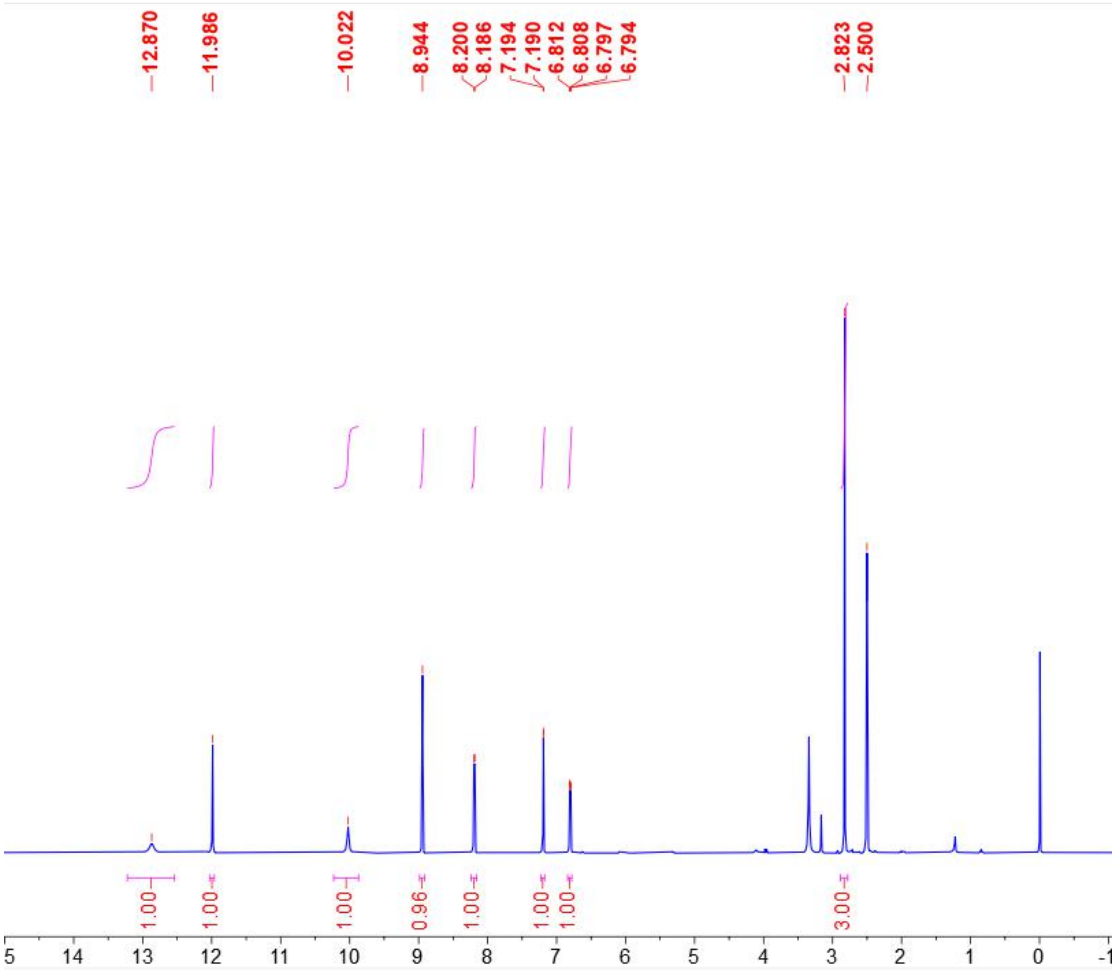


Figure S14. ^{1}H-NMR spectrum of compound **2**

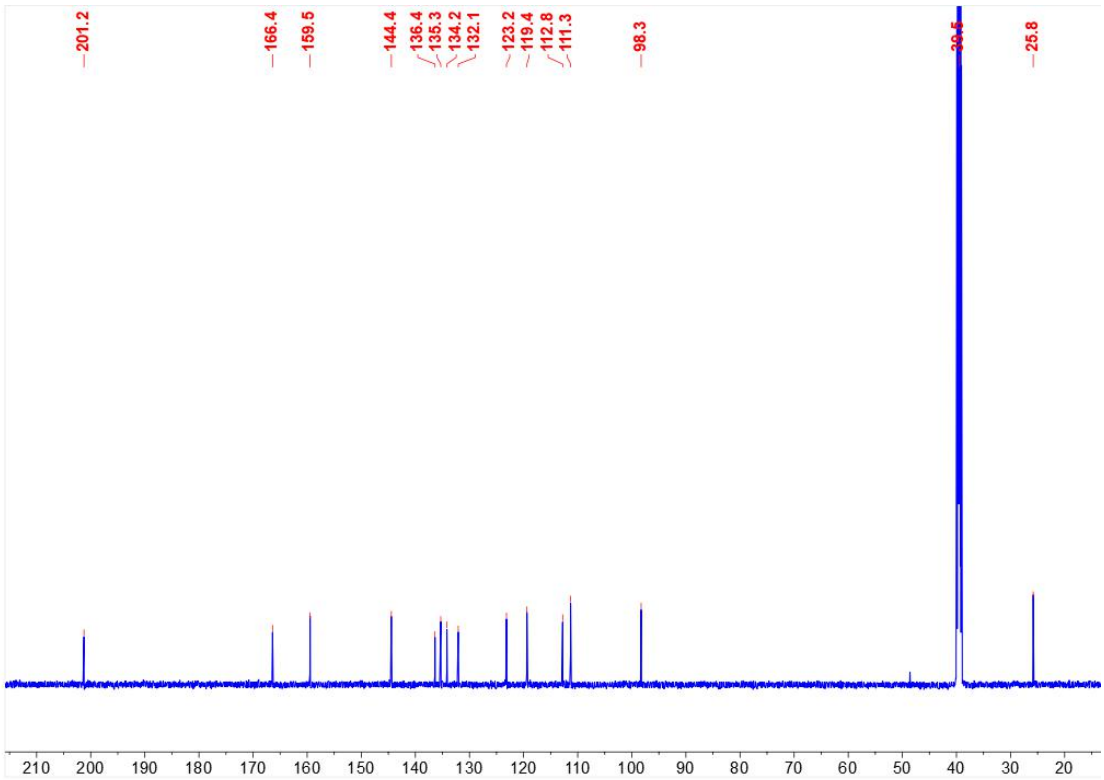


Figure S15. ^{13}C-NMR spectrum of compound **2**

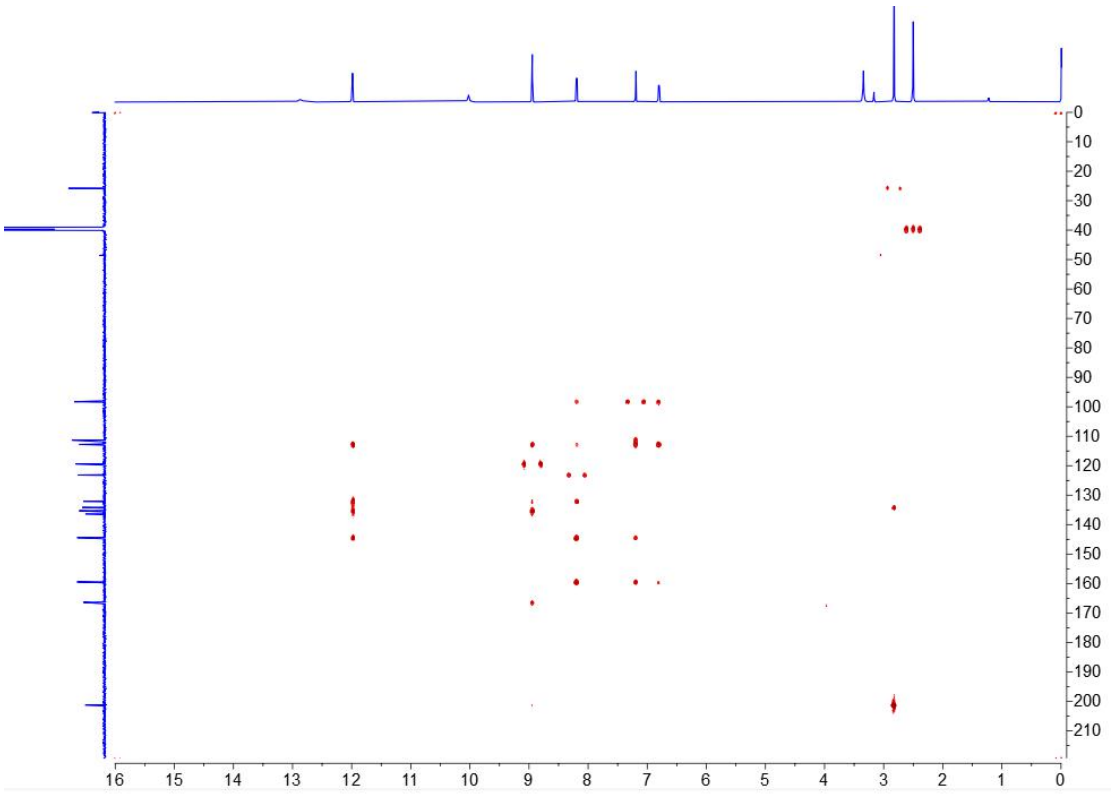


Figure S16. HMBC spectrum of compound **2**

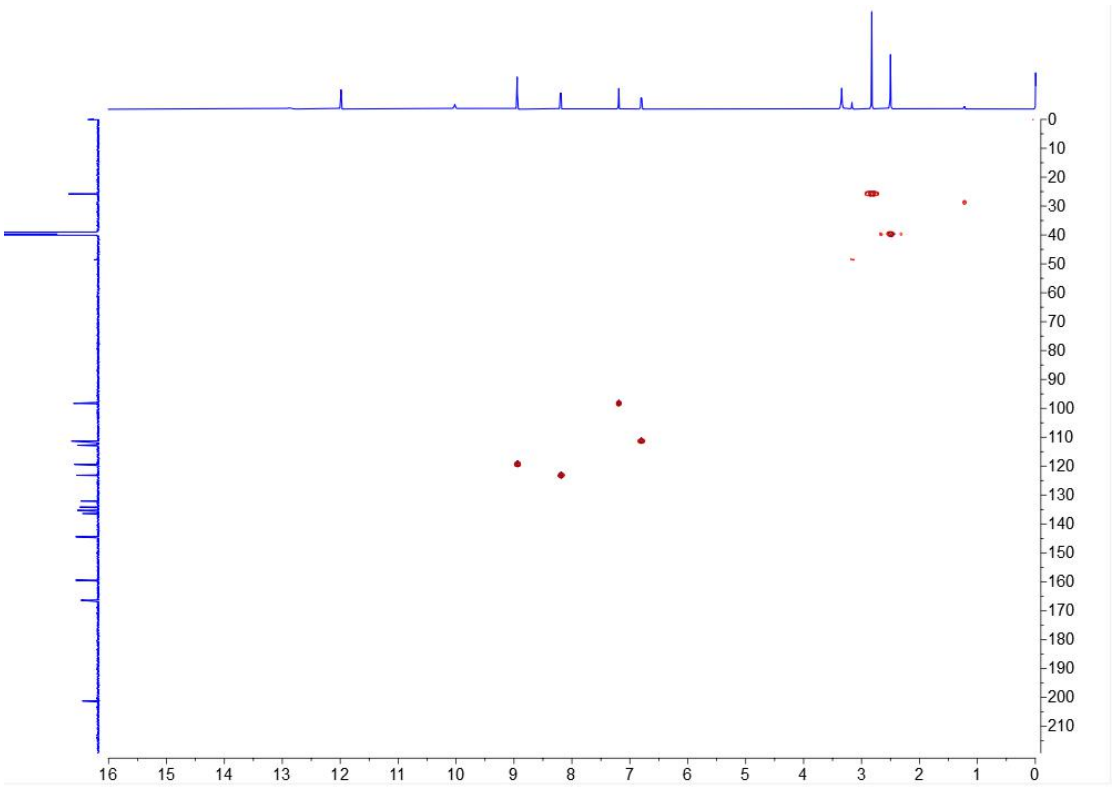


Figure S17. HSQC spectrum of compound **2**

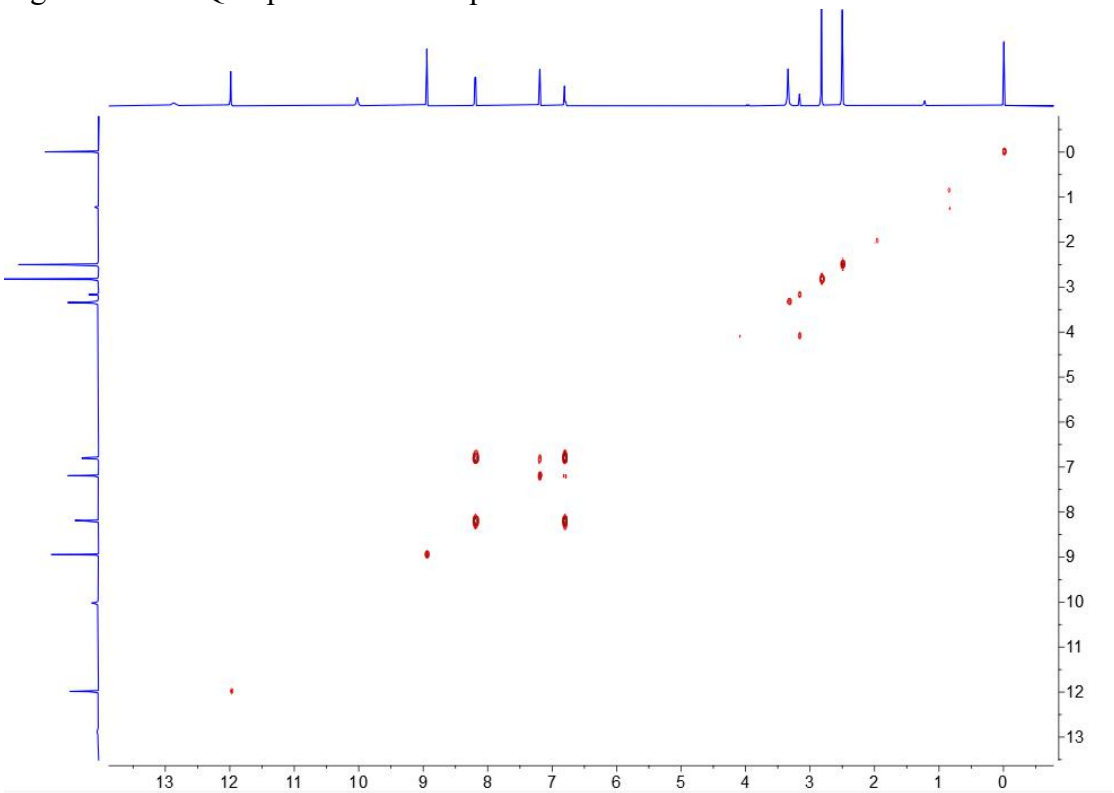


Figure S18. ^{1}H-^{1}H COSY spectrum of compound **2**

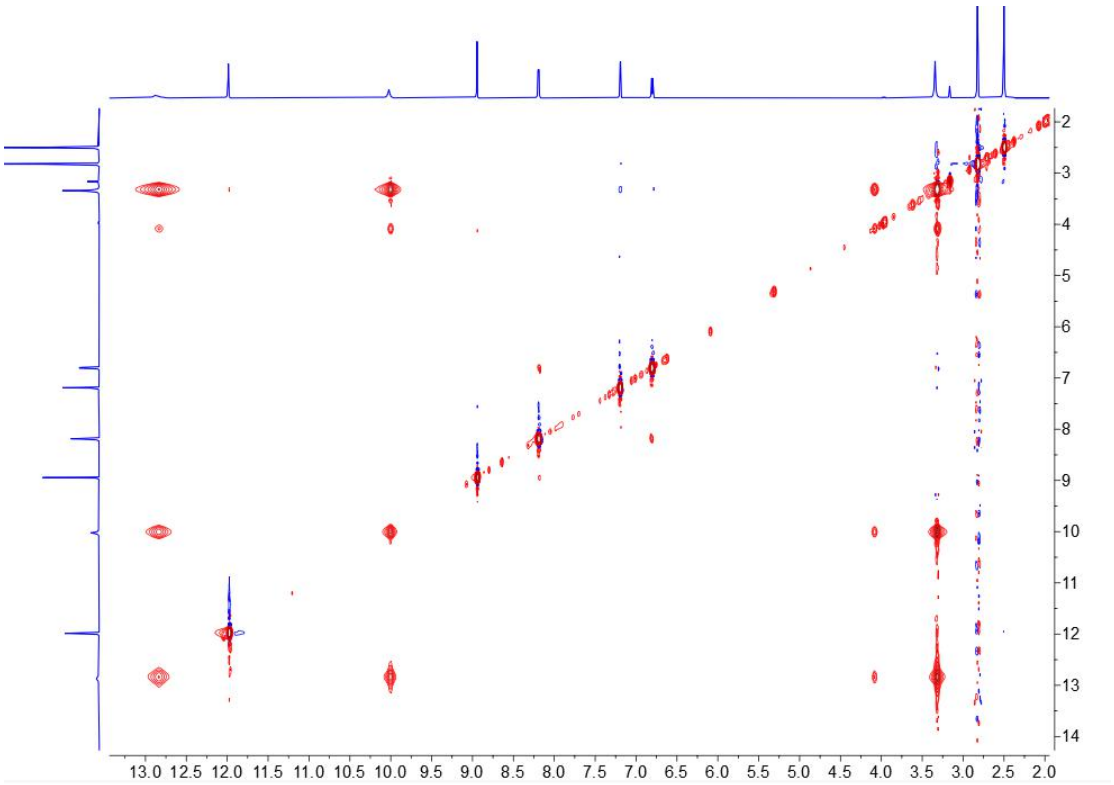


Figure S19. NOESY spectrum of compound **2**

Table S1. The ^{1}H and ^{13}C NMR spectral data for **1** in CD_3OD.

No.	^{1}H NMR	^{13}C NMR
1		172.5 (s)
2	5.79 (1H, br s)	111.0 (d)
3		161.0 (s)
4		152.1 (s)
5	5.73 (1H, ddd, 6.9, 2.4, 1.9)	109.5 (d)
6	2.37 (1Hα, dd, 17.6, 1.9)	40.0 (t)
	2.65 (1Hβ, dd, 17.6, 6.9)	
7		47.9 (s)
8	2.80 (1H, ddd, 11.5, 7.4, 2.2)	50.1 (d)
9	1.58 (1Hβ, m)	22.9 (t)
	1.97 (1Hα, m)	
10	1.27–1.38 (2H, m)	30.2 (t)
11	1.57 (1H, m)	55.8 (d)
12	0.76 (3H, s)	12.4 (q)
13	2.11 (1H, m)	41.4 (d)
14	1.07 (3H, d, 6.6)	21.3 (q)
15	5.22 (1H, dd, 15.3, 8.4)	136.4 (d)
16	5.29 (1H, dd, 15.3, 7.8)	133.9 (d)
17	1.87 (1H, m)	44.3 (d)
18	1.48 (1H, m)	34.3 (d)
19	0.84 (3H, d, 6.8)	20.1 (q)
20	0.86 (3H, d, 6.8)	20.5 (q)
21	0.94 (3H, d, 6.9)	18.2 (q)

Table S2. The ^{1}H and ^{13}C NMR spectral data for **2** in DMSO-d_6.

No.	^{1}H NMR	^{13}C NMR
1		134.2 (s)
3		136.4 (s)
4	8.94 (1H, s)	119.4 (d)
5	8.19 (1H, d, 8.6)	123.2 (d)
6	6.80 (1H, dd, 8.6, 2.0)	111.3 (d)
7		159.5 (s)
8	7.19 (1H, d, 2.0)	98.3 (d)
10		135.3 (s)
11		132.1 (s)
12		112.8 (s)
13		144.4 (s)
14		201.2 (s)
15	2.82 (3H, s)	25.8 (q)
16		166.4 (s)
7-OH	10.02 (1H, br s)	
9-NH	11.99 (1H, s)	
16-OH	12.87 (1H, br s)	

Table S3. Inhibition of LPS-induced NO production in RAW264.7 cells of new compounds **1** and **2**

Compounds	IC_{50} (μM)	Cytotoxicity (μM)
1	71.36 ± 5.11	> 80
2	9.88 ± 0.48	> 80
aminoguanidine[a]	8.92± 0.66	> 80

[a]aminoguanidine was used as a positive control.