Supplementary Material

Coumarin-pi, a new antioxidant coumarin derivative from *Paxillus involutus*

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ABSTRACT

Chemical investigation of *Paxillus involutus* lead to the isolation of a new coumarin derivative coumarin-pi (**1**), and three known compounds (**2**-**4**). The structure of the new compound was elucidated by interpretation of 1D and 2D NMR data. Compound **1** possesses a rare benzofuranylcoumarin skeleton. The isolated compounds were evaluated for antioxidant activities and coumarin-pi (**1**) exhibited significant activity with IC_{50} value of 16.3 µg/mL.

Keywords

Paxillus involutus; benzofuranylcoumarin; antioxidant activity

1. Extraction and isolation

The fruiting bodies of *Paxillus involutus* were collected from Yanshan Mountain, Hebei Province, and identified by Prof. Li-an Wang.

The fresh fruiting bodies were dried in a constant temperature drying box at 42°C until a constant weight was reached, and then the material was powdered in a grinder. The powdered dried *Paxillus involutus* fruit (3 kg) was stirred in 30 L of ethyl acetate for 2 h and left to stand overnight at 4°C. The remaining precipitate was re-extracted 3-5 times following the protocol described above. The organic layer was concentrated under vacuum to yield the crude extract (94 g).

This residue was separated by silica gel CC by gradient elution with petroleum ether–ethyl acetate (1:0 to 0:1). The fraction eluted by petroleum ether–ethyl acetate at 5:1 was separated by silica gel CC to afford compound **2** and compound **3**.The fraction eluted by ethyl acetate was concentrated under vacuum and further separated by silica gel CC with $CHCl_3$–MeOH (1:0 to 0:1). The fraction eluted by $CHCl_3$–MeOH at 10:1 was separated by Sephadex LH-20 (MeOH) to afford compound **1.**The fraction eluted by $CHCl_3$–MeOH at 5:1 was separated by silica gel CC to afford compound **4.**

2. DPPH radical scavenging activity

All reactions were conducted in 96-well microplates. The coumarin-pi was re-dissolved in methanol. Coumarin-pi at defined concentrations was mixed with 100 μL (2×10^{-4} mol/L) of DPPH solution. BHA was used as the positive control. Additionally, 100 μL of coumarin-pi at defined concentrations was mixed with 100 μL

of ethanol, and these samples were used as *blanks*. Finally, 100 μL of methanol and 100 μL (2×10^{-4} mol/L) of the DPPH solution were mixed, and this sample was used as the *control*. After 30 min of incubation at ambient temperature in darkness, the absorbance was determined at 517 nm with a spectrophotometer. Each experiment was repeated 6 times.

The scavenging activity was calculated according to the formula below:

DPPH Scavenging (%) = $[1-(A_{sample}-A_{blank})/A_{control}] \times 100$

where A_{sample} was the absorbance of coumarin-pi or BHA mixed with DPPH at a particular concentration, and A_{blank} and $A_{control}$ were the samples mentioned above.

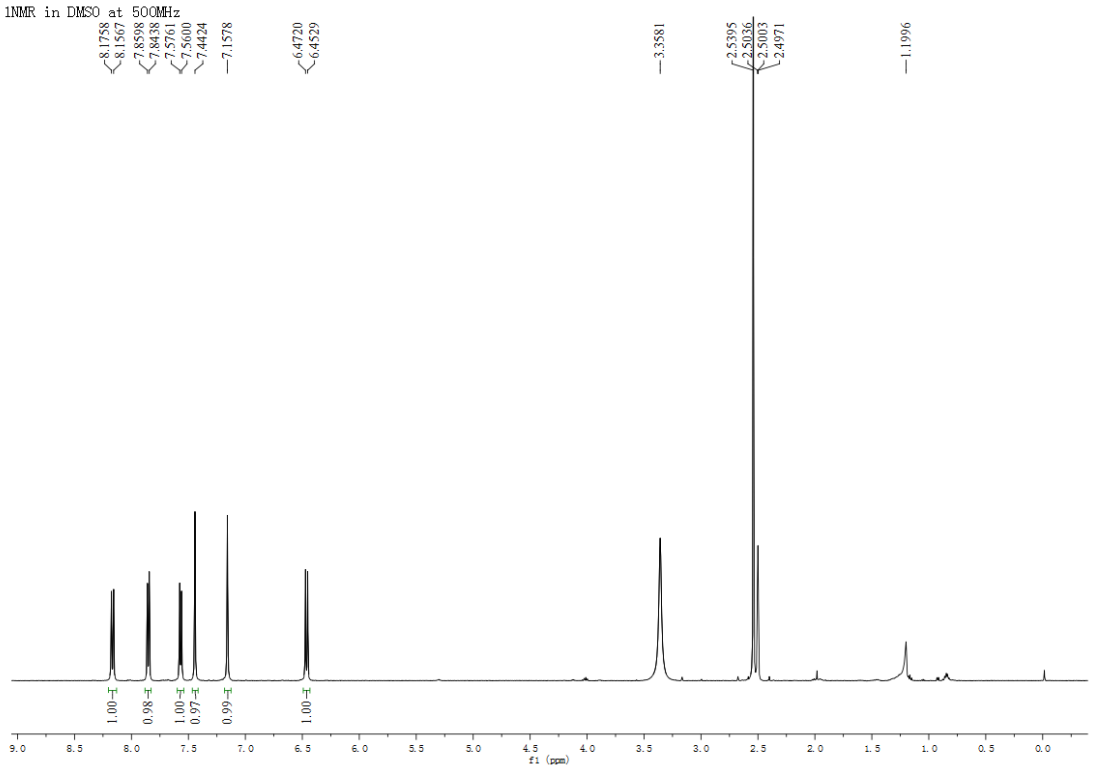


Figure S1.[1]H-NMR spectrum of compound **1**

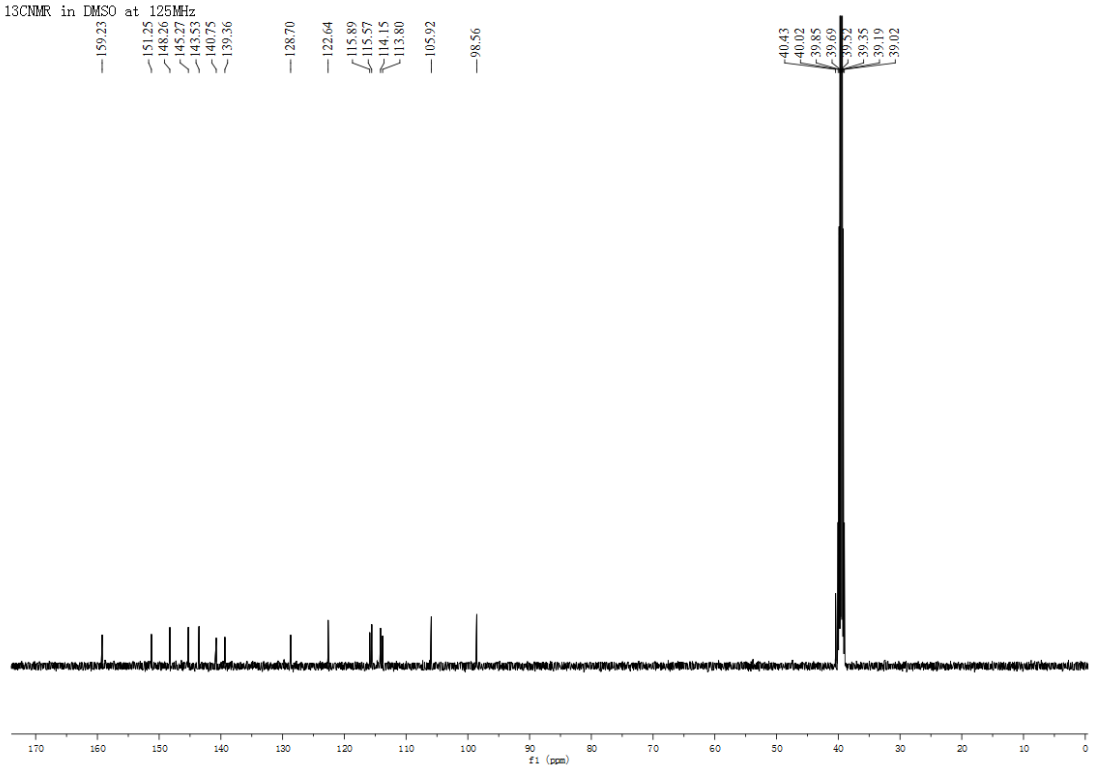


Figure S2.[13]C-NMR spectrum of compound **1**

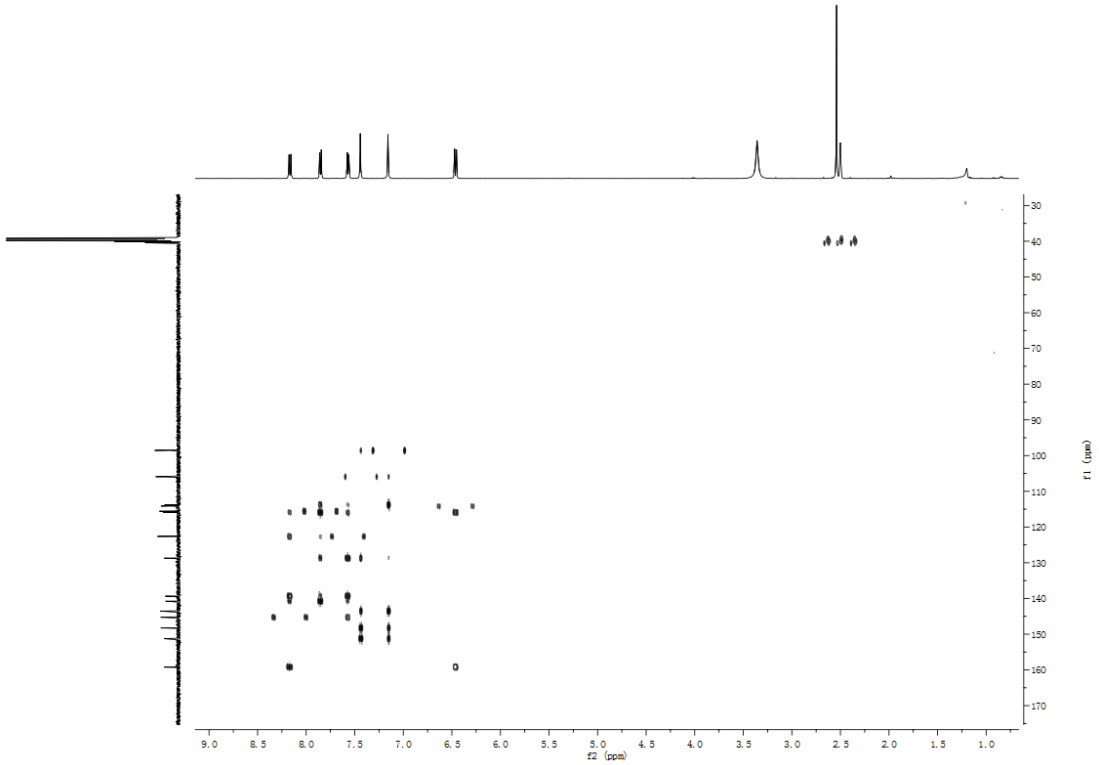


Figure S3.HMBC spectrum of compound **1**

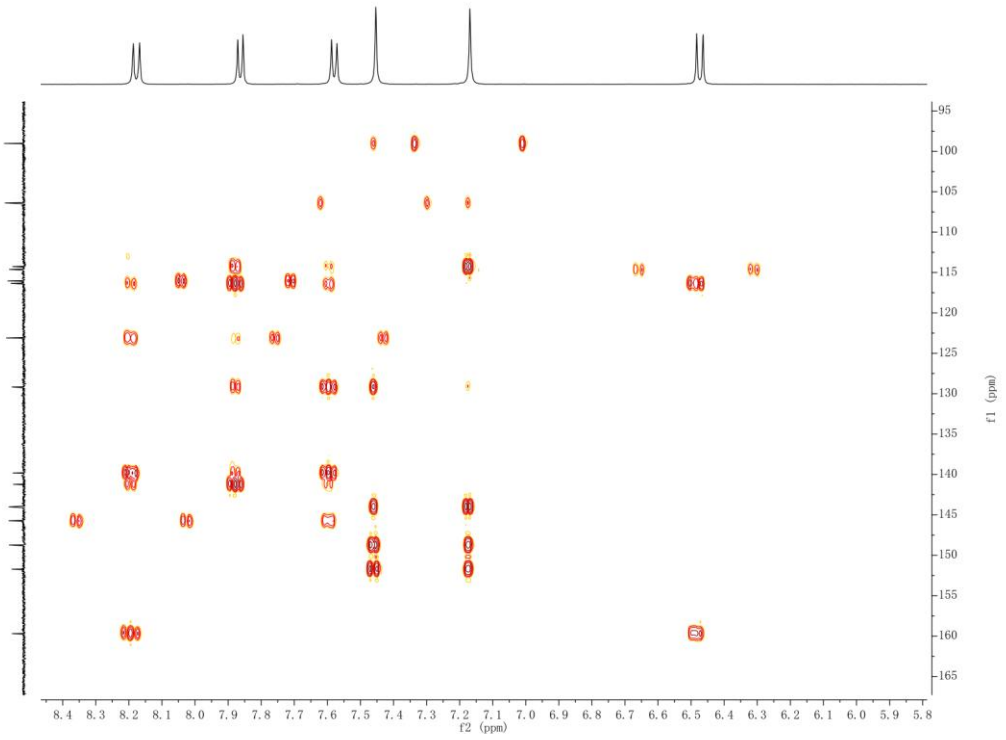


Figure S4.HMBC spectrum of compound **1**

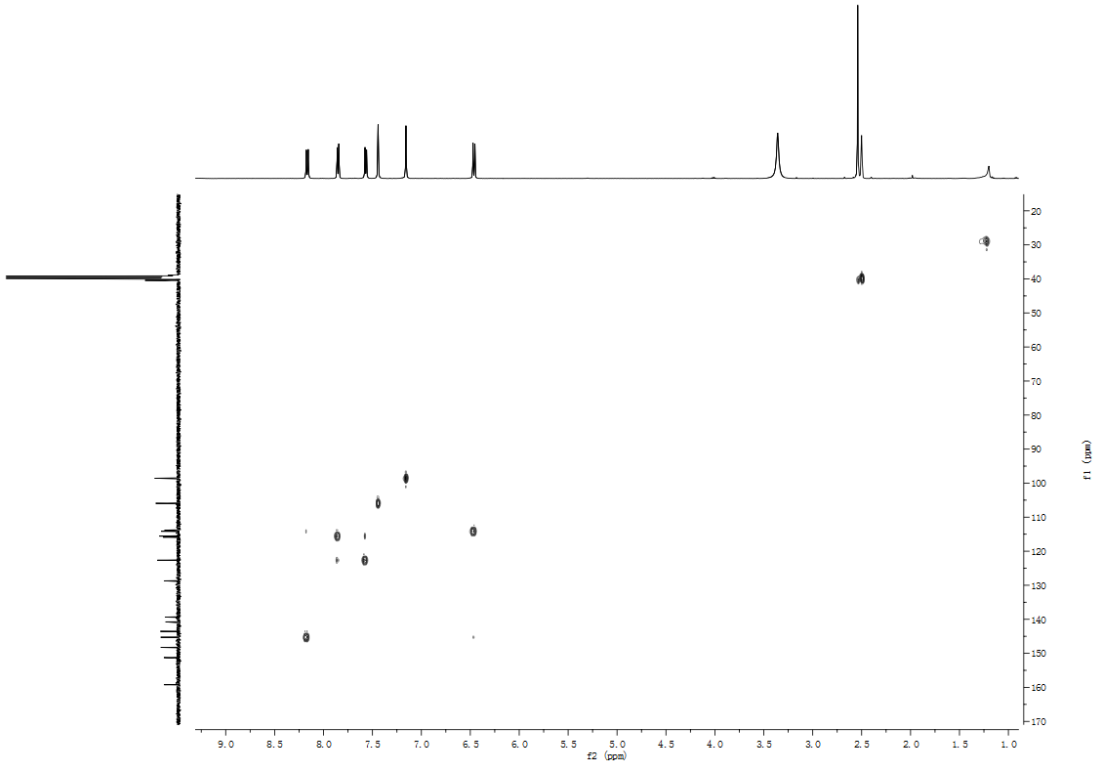


Figure S5.HSQC spectrum of compound **1**

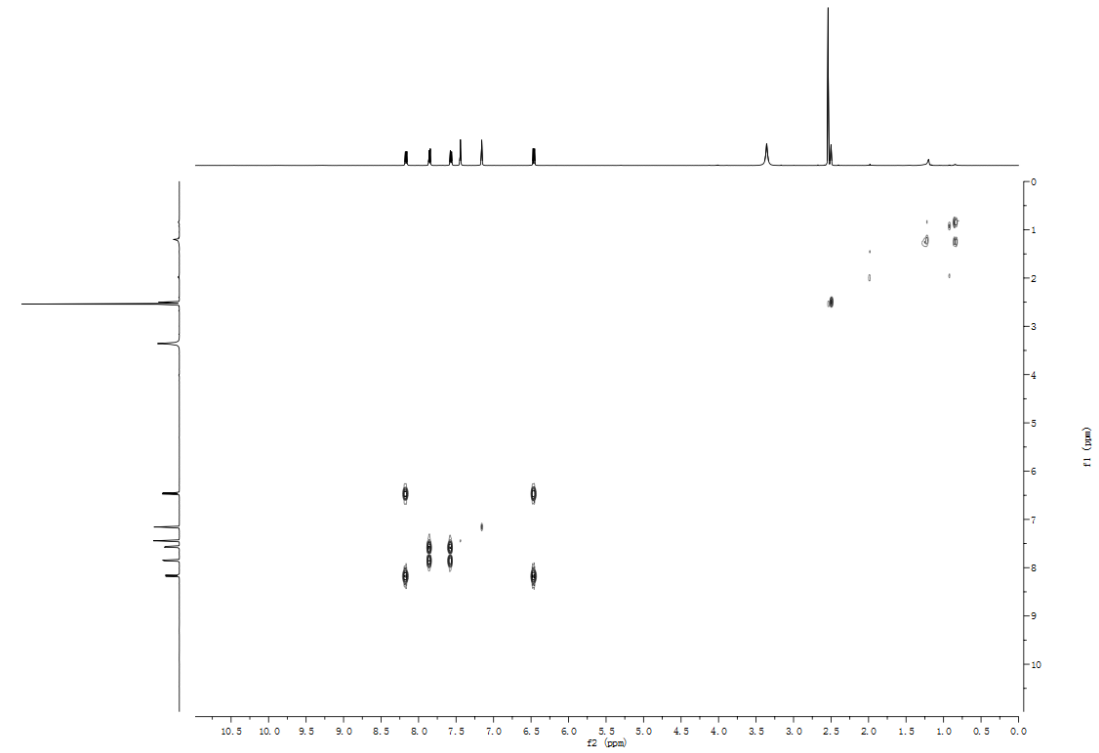


Figure S6.^{1}H-^{1}H COSY spectrum of compound **1**

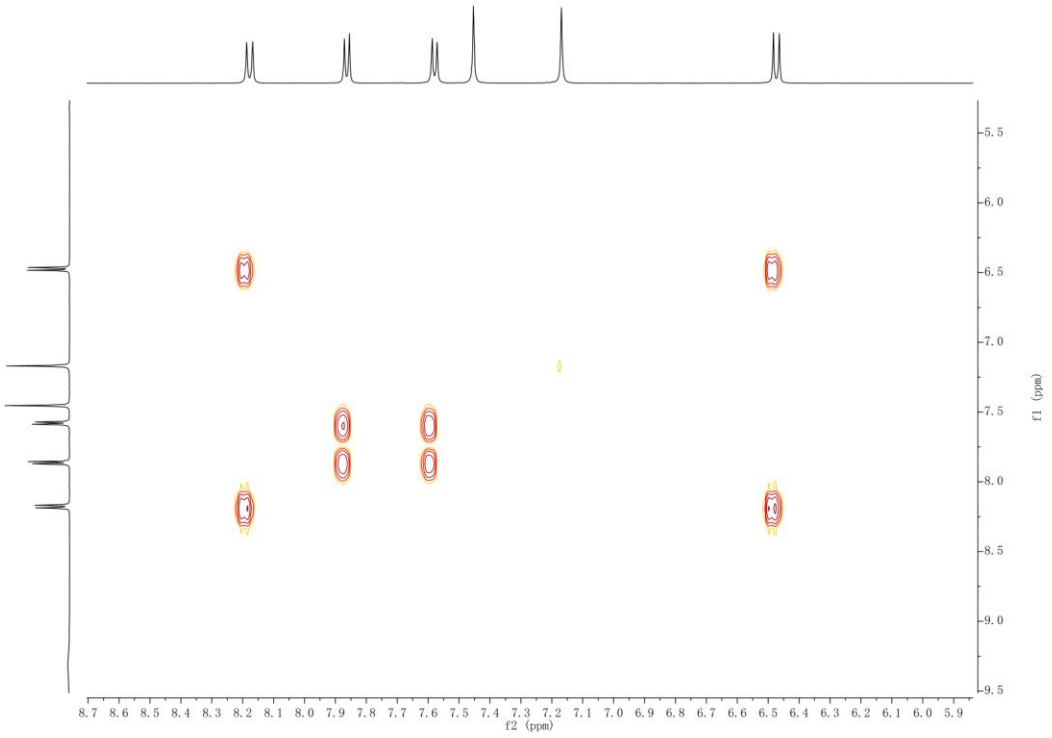
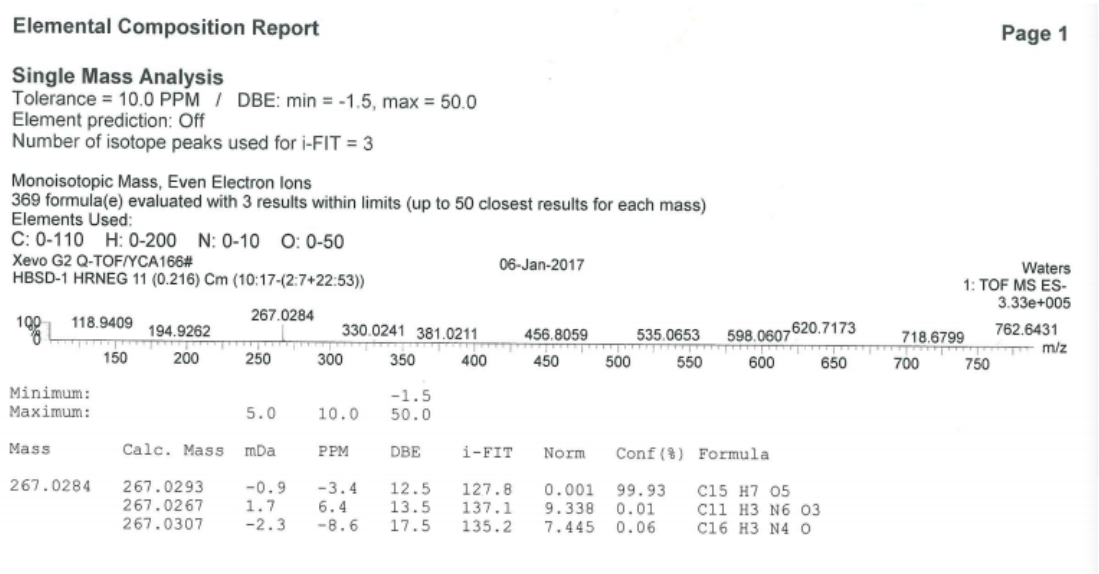


Figure S7.^{1}H-^{1}H COSY spectrum of compound **1**



Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
369 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-110 H: 0-200 N: 0-10 O: 0-50

Xevo G2 Q-TOF/YCA166#
HBSD-1 HRNEG 11 (0.216) Cm (10:17-(2:7+22:53))

06-Jan-2017

Waters
1: TOF MS ES-
3.33e+005

Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
267.0284	267.0293	-0.9	-3.4	12.5	127.8	0.001	99.93	C15 H7 O5
	267.0267	1.7	6.4	13.5	137.1	9.338	0.01	C11 H3 N6 O3
	267.0307	-2.3	-8.6	17.5	135.2	7.445	0.06	C16 H3 N4 O

Figure S8.HRESI-MS spectrum of compound **1**

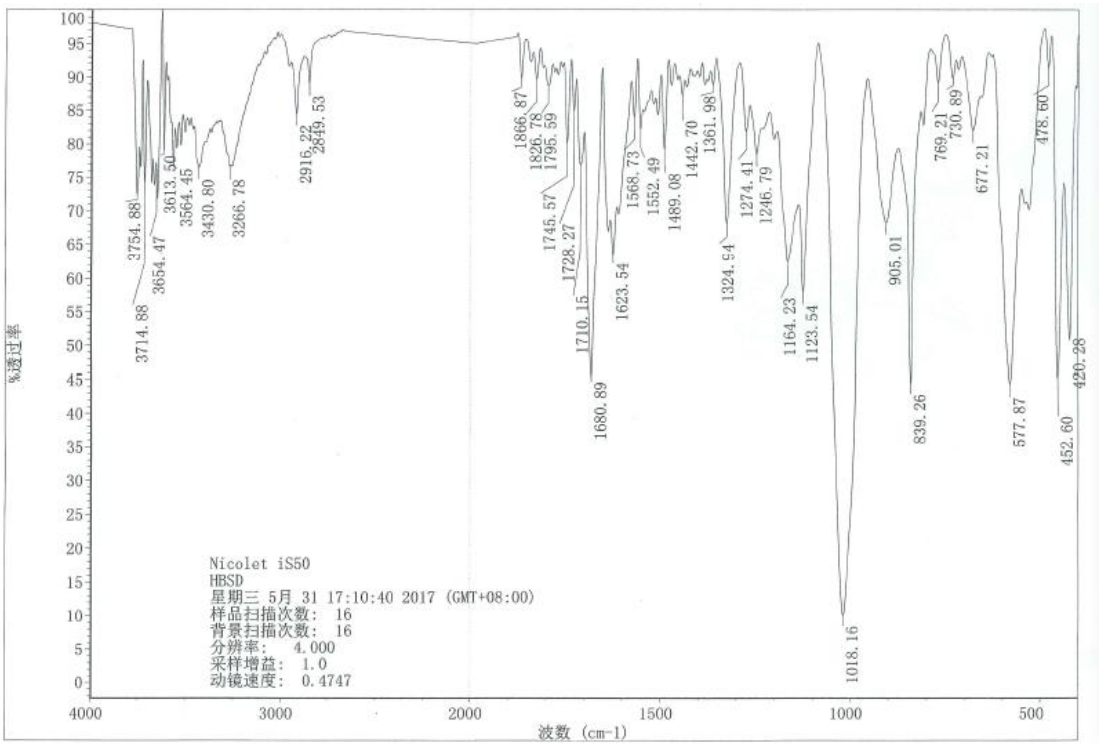


Figure S9.IR spectrum of compound **1**

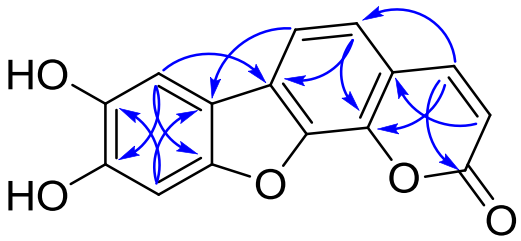


Figure S10. Key HMBC correlations of compound **1**.

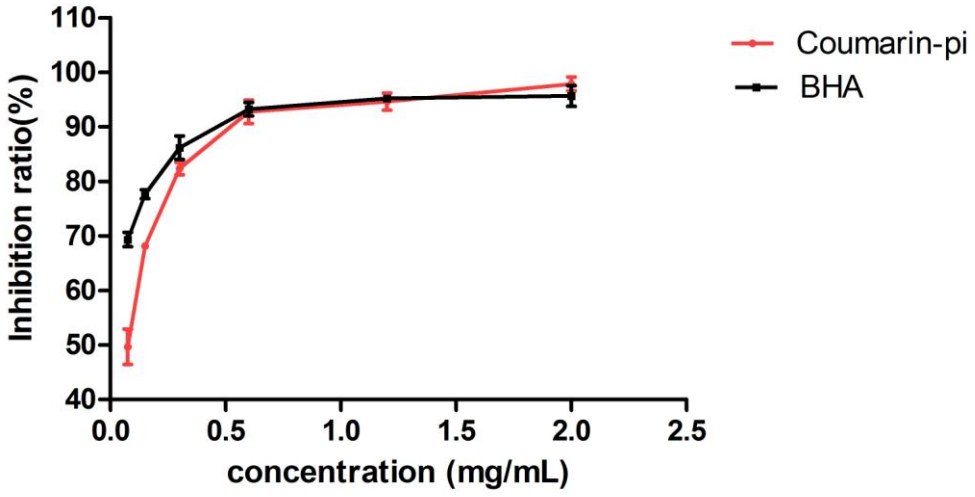


Figure S11. The antioxidant activity of compound **1**.

Table S1. ^{1}H (500 MHz) and ^{13}C (150 MHz) NMR data of compound **1** in DMSO-d_6.

position	δ_C, type	δ_H (J in Hz)
2	159.2, C	
3	114.1, CH	6.46 (1H, d, J = 9.5 Hz)
4	145.3, CH	8.17 (1H, d, J = 9.5 Hz)
4a	115.9, C	
5	122.6, CH	7.57 (1H, d, J = 8.0 Hz)
6	115.6, CH	7.85 (1H, d, J = 8.0 Hz)
7	128.7, C	
8	140.8, C	
8a	139.4, C	
9	113.8, C	
10	151.3[a], C	
11	98.6, CH	7.16 (1H, s)
12	148.3[b], C	
13	143.5[c], C	
14	105.9, CH	7.44 (1H, s)

[a, b, c] maybe interchanged.