SUPPLEMENTARY MATERIAL

Chromone and isocoumarin derivatives from the endophytic fungus *Xylomelasma* sp. Samif07, and their antibacterial and antioxidant activities

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Abstract: Five chromone derivatives, including 2,6-dimethyl-5-methoxyl-7-hydroxylchromone (**1**), 6-hydroxymethyleugenin (**2**), 6-methoxymethyleugenin (**3**), chaetoquadrin D (**4**), and isoeugenitol (**5**), and three isocoumarin congeners, namely diaporthin (**6**), 8-hydroxy-6-methoxy-3-methylisocoumarin (**7**), and 6-methoxymellein (**8**), were isolated from the culture of the endophytic fungus *Xylomelasma* sp. Samif07 derived from the medicinal plant *Salvia miltiorrhiza* Bunge. Among them, compound **1** was a new natural product. Their structures were determined by spectroscopic methods and comparison with the literature. The isolated compounds were evaluated for their antibacterial and antioxidant activities. Compound **5** showed notable antitubercular activity against *Mycobacterium tuberculosis* with MIC value of 10.31 μg/mL, while compounds **1**-**3**, and **5**-**7** displayed inhibitory activities against the other bacteria with MIC range of 25~100 μg/mL. Meanwhile, compound **6** showed potent hydroxyl radical-scavenging activity with EC_{50} value of 15.1 μg/mL, while compounds **5**-**7** showed certain ferric reducing ability.

Keywords: Endophytic fungus; *Xylomelasma* sp. Samif07; chromone; isocoumarin; antibacterial activity; antioxidant activity

Contents

Experimental section

General experimental procedures

High resolution electrospray ionization mass spectrometry (HRESIMS) spectra were recorded on an LC1260-Q-TOF/MS 6520 machine (Agilent Technologies, CA, USA). ^{1}H, ^{13}C, and 2D NMR spectra were measured on an Avance 400 NMR spectrometer (Bruker BioSpin, Zurich, Switzerland). Chemical shifts were expressed in δ (ppm) referenced to the solvent residual peaks (δ_H 2.05/δ_C 29.84, δ_H 3.31/δ_C 49.0 for acetone-d_6 and CD_3OD, respectively), and coupling constants (J) in hertz. Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden), and silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China) were used for column chromatography. Semi-preparative HPLC separation was carried out on a Lumtech K-501 pump (Lumiere Tech. Ltd., Beijing, China) with a K-2501 UV detector using a Luna-C18 column (250 mm × 10 mm i.d., 5 μm, Phenomenex Inc., Torrance, CA, USA). High performance liquid chromatography (HPLC) analysis was performed using a Shimadzu LC-20A instrument with a SPD-M20A photodiode array detector (Shimadzu Corp., Tokyo, Japan) and an analytic C_{18} column (250 mm × 4.6 mm i.d., 5 μm; Phenomenex Inc., Torrance, California, USA). The precoated silica gel GF-254 plates (Qingdao Marine Chemical Inc., China) on glass were used for analytical thin layer chromatography (TLC). Spots were visualized under UV light (254 or 356 nm) or by spraying with 10% H_2SO_4 in 95% ethanol followed by heating.

Fungal material and fermentation

The fungus (strain No. Samif07) was isolated from the healthy root of the medicinal plant *Salvia miltiorrhiza* Bunge (Labiatae), which was collected from the Beijing Medicinal Plant Garden, P. R. China, in 2013. This fungus was identified as X*ylomelasma* sp. by morphological inspection and by comparing its ITS sequence (GenBank accession No. KC878701) to the closet species through BLAST analysis (Lou et al. 2013). A voucher specimen was deposited at the Department of Plant Pathology, China Agricultural University.

The fungus was grown on potato dextrose agar (PDA) plates at 25 °C for 10 days. Then, the fungal hyphae were transferred to several 250 mL-Erlenmeyer flasks each

containing 150 mL potato dextrose broth (PDB) medium, and incubated on a rotary shaker at 120 rpm and 25 °C for 9 days, to prepare the seed culture.

The scale-up liquid fermentation was carried out in 1 L-Erlenmeyer flasks, each containing 500 mL PDB, while the solid fermentation was carried out in 1 L-Erlenmeyer flasks, each containing 120 g rice with 120 mL distilled water. Each flask was inoculated with a seed culture. The liquid fermentation (6.0 L) was incubated on a rotary shaker at 120 rpm and 25 °C for 19 days, while the static fermentation (2.4 kg of rice) was kept at 28 °C for 60 days before harvest.

Extraction and isolation

The liquid cultures were filtrated to separate the mycelia and filtrate. The mycelia were extracted with methanol, followed by concentration in a rotary evaporator under vacuum to remove the solvent, which resulted in 5.33 g of methanol extract.

The rice fermentations were extracted with EtOAc for three times. Then, the EtOAc extracts were combined and concentrated under vacuum to obtain a brownish residue (35.0 g).

The methanol extract was subjected to vacuum liquid chromatography (VLC) over silica gel eluting with a gradient of CH_2Cl_2−MeOH (100:0–0:100) to obtain six fractions (Frs. M1−M6). Fraction M2 was subjected to medium pressure liquid chromatography (MPLC) over silica gel using a gradient of CH_2Cl_2−MeOH (100:0–0:100) as the mobile phase to give four subfractions (Frs. M2a-d). Fr. M2b was chromatographed over Sephadex LH-20 ($CHCl_3$/MeOH=1:1), followed by purification using semi-preparative HPLC (65% MeOH/H_2O) to afford compound **3** (6.6 mg). Likewisely, fractions M3 and M4 were processed in the same way as Fr. M2 to give five (Frs. M3a-e) and six (Frs. M4a-f) subfractions, respectively. Compound **8** (0.6 mg) was isolated from Fr. M3c by column chromatography (CC) over Sephadex LH-20 ($CHCl_3$/MeOH=1:1) and semi-preparative HPLC (55% MeOH/H_2O). Similarly, compound **6** (3.4 mg) was isolated from Fr. M4e by the same token except a different eluent was used for the HPLC (42% MeOH/H_2O).

The EtOAc extract was subjected to VLC over silica gel eluting with a gradient of CH_2Cl_2−MeOH (100:0–0:100) to obtain eight fractions (Frs E1−E8). Fraction E4 was subjected to MPLC using the same procedure described for Fr. M2 to obtain seven subfractions (Frs. E4a-g). Fr. E4b was subjected to gel filtration over Sephadex LH-20 ($CHCl_3$/MeOH=1:1), followed by semi-preparative HPLC (60% MeOH/H_2O)

to give compounds **3** (3.5 mg) and **5** (2.7 mg). Fr. E4d was processed in a similar manner except the eluent for HPLC (52% MeOH/H_2O), to yield **7** (2.5 mg), **1** (10.7 mg), and **2** (8.4 mg). Similarly, compound **4** (0.8 mg) was purified from Fr. E4e using the same procedure (68% MeOH/H_2O for HPLC).

2,6-Dimethyl-5-methoxyl-7-hydroxylchromone (**1**): yellow amorphous powder; UV (online PDA) λ_{max} 211, 227, 245, 292 nm; ^{1}H (in acetone-d_6, or CD_3OD, 400 MHz) and ^{13}C (in acetone-d_6, or CD_3OD, 100 MHz) NMR data, see Table S1; HRESIMS *m/z* 219.0661 $[M-H]^-$ (calcd. for $C_{12}H_{11}O_4$, 219.0663).

Antibacterial assay

The antitubercular activities of compounds **1-8** were tested against *Mycobacterium tuberculosis* as described previously (Daletos et al. 2015), and rifampicin was used as the positive control.

Meanwhile, the antibacterial activities of the isolated compounds except **4** and **8** were tested against *Bacillus subtilis*, *Staphylococcus haemolyticus*, *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Ralstonia solanacearum*, and *Xanthomonas vesicatoria* as described previously (Shan et al. 2014). Streptomycin was used as the positive control.

Hydroxyl radical-scavenging activity assay

The hydroxyl radical-scavenging activity of compounds **1-3** and **5-7** was determined according to the method described previously (Li et al. 2012). Ascorbic acid was used as the positive control.

Fe^{3+} reducing activity assay

The Fe^{3+} reducing capability of compounds **1-3** and **5-7** was determined following the method outlined by Li *et al* (Li, et al. 2012). Butylated hydroxytoluene (BHT) was used as the positive control.

Table S1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of **1**.

Position	In acetone-d_6		In CD_3OD	
	δ_C, type	δ_H, multi. (J in Hz)	δ_C, type	δ_H, multi. (J in Hz)
2	164.3, C	-	166.5, C	-
3	111.56, CH	5.92, s	111.4, CH	6.01, s
4	176.9, C	-	179.6, C	-
4a	111.64, C	-	111.4, C	-
5	159.0, C	-	159.2, C	-
6	117.4, C	-	118.8, C	-
7	161.6, C	-	163.1, C	-
8	99.0, CH	6.71, s	99.3, CH	6.64, s
8a	158.3, C	-	159.1, C	-
2-CH_3	19.6, CH_3	2.26, s	19.8, CH_3	2.32, s
6-CH_3	8.3, CH_3	2.10, s	8.4, CH_3	2.14, s
5-OCH_3	61.4, CH_3	3.75, s	61.8, CH_3	3.77, s

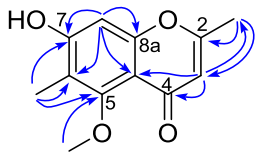


Figure S1. Selected HMBC correlations of **1**.

Table S2. Antibacterial activities of the isolated compounds (MIC, μg/mL).

Bacterium	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Positive control [a]
M. tuberculosis	>40	>40	>40	>40	10.31	>40	>40	>40	1.78
B. subtilis	>100	75	>100	nt [b]	>100	50	25	nt	25
S. haemolyticus	>100	75	75	nt	>100	>100	>100	nt	50
A. tumefaciens	>100	>100	>100	nt	75	>100	75	nt	25
E. Carotovora	100	>100	75	nt	75	>100	>100	nt	50
R. solanacearum	>100	>100	>100	nt	>100	>100	>100	nt	50
X. vesicatoria	>100	>100	>100	nt	>100	>100	25	nt	50

[a] Positive control was rifampicin (against *M. tuberculosis*) or streptomycin sulfate (against the other bacteria).

[b] nt: not tested.

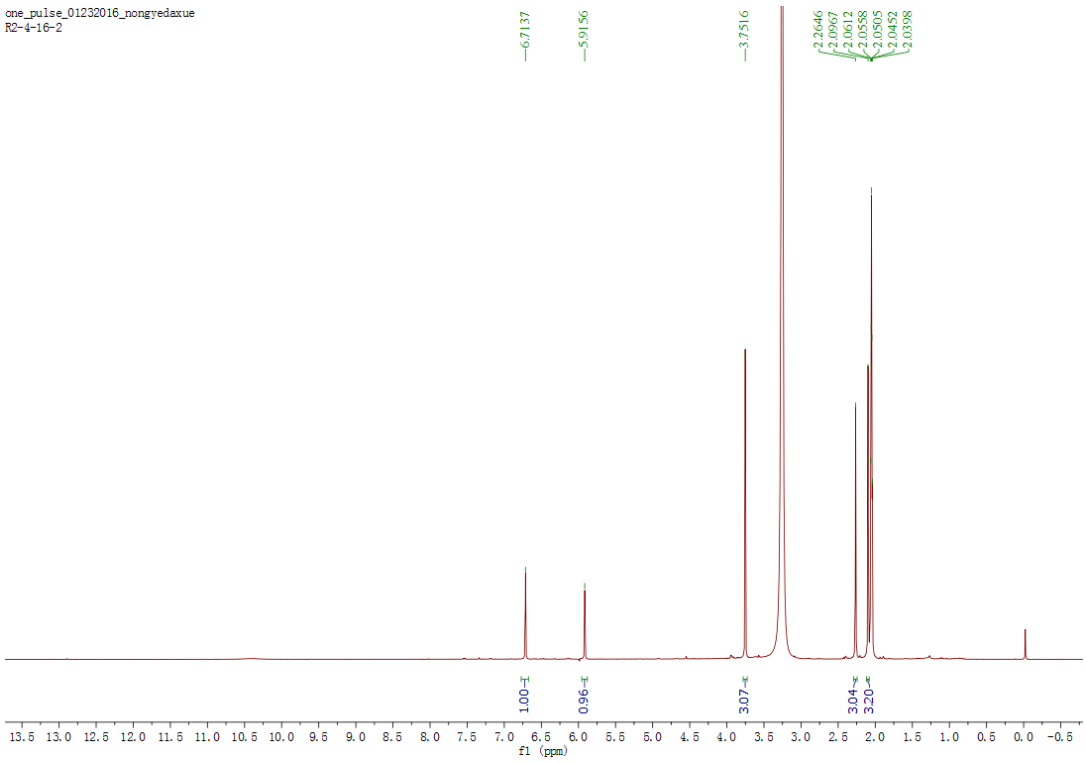


Figure S2. ^{1}H NMR spectrum of **1** (400 MHz, acetone-d_6).

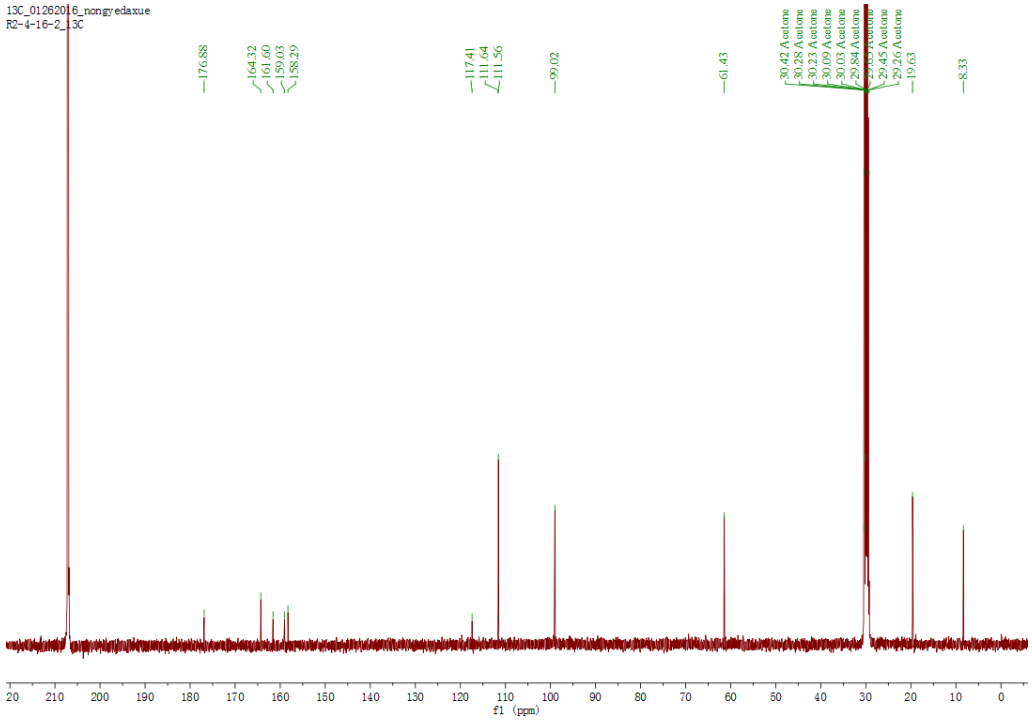


Figure S3. ^{13}C NMR spectrum of **1** (100 MHz, acetone-d_6).

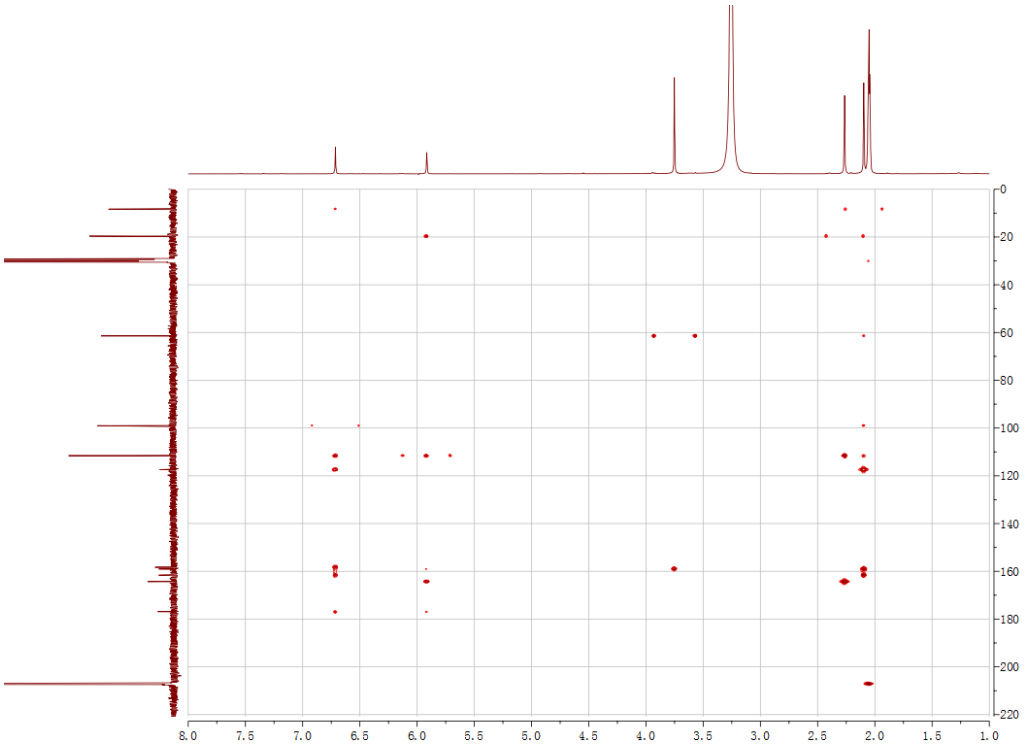


Figure S4. HMBC spectrum of **1** (acetone-d_6).

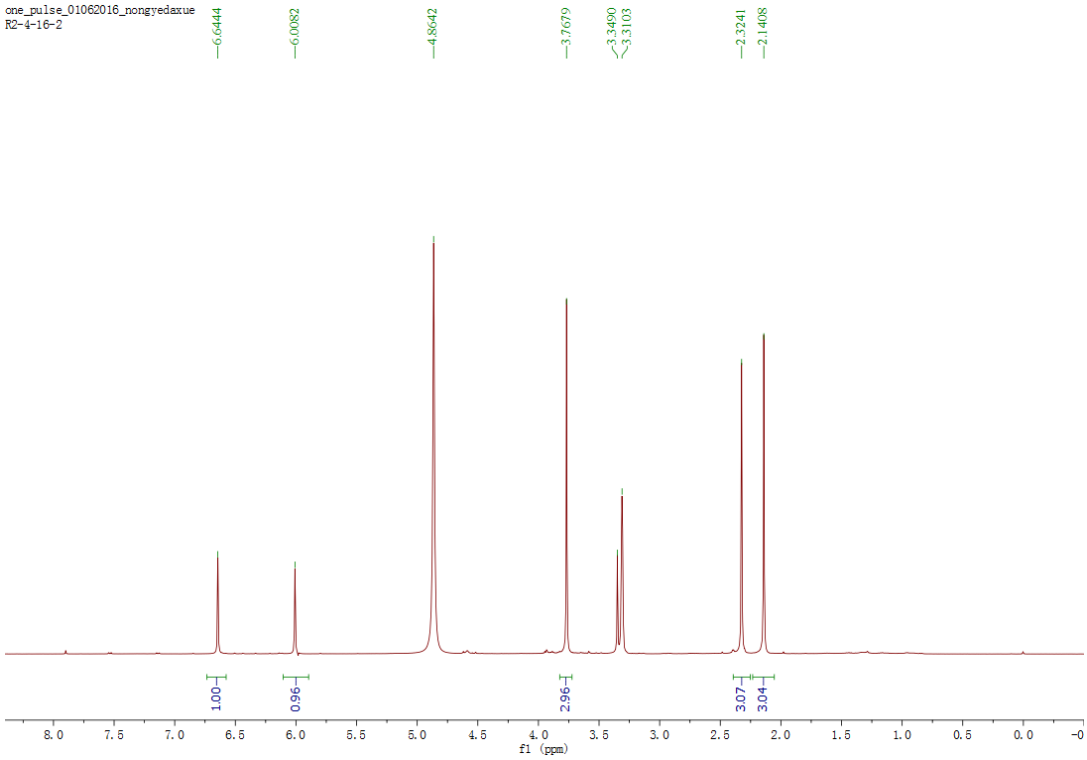


Figure S5. ^{1}H NMR spectrum of **1** (400 MHz, CD_3OD).

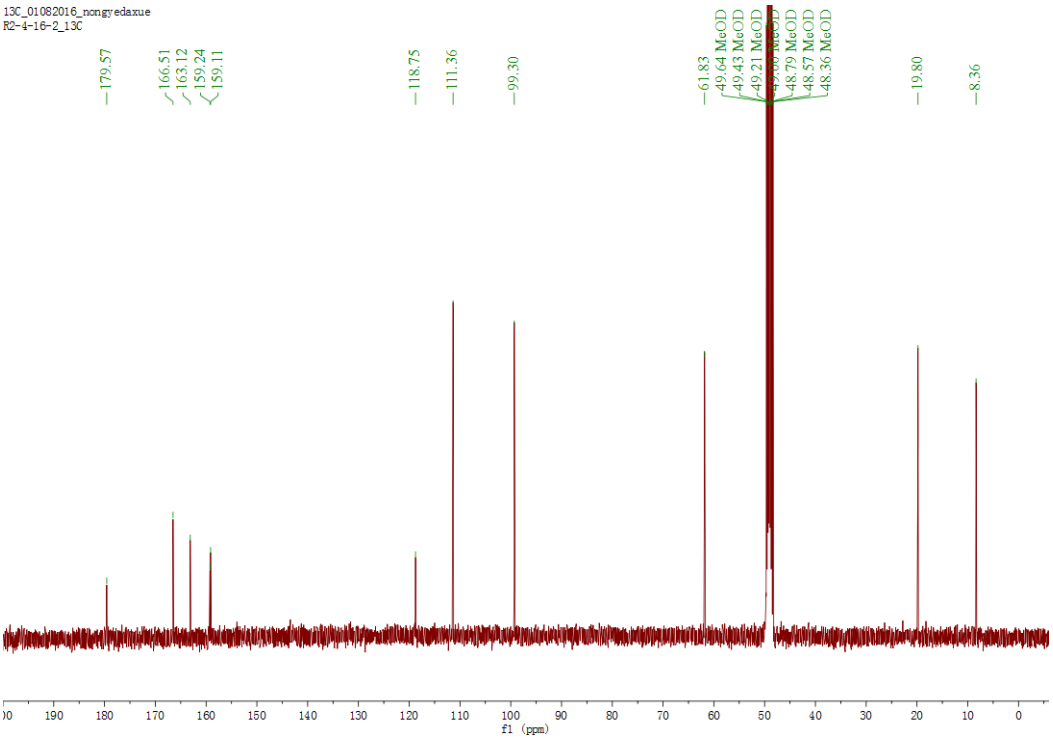
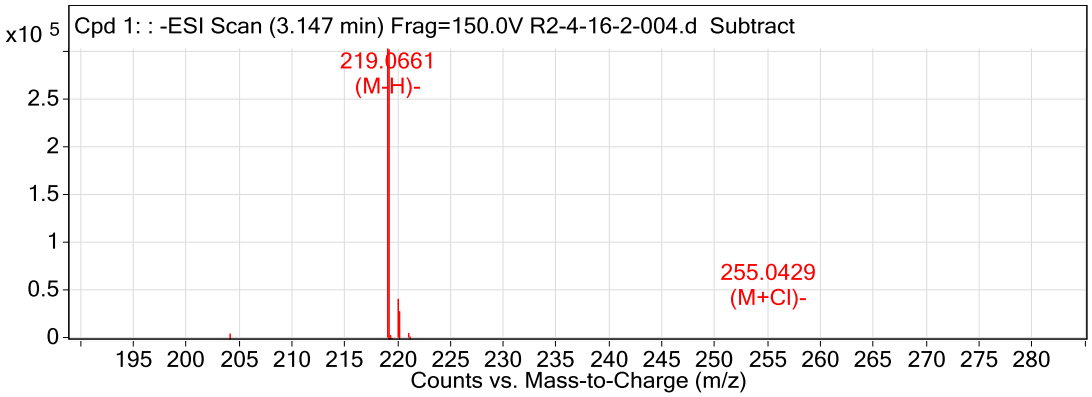


Figure S6. ^{13}C NMR spectrum of **1** (100 MHz, CD_3OD).



m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
219.0661	219.0663	-0.97	-1	302812.7	C12H11O4	$(M-H)^-$
255.0429	255.043	-0.2	-1	210.1	C12H12ClO4	$(M+Cl)^-$

Figure S7. HRESIMS spectrum of **1**.

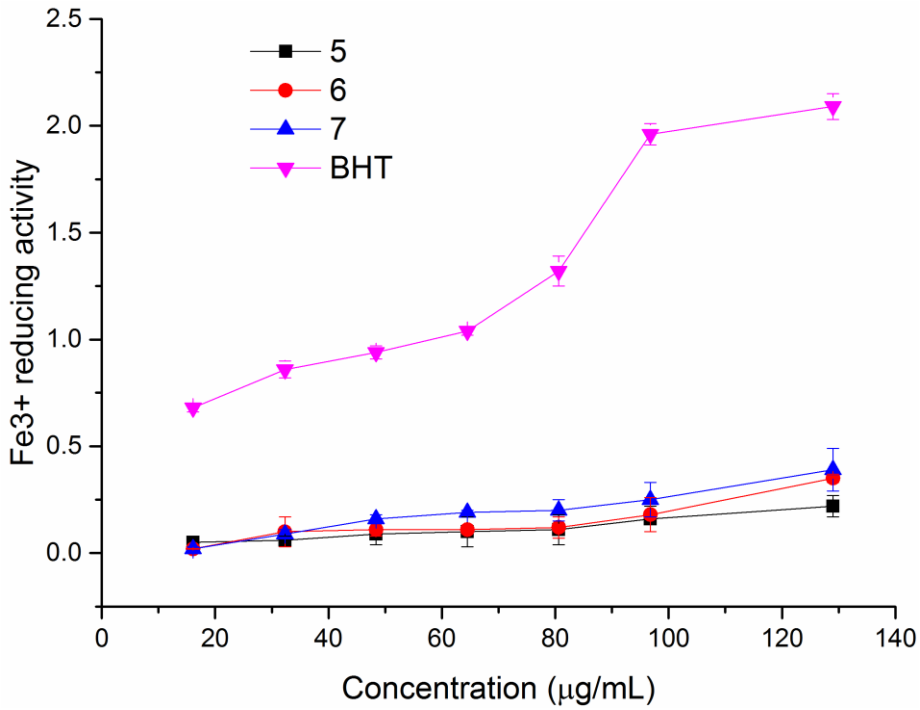


Figure S8. Ferric reducing activities of the isolated compounds.

Supplementary references

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