SUPPLEMENTARY MATERIAL

Characterization and bioactive potentials of secondary metabolites from *Fusarium chlamydosporum*

Zhao-Fu Wang[a,†], Wei Zhang[a,†], Lin Xiao[a], Yuan-Ming Zhou[b] and Feng-Yu Du[a,c,*]

[a] *College of Chemistry and Pharmacy, Qingdao Agricultural University, Qingdao, China*

[b] *Analytical and Testing Center, Qingdao Agricultural University, Qingdao, China*

[c] *Shandong Key Laboratory of Applied Mycology, Qingdao Agricultural University, Qingdao, China*

Correspondence: Feng-Yu Du, fooddfy@126.com

[†]Both authors contributed equally to the work.

ABSTRACT

A search for bioactive secondary metabolites from the endophytic fungus *Fusarium chlamydosporum*, isolated from the root of *Suaeda glauca*, led to the isolation of three indole derivatives (**1**–**3**), three cyclohexadepsipeptides (**4**–**6**), and four pyrones (**7**–**10**). The structures of new (**1**) and known compounds (**2**–**10**) were elucidated on the basis of extensive spectroscopic analysis. All these compounds were evaluated for phytotoxic, antimicrobial activities, and brine shrimp lethality. Compound **1** showed significant phytotoxic activity against the radicle growth of *Echinochloa crusgalli*, even better than the positive control of 2,4-D. Cyclohexadepsipeptides (**4**–**6**) and pyrones (**7**–**10**) exhibited brine shrimp lethality, especially **4** and **7** with the LD_{50} values of 2.78 and 7.40 μg mL^{-1}, respectively, better than the positive control.

KEYWORDS

Endophytic fungus; Secondary metabolites; Phytotoxic activity; Brine shrimp lethality; Antimicrobial activity

Experimental

Chemicals and instruments

NMR spectra were recorded at 500 MHz and 125 MHz for ^{1}H and ^{13}C, respectively, on a Bruker Avance III spectrometer. HR-ESI-MS were determined on an Agilent Q-TOF mass spectrometer. Column chromatography (CC) was performed with Silica gel (48-75 μm, Qingdao Haiyang Chemical Co.), Lobar LiChroprep RP-C18 (40-63 μm, Merck), and Sephadex LH-20 (18-110 μm, Merck). Semi-preparative HPLC (semi-pHPLC) was performed using a Dionex HPLC system equipped with a P680 pump (flow rate: 3 mL min^{-1}) and a UVD340U multiple wavelength detector (detection wavelength: 230 nm). Solvents were distilled prior to use for extraction and purification procedures. The deuterated solvents (CD_3OD, Deuterated ratio, 99.8 %) with TMS as the internal referent were purchased from Cambridge Isotope Laboratories, Inc (Andover, USA).

Microorganism materials

The endophytic fungus of *Fusarium chlamydosporum* QJP1 was isolated from the root of *Suaeda glauca* collected from the Yellow River Delta of Dongying, China, in November 2015. The fungus was identified by analysis of the ITS region of the rDNA, as described in our previous report (Du, Zhang et al. 2014). The sequence data derived from the fungal strain was deposited at GeneBank, with the accession No. KY290716. The strain is preserved at China General Microbiological Culture Collection Center with the preservation No. CGMCC13198. Three pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enterica*, as well as two plant pathogenic fungi of *Rhizoctonia cerealis* and *Colletotrichum gloeosporioides*, were obtained from Institution of Food and Biotechnology, Qingdao Agricultural University.

Fermentation, extraction and isolation

The fungal strain was statically fermented at room temperature for 30 days on sterilized solid medium containing rice (100 g per flask), peptone (0.6 g per flask), and sea water (100 mL per flask) in 1 L flasks (×100). The rice culture was exhaustively extracted with EtOAc to give a crude extract, which was dried and fractionated by Silica gel vacuum liquid chromatography (VLC) using different solvents of increasing polarity from petroleum ether (PE) to methanol (MeOH) to yield 12 fractions (Fractions (Frs.)

1–12) based on HPLC analysis.

Fr. 6 (eluted with PE : EtOAc = 1:1) was purified by CC over RP-C18 eluting with a MeOH-H_2O gradient (from 1:9 to 1:0) to afford three subfractions (Frs. 6-1 to 3). Fr. 6-1 was further separated by semi-pHPLC (15% MeCN-H_2O) to afford compounds **7** (30.2 mg, t_R 6.4 min) and **8** (3.1 mg, t_R 10.2 min); While Fr. 6-2 was also purified by semi-pHPLC (30% MeOH-H_2O) to obtain compounds **1** (4.8 mg, t_R 10.9 min), **2** (8.4 mg, t_R 13.4 min), and **3** (17.2 mg, t_R 15.8 min). Compounds **4–6** were obtained from Fr. 6-3 by CC over sephadex LH-20 eluted with MeOH and then by semi-pHPLC (65 MeCN-H_2O). Fr. 7 (eluted with Chloroform : MeOH = 40:1) was purified by CC over RP-C18 eluting with a MeOH-H_2O gradient to afford compounds **9** (43.8 mg) and **10** (5.0 mg).

The two known indole derivatives of methyl indol-3-ylacetate (**2**) (Nieman et al. 2003) and N_b-acetyltryptamine (**3**) (Pedras et al. 2005), three cyclohexadepsipeptides of destruxin A4 (**4**) (Krasnoff SB et al. 1996), trichomide B (**5**) (Zhang et al. 2013), and homodestcardin (**6**) (Zhang et al. 2013), as well as four pyrones of kojic acid (**7**) (Wei et al. 2014), kojic acid monomethyl ether (**8**) (Li et al. 2003), 5-hydroxy-4-oxo-4*H*-pyran-2-ethyl formate (**9**) (Wei et al. 2014) and phomapyrone C (**10**) (Pedras et al. 1994), were determined by detailed analysis of their spectroscopic data and by comparison with those reported in the literatures.

Phytotoxic bioassay

The isolated compounds were evaluated for the phytotoxic effects on the radicle growth of *E. crusgalli* and *A. retroflexus* using the grinded plant tissue powders mixed with agar (PPA) method, with a slight modification (Su et al. 2014; Zhang et al. 2016). Briefly, seeds were firstly pretreated with sodium hypochlorite (0.2%) for 15 minutes and soaked with flowing water for 4-6 hours. Then, the wet seeds were put on the moist filter paper to germinate under the dark condition for 12 hours. The isolated compounds were dissolved in 1 mL dimethyl sulfoxide (DMSO) and diluted with 99 mL water (containing 0.5 g agar) to obtain the agar solution, which was further divided into three Bunsen beakers. Subsequently, five germinated seeds with same radicle lengths were planted and further cultivated in the artificial climate box under the 25 ℃,

light-avoidance condition. After 24 hours, the radicle lengths were measured and compared to the untreated control. The inhibition rate (Li et al. 2014) was calculated using the formula as followed:

$$\text{Inhibition rate (\%)} = (L_{\text{untreated control}} - L_{\text{treatment}})/L_{\text{untreated control}} \times 100$$

The $L_{\text{untreated control}}$ and $L_{\text{treatment}}$ were the radicle lengths of seedlings in the untreated and treated samples, respectively. The widely-used herbicide of 2,4-D was used as the positive control.

Brine shrimp lethality and antimicrobial bioassay

Evaluation of brine shrimp lethality were performed as previously reported, and the positive control was colchicine (Du, Li et al. 2014; Du, Zhang et al. 2014; Du et al. 2017). While antimicrobial bioassay were determined using the micro-broth dilution method in 96-well microtiter plates (Li et al. 2014; Zhang et al. 2010). Chloramphenicol and tebuconazole were used as positive controls against bacteria and fungi, respectively.

Table S1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **1** ($CDCl_3$, δ: ppm).

Position	δ_C (type)	δ_H (mult., *J* in Hz)	Position	δ_C (type)	δ_H (mult., *J* in Hz)
1-*NH*		8.14 (br s, 1H)	8	31.8, CH_2	3.81 (m, 2H)
2	123.0, CH	7.20 (m, 1H)	9	171.7, C	
3	108.5, C		1'	18.9, CH_3	1.08 (d, 6.0, 3H)
3a	127.1, C		2'	70.1, CH	3.68 (m, 1H)
4	118.6, CH	7.62 (d, 7.9, 1H)	3'	75.5, CH	4.74 (m, 1H)
5	119.8, CH	7.15 (m, 1H)	4'	16.4, CH_3	1.22 (d, 5.2, 3H)
6	122.3, CH	7.15 (m, 1H)			
7	111.4, CH	7.35 (d, 8.1, 1H)			
7a	136.1, C				

Table S2. Brine shrimp lethality of compounds **4**−**10** (LD_{50}: μg/mL).

	4	**5**	**6**	**7**	**8**	**9**	**10**	colchicine[a]
LD_{50}	2.78	18.62	17.55	7.40	217.56	314.37	492.41	7.75

[a]Positive control: colchicine

Table S3. Antimicrobial activities of compounds **1, 3, 9,** and **10** (MIC: μg/mL).

	S. aureus	*E. coli*	*S. enterica*	*R. cerealis*	*C. gloeosporioides*
1	128	128	64	—	—
3	—	—	128	128	—
9	—	—	—	128	128
10	—	—	—	—	128
chloramphenicol[a]	4	4	8		
tebuconazole[b]				16	8

[a,b]Positive control for the antibacterial and antifungal bioassay, respectively.

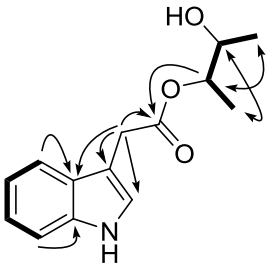

Figure S1. Key COSY (bold lines) and HMBC (arrows) correlations of compound **1**.

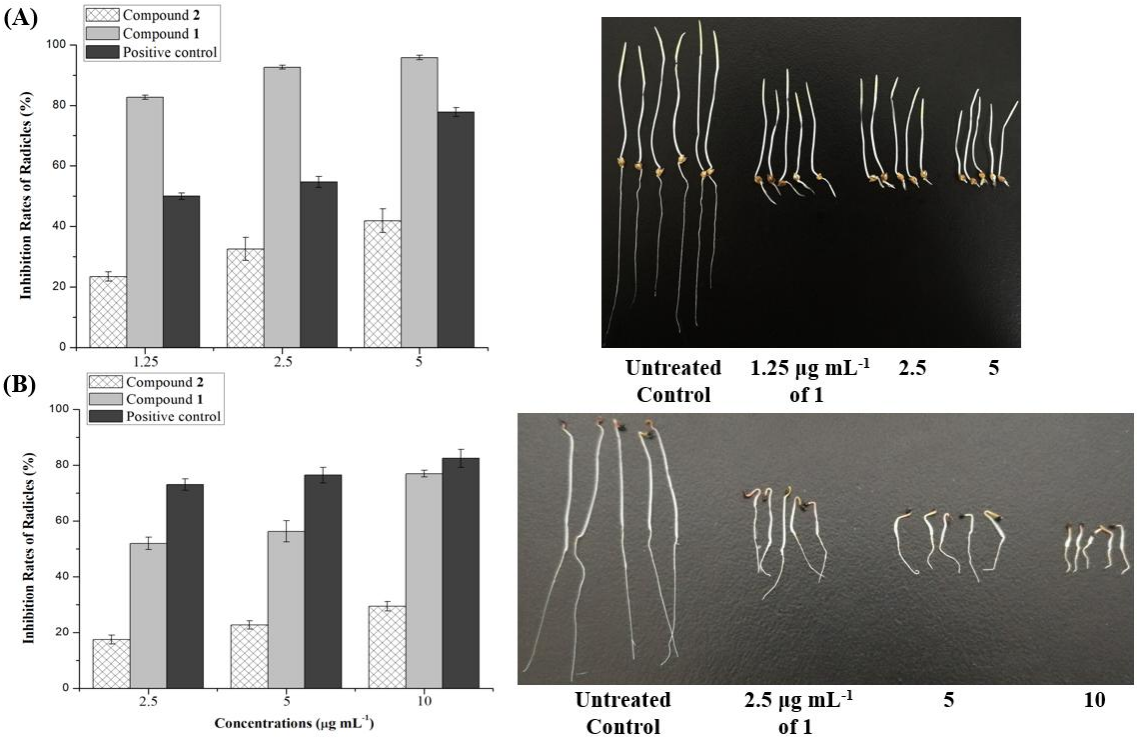

Figure S2. Phytotoxic activities of compounds **1** and **2** against the radicle growth of *E. crusgalli* (A) and *A. retroflexus* (B).

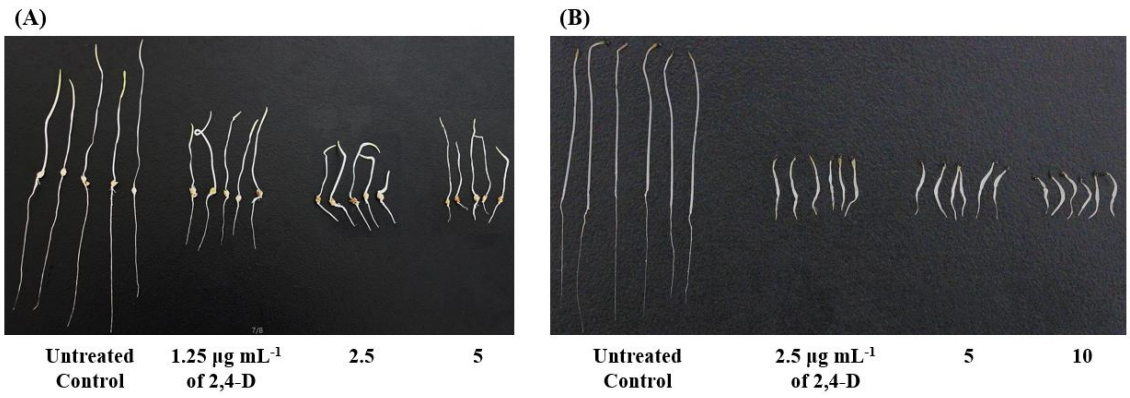

Figure S3. Phytotoxic activities of the positive control of 2,4-D against *E. crusgalli* (A) and *A. retroflexus* (B).

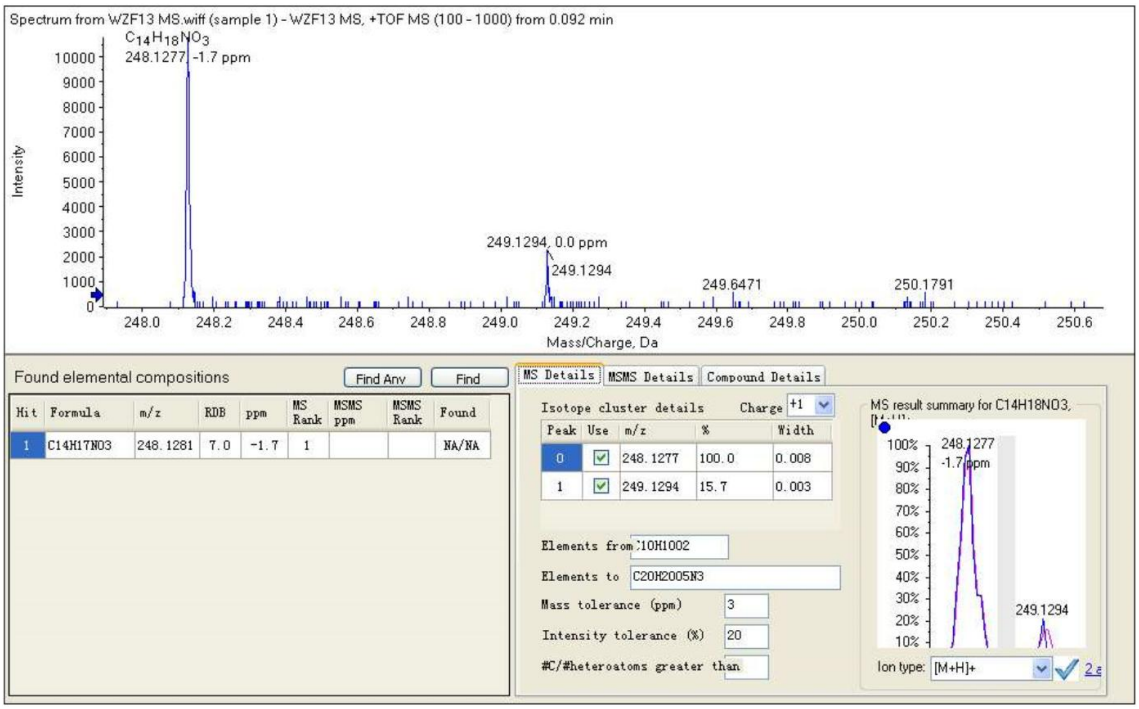

Figure S4. HRESIMS of compound **1**.

m/z 248.1277 $[M + H]^+$ (calcd for $C_{14}H_{18}NO_3^+$, 248.1281)

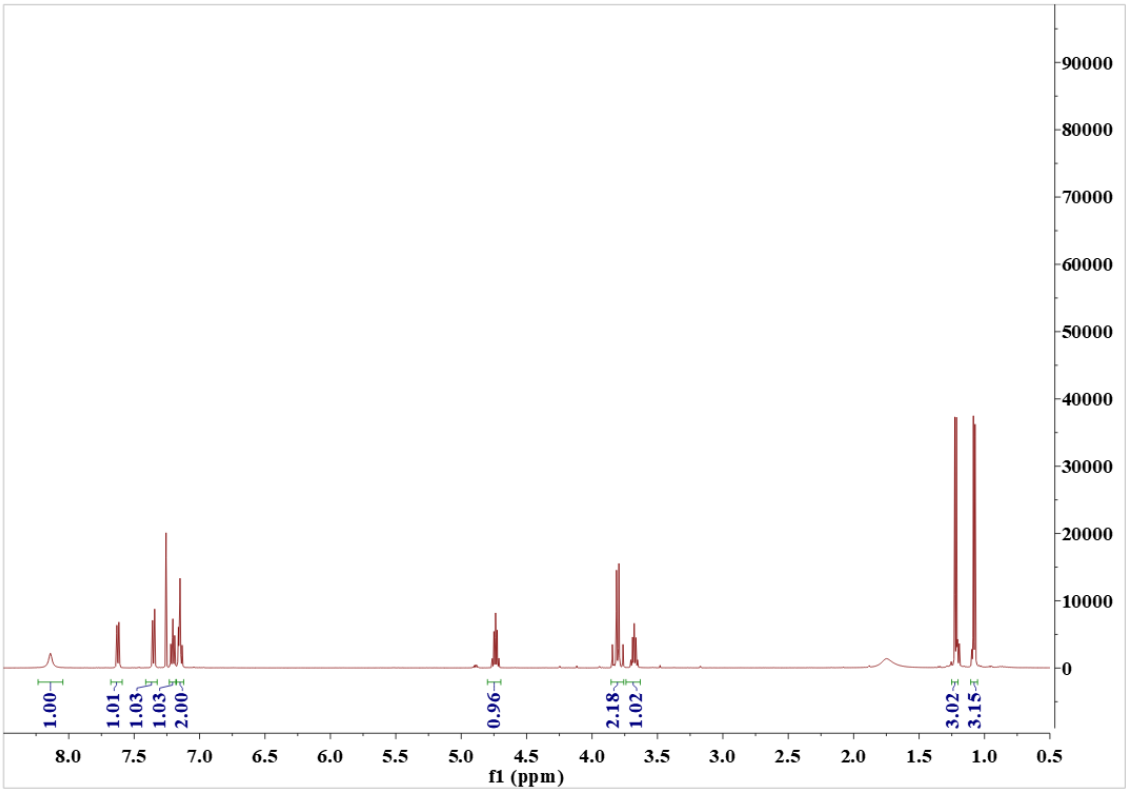

Figure S5. ^1H NMR (500 MHz, $CDCl_3$) spectrum of compound **1**.

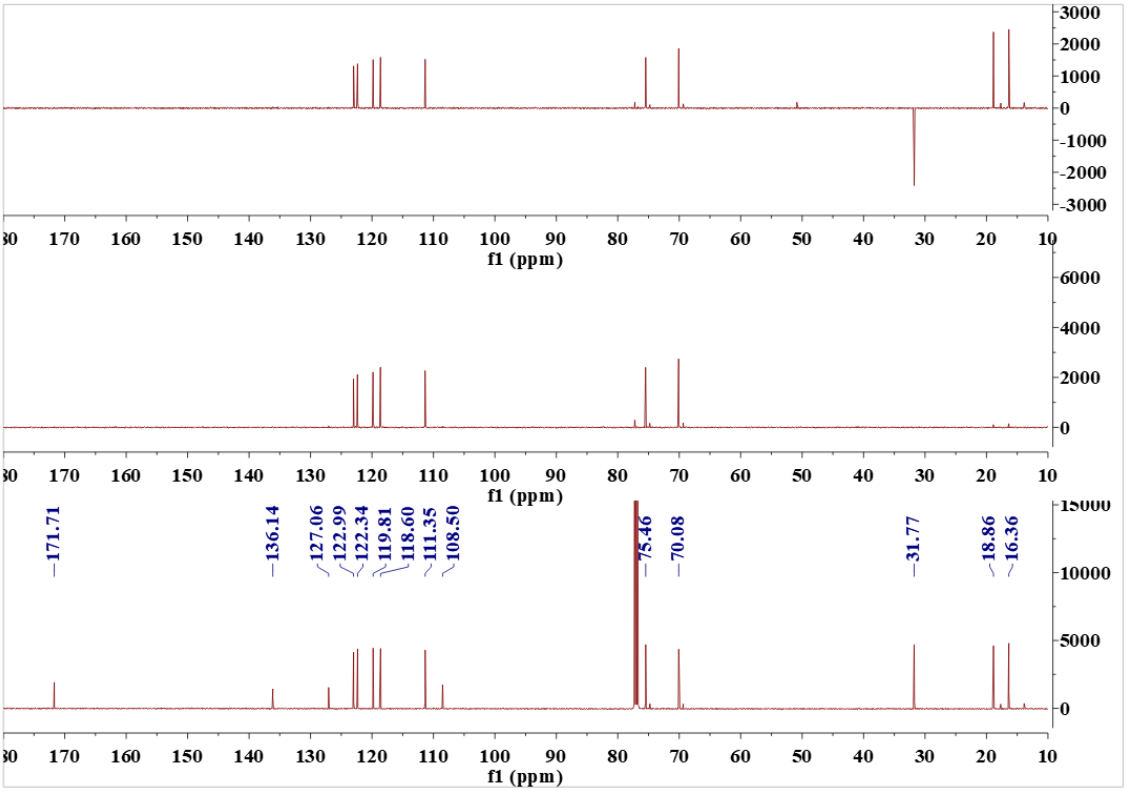

Figure S6. DEPT spectra of compound **1**.

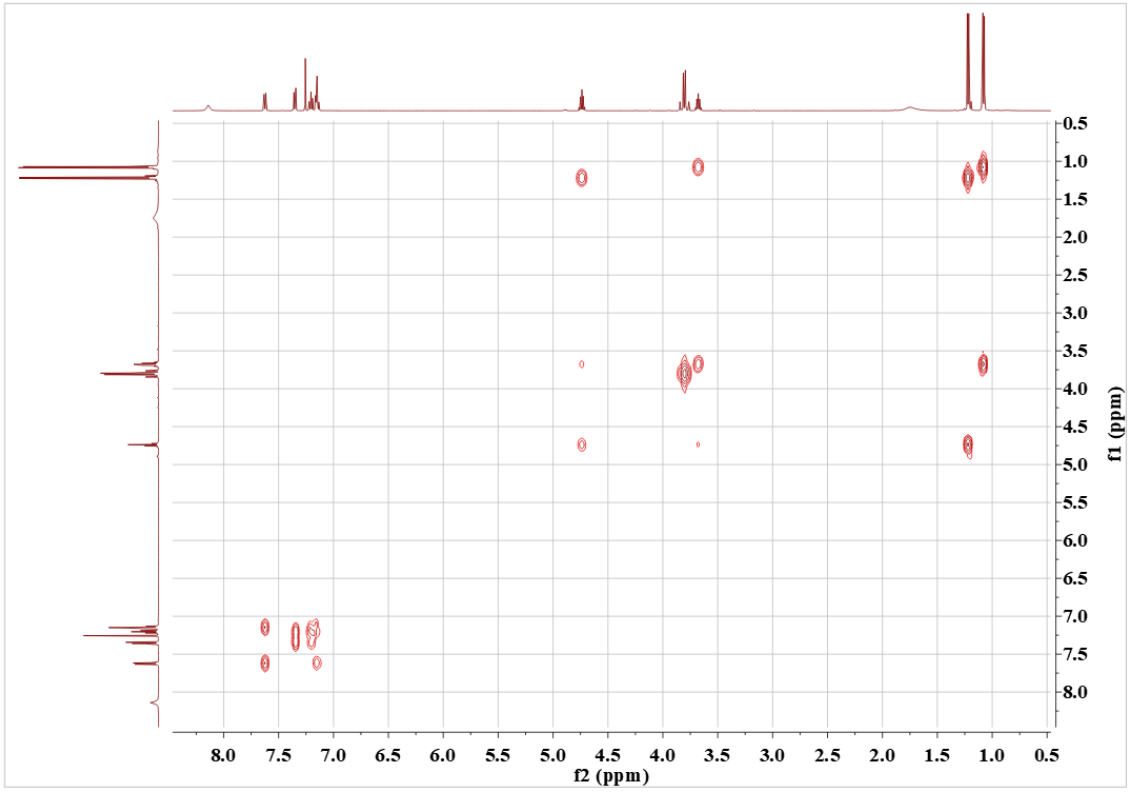

Figure S7. ^{1}H-^{1}H COSY spectrum of compound **1**.

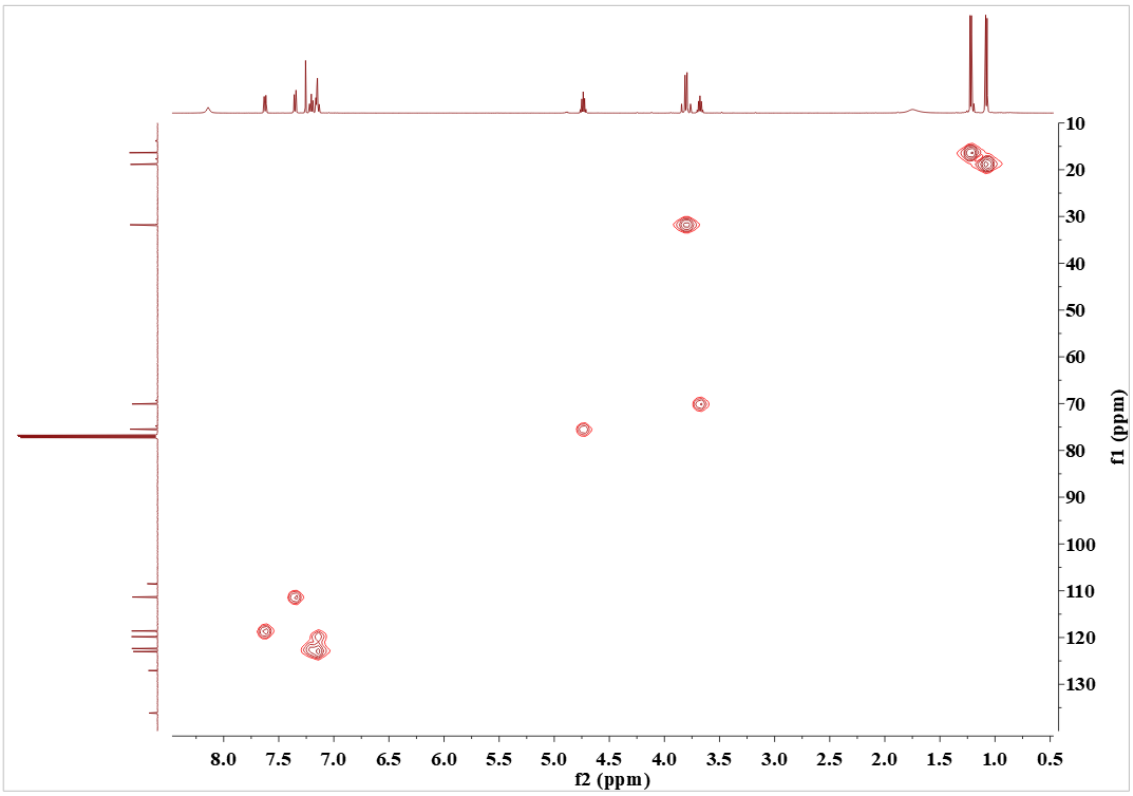

Figure S8. HSQC spectrum of compound **1**.

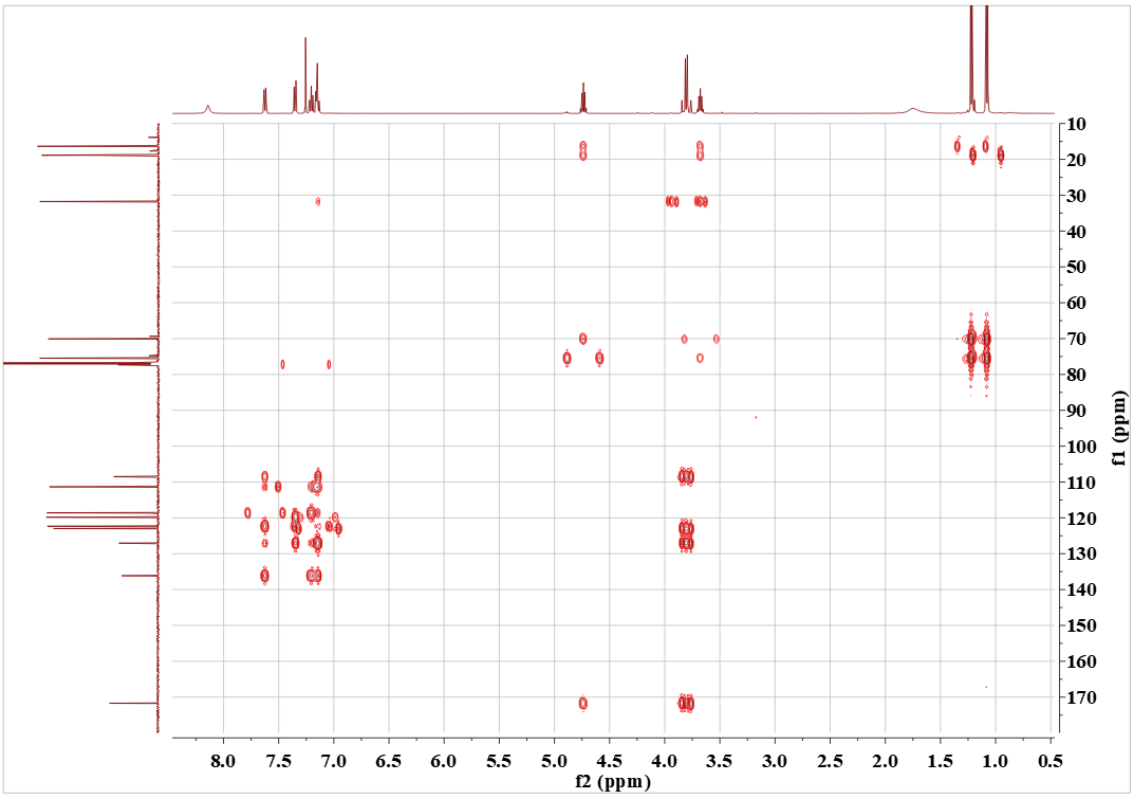

Figure S9. HMBC spectrum of compound **1**.

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