SUPPLEMENTARY MATERIAL

**New phenyl derivatives from endophytic fungus
Botryosphaeria sp. SCSIO KcF6 derived of mangrove plant
*Kandelia candel***

Zhi-ran Ju^{a,b,1}, Xiaochu Qin^{c,1}, Xiu-ping Lin^b, Jun-feng Wang^b, Kumaravel
Kaliyaperumal^b, Yong-qi Tian^b, Juan Liu^b, Fen Liu^a, Zhengchao Tu^{c,*}, Shi-hai Xu^{a,*},
Yonghong Liu^{b,*}

^aDepartment of Chemistry, Jinan University, Guangzhou 510632, China;

^bCAS Key Laboratory of Tropical Marine Bio-resources and Ecology/Guangdong
Key Laboratory of Marine Materia Medica/ RNAM Center for Marine Microbiology,
South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou
510301, China;

^cLaboratory of Molecular Engineering and Laboratory of Natural Product Synthesis,
Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences,
Guangzhou 510530, China

¹ The first two authors contributed equally to this work.

*Corresponding author

*Correspondence to Y. Liu, CAS Key Laboratory of Tropical Marine Bio-resources
and Ecology/Guangdong Key Laboratory of Marine Materia Medica/ RNAM Center
for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy
of Sciences, Guangzhou 510301, China. Tel./Fax: +86-020-8902-3244.

E-mail address: yonghongliu@scsio.ac.cn (Y. Liu).

Dr. Shihai Xu

Department of Chemistry, Jinan University, Guangzhou 510632, China

E-mail: txush@jnu.edu.cn

Dr. Zhengchao Tu, Guangzhou Institutes of Biomedicine and Health, Chinese
Academy of Sciences, Guangzhou 510530, China, E-mail: tu_zhengchao@gibh.ac.cn

ABSTRACT

Two new phenyl derivatives (**1** and **3**), along with two new natural products (**4** and **5**), and three known compounds (**2**, **6**, and **7**), were isolated from an endophytic fungus *Botryosphaeria* sp. SCSIO KcF6. The structures of these compounds **1–7** were elucidated by the extensive 1D and 2D-NMR and HRESIMS Data analysis, and compared with those of reported data. The absolute configuration of the compounds **1** and **3** were assigned by optical rotation and CD data. The isolated compounds were evaluated for their cytotoxic, anti-inflammatory (COX-2), and antimicrobial activities. Compound **3** exhibited a specific COX-2 inhibitory activity with the IC₅₀ value of 1.12 μ M.

Keywords: Endophytic fungus, *Botryosphaeria* sp. SCSIO KcF6, COX-2 inhibitory activity

Contents

1. 1D- and 2D-NMR spectra data	4
1.1 Fig. 1 The ^1H -NMR spectrum of compound 1	4
1.2 Fig. 2 The ^{13}C -NMR (DEPT) spectrum of compound 1	5
1.3 Fig. 3 The HSQC spectrum of compound 1	6
1.4 Fig. 4 The HMBC spectrum of compound 1	7
1.5 Fig. 5 The ^1H - ^1H COSY spectrum of compound 1	8
1.6 Fig. 6 The ^1H -NMR spectrum of compound 3	9
1.7 Fig. 7 The ^{13}C -NMR (DEPT) spectrum of compound 3	10
1.8 Fig. 8 The HSQC spectrum of compound 3	11
1.9 Fig. 9 The HMBC spectrum of compound 3	12
1.10 Fig. 10 The ^1H - ^1H COSY spectrum of compound 3	13
2. The chemical structure of compounds 1-7	14
2.1 Fig. 11 The chemical structure of compounds 1-7	14
2.2 Fig. 12 HMBC correlations of compound 1	15
2.3 Fig. 13 HMBC correlations of compound 3	15
3. The CD spectra of compound 3	16
3.1 Fig. 14 The CD spectra of compound 3	16
4. Spectroscopic data of the isolated compounds	17
4.1 Compound 2	17
4.2 Compound 4	17
4.3 Compound 5	17
4.4 Compound 6	17
4.5 Compound 7	17
5. Table. ^1H and ^{13}C -NMR data for 1- 3	18
5.3 Table 1. ^1H and ^{13}C -NMR data for 1 and 2	18
5.3 Table 2. ^1H , ^{13}C -NMR and HMBC data for compound 3	19
6. COX-2 inhibitory value of Compound 3	20
6.1. Graphics of COX-2 inhibitory value of Compound 3	20

1. 1D- and 2D-NMR spectra data

Fig. 1 The ^1H -NMR spectrum of compound **1** (500 MHz in MeOD)

Fig. 2 The ^{13}C -NMR (DEPT) spectrum of compound **1** (125 MHz in MeOD)

Fig. 3 The HSQC spectrum of compound **1**

Fig. 4 The HMBC spectrum of compound **1**

Fig. 5 The ^1H - ^1H COSY spectrum of compound **1**

Fig. 6 The ^1H -NMR spectrum of compound **3** (500 MHz in CDCl_3)

Fig. 7 The ^{13}C -NMR (DEPT) spectrum of compound **3** (125 MHz in CDCl_3)

Fig. 8 The HSQC spectrum of compound **3**

Fig. 9 The HMBC spectrum of compound **3**

Fig. 10 The ^1H - ^1H COSY spectrum of compound **3**

2. The chemical structure of compounds 1-7

Fig.11 The chemical structure of compounds 1-7

Fig.12. HMBC correlations of compound 1

Fig.13 HMBC correlations of compound 3

3. The CD spectra of compound 3

Fig.14 The CD spectra of compound 3

4. Spectroscopic data of the isolated compounds

Compound **2**: Needles, $[\alpha]_D^{25} +67.1$ (c 1.0, CH₃OH), IR (KBr) $\nu_{\max}$: 3333, 1680, 694 cm⁻¹; ¹H-NMR (500MHz, MeOD), δ : 6.34 (1H, s, H-4), 6.27 (1H, s, H-7), 6.26 (1H, s, H-5), 4.15 (1H, q, H-10), 2.58 (2H, m, H-9), 1.25 (3H, d, J = 6.2 Hz, H-11); ¹³C-NMR (125MHz, MeOD), δ : 23.3 (C-11), 43.8 (C-9), 66.2 (C-10), 99.0 (C-8a), 102.9 (C-7), 104.4 (C-5), 107.1 (C-4), 141.1 (C-5a), 155.8 (C-3), 164.8 (C-8), 167.9 (C-6), 169.1 (C-1). HREIMS at m/z 259.0573 [M+Na]⁺ (calcd for C₁₂H₁₂NaO₅).

Compound **4**: Brown oil; ¹H -NMR (500 MHz, MeOD), δ : 1.85 (1H, ddd, J = 12.5, 10.4, 9.1 Hz, CH₂ (3)), 2.36 (1H, ddd, J = 12.5, 4.9, 3.2 Hz, CH₂ (3)), 3.94 (1H, ddd, J = 10.3, 6.8, 3.2 Hz, H-2), 4.78 (1H, dd, J = 9.0, 5.0 Hz, H-1), 4.83 (1H, d, J = 6.8 Hz, H-4), 6.74 (1H, d, J = 7.9 Hz, H-8), 7.02 (1H, d, J = 7.7 Hz, H-6), 7.17 (1H, t, J = 7.9 Hz, H-7); ¹³C-NMR (125MHz, MeOD), δ : 39.2 (C-3), 68.6 (C-4), 72.2 (C-2), 73.7 (C-1), 115.7 (C-8), 119.3 (C-6), 123.0 (C-5a), 129.9 (C-7), 141.9 (C-8a), 157.9 (C-5).

Compound **5**: Brown oil; ¹H -NMR (500 MHz, MeOD), δ : 1.93 (1H, m, CH₂ (3)), 2.14 (1H, m, CH₂ (3)), 4.19 (1H, ddd, J = 9.1, 6.0, 3.1 Hz, H-2), 4.73 (1H, d, J = 6.0 Hz, H-1), 4.82 (1H, m, H-4), 6.73 (1H, m, H-8), 6.93 (1H, d, J = 7.6 Hz, H-6), 7.13 (1H, t, J = 7.8 Hz, H-7); ¹³C-NMR (125MHz, MeOD), δ : 37.4 (C-3), 67.7 (C-4), 70.3 (C-2), 72.2 (C-1), 115.7 (C-8), 120.5 (C-6), 123.7 (C-5a), 129.8 (C-7), 140.9 (C-8a), 158.1 (C-5).

Compound **6**: Yellow oil; ¹H -NMR (500 MHz, CDCl₃), δ : 0.88 (3H, s, H-19), 1.19 (3H, d, J = 6.5 Hz, H-25), 1.48 (3H, s, H-22), 1.54 (6H, s, H-20, H-21), 1.66 (2H, dd, J = 27.1, 9.9 Hz, H-2), 1.83 (1H, m, H-10), 1.91 (1H, t, J = 11.3 Hz, H-14), 1.99 (2H, d, J = 10.7 Hz, H-9), 2.17 (2H, d, J = 10.3 Hz, H-5), 2.25 (1H, m, H-5), 2.26 (1H, t, J = 11.9 Hz, H-14), 2.34 (1H, d, J = 16.5 Hz, H-10), 2.59 (2H, dd, J = 22.1, 8.5 Hz, H-6), 3.76 (2H, dd, J = 26.2, 19.5 Hz, H-24), 3.96 (1H, d, J = 9.0 Hz, H-11), 5.13 (1H, s, H-7), 5.25 (1H, s, H-3), 5.38 (1H, m, H-13); ¹³C-NMR (125MHz, CDCl₃), δ : 10.3 (C-22), 14.4 (C-25), 15.2 (C-21), 15.5 (C-20), 16.3 (C-19), 23.8 (C-6), 28.8 (C-14), 29.7 (C-10), 34.9 (C-9), 37.12 (C-23), 39.3 (C-2), 40.3 (C-5), 49.1 (C-1), 49.4 (C-15), 65.6 (C-24), 76.4 (C-11), 121.7 (C-3), 124.3 (C-7), 129.2 (C-13), 132.7 (C-8), 136.1 (C-4), 137.8 (C-12), 147.5 (C-16), 151.1 (C-17), 209.1 (C-18).

Compound **7**: Yellow oil; ¹H-NMR (500 MHz, DMSO), δ : 0.88 (3H, s, H-19), 1.22 (3H, t, J = 8.5 Hz, H-25), 1.48 (3H, d, J = 13.2 Hz, H-22), 1.59 (6H, s, H-20, H-21), 1.70 (1H, t, J = 13.0 Hz, H-10), 1.84 (1H, m, H-10), 1.92 (1H, m, H-14), 2.00 (3H, s, COCH₃), 2.05 (1H, d, J = 13.7 Hz, H-5), 2.25 (1H, m, H-5), 2.26 (2H, m, H-6), 2.51 (1H, s, H-14), 2.62 (1H, d, J = 11.2 Hz, H-23), 2.72 (1H, m, H-15), 4.18 (2H, d, J = 7.6 Hz, H-24), 4.44 (1H, t, J = 7.8 Hz, H-11), 5.11 (1H, s, H-7), 5.23 (1H, s, H-13), 5.28 (1H, m, H-3); ¹³C-NMR (125MHz, DMSO), δ : 10.3 (C-22), 14.2 (C-25), 15.0 (C-20), 15.7 (C-21), 15.9 (C-19), 20.7 (COCH₃), 23.3 (C-6), 28.5

(C-14), 30.3 (C-10), 33.3(C-23), 35.3 (C-9), 39.6 (C-2), 40.7 (C-5), 48.3 (C-1), 48.5 (C-15), 65.7 (C-24), 74.2 (C-11), 121.9 (C-3), 123.3 (C-7), 126.8 (C-13), 132.5 (C-8), 136.8 (C-4), 136.9 (C-12), 147.8 (C-17), 147.8 (C-16), 170.2 (COCH₃), 207.3 (C-18).

5. Table. ¹H and ¹³C-NMR data for 1- 3

Table 1. ¹H and ¹³C-NMR data (500/125 MHz) for **1** and **2** in MeOD

No.	1		2	
	δ_{H} (<i>J</i> in Hz)	δ_{C}	δ_{H} (<i>J</i> in Hz)	δ_{C}
1		167.9		169.1
3		156.0		155.8
4	6.61 s	101.7	6.34 s	107.1
5	6.38 s	103.9	6.26 s	104.4
5a		132.4		141.1
6		161.0		167.9
7		136.0	6.27 s	102.9
8		161.7		164.8
8a		98.3		99.0
9	2.62 m	44.2	2.58 m	43.8
10	4.16 m	66.4	4.15 q	66.2
11	1.26 d, (6.2)	23.5	1.25 d, (6.2)	23.3
12	3.76 s	61.8		

Table 2. ^1H , ^{13}C -NMR (500/125 MHz) and HMBC data for compound **3** in CDCl_3

No.	3		
	δ_{H} (<i>J</i> in Hz)	δ_{C}	HMBC(H $\rightarrow$ C)
1		156.8	
2			
2a	6.85 <i>d</i> (8.64)	116.0	C-4, C-1
3			
3a	7.56 <i>d</i> (8.62)	132.0	C-5, C-1
4		125.3	
5	6.43 <i>s</i>	110.0	C-7, C-3, C-3a, C-6
6		134.3	
7		163.4	
8		108.6	
9	2.64 <i>dt</i> (6.85, 6.85, 13.72)	33.2	C-8, C-10, C-11
10		15.5	
11	1.03 <i>t</i> (6.39, 6.39)	14.7	C-8, C-9
12		166.5	
13	3.82 <i>s</i>	53.6	C-12

6. COX-2 inhibitory value of Compound 3

	WJF-53	WJF-55	Compound 3	BD11
IC ₅₀	1.052E-05	1.92E-06	1.118E-05	8.483E-06

Graphics of COX-2 inhibitory value of Compound 3