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# Three new napthalene derivatives from the endophytic fungus *Phomopsis fukushii*

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ABSTRACT

An endophytic *Phomopsis fukushii* was isolated from the rhizome of *Paris polyphylla* var. *yunnanensis*, Diaporthaceae family, and three new (**1–3**) and four known napthalene derivatives (**4–7**) were isolated from the fermentation products of this fungus. Their structures were elucidated by spectroscopic methods including extensive 1D- and 2D-NMR techniques. Compounds **1–3** were evaluated for their anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) activity with vancomycin as positive control. The results showed that compounds **1–3** showed good inhibitions with MCI valves of 4, 4 and 6 *m*g/mL, respectively.

## 1. Introduction

The *Phomopsis* is a genus of ascomycete fungi in the Diaporthaceae family. This genus contains more than 900 species named from a wide range of hosts (Udayanga et al., 2011), and some of which can produce a number of secondary metabolites with various biological activities, such as xanthones (Meng et al., 2015; Yang et al., 2013), benzofurans (Du et al., 2017), lactones (Huang et al., 2008; Erbert et al., 2012) epoxycyclohexenes (Hussain et al., 2009), isocoumarins (Hussain et al., 2009), Chromones (Huang et al., 2016), biphenyls (Li et al., 2017), polyketides (Tang et al., 2017), steroids (Hu et al., 2017), napthalene derivatives (Hermawati et al., 2017; Jalgaonwala et al., 2011; Buckingham, 2009), and the like.

Among them, naphthalenes derivatives are important drug resources. Several naphthalene containing drugs are available, such as nafacillin, naftifine, tolnaftate, terbinafine, etc. They play a vital role in the control of microbial infection (Wilson and Gisvolds, 2004). Naphthalenes from natural products also have a variety of biological properties, such as antimicrobial, antioxidant, cytotoxic, anti-inflammatory, and antiprotozoal (Ibrahim and Mohamed, 2016). They are of great interest as potent lead compounds for medicinal chemistry researches.

Motivated by a search for new bioactive metabolites from the fermentation products of microbe, an endophytic *Phomopsis fukushii* was isolated from the rhizome of *Paris polyphylla* var. *yunnanensis*, collected in Kunming, Yunnan, PR China, and the chemical constituents of its fermentation products were investigated. As a result, three new (**1-3**) and four known (**4–7**) naphthalenes derivatives were isolated. This paper deals with the isolation and structural characterization of these compounds, and their anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) activity.

## 2. Results and discussion

The fermented substrate was extracted with 70% aqueous acetone. The extract was subjected repeatedly to column chromatography on silica gel, RP-18, and semi-preparative RP-HPLC separation to afforded compounds **1–7.** Their structures were shown in Fig. 1, and the $^{1}H$ and $^{13}C$ NMR data of the compounds **1–3** were listed in Table 1. By comparing with the literature, the known compounds were identified as 2-acetyl-1,3,6,8-tetrahydroxynaphthalene (**4**) (Watanabe et al., 2000), 1-(3,8-dihydroxy-1,6- dimethoxynaphthalen-2-yl)ethanone (**5**) (Tsuboi et al., 1977), methyl (4-hydroxy- naphthaten-1-yl)acetate (**6**) (Pedras et al., 1994), and (3*R*)-3,4-dihydro-3-hydroxy-4-oxo-8-methoxylapachenole (**7**) (Scharf et al., 2016).

Compound **1** was obtained as a yellow gum. The molecular formula of $C_{15}H_{16}O_6$ was determined from the HRESIMS spectra showing the quasi-molecular ion at *m/z* 315.0852 $[M + Na]^+$ (calcd $C_{15}H_{16}NaO_6$). The UV absorptions at 332, 268, and 215 nm indicated the presence of an extended chromophore and a substituted aromatic ring. Its IR spectral data showed the presence of hydroxy (3415 $cm^{-1}$), carbonyl (1687 $cm^{-1}$) and aromatic rings (1624, 1530, and 1442 $cm^{-1}$). The $^{1}H$ and $^{13}C$ NMR spectra of **1** (Table 1) along with analysis of the DEPT

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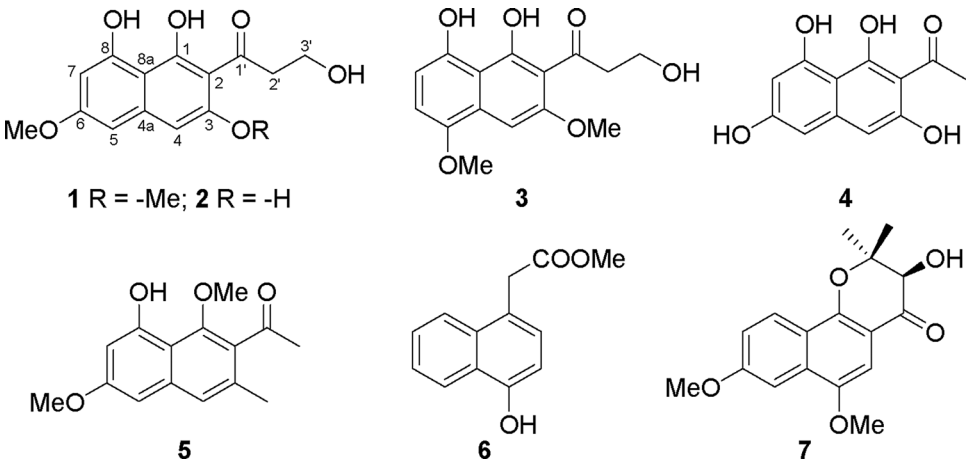


**Fig. 1.** The structures of napthalene derivatives from endophytic fungus *Phomopsis fukushii.*

**Table 1**
$^{1}H$ and $^{13}C$ NMR data for compounds **1–3** ($CDCl_3$, 125 and 500 MHz).

| No. | $\delta_C$ (mult.) | $\delta_H$ (mult, *J*, Hz) | $\delta_C$ (mult.) | $\delta_H$ (mult, *J*, Hz) | $\delta_C$ (mult.) | $\delta_H$ *(mult, J, Hz)* |
|---|---|---|---|---|---|---|
| 1 | 166.3 s | | 166.9 s | | 165.5 s | |
| 2 | 108.3 s | | 106.8 s | | 108.7 s | |
| 3 | 153.8 s | | 156.2 s | | 155.0 s | |
| 4 | 98.3 d | 6.43 s | 101.5 d | 6.49 s | 94.9 d | 7.09 s |
| 4a | 138.0 s | | 138.6 s | | 131.3 s | |
| 5 | 98.5 d | 6.59 (d) 2.2 | 98.8 d | 6.55 (d) 2.2 | 148.7 s | |
| 6 | 164.4 s | | 164.3 s | | 106.5 d | 6.76 (d) 8.2 |
| 7 | 103.1 d | 6.29 (d) 2.2 | 103.5 d | 6.31 (d) 2.2 | 109.9 d | 6.43 (d) 8.2 |
| 8 | 158.6 s | | 159.0 s | | 145.8 s | |
| 8a | 104.6 s | | 105.4 s | | 112.2 s | |
| 1′ | 205.6 s | | 205.7 s | | 205.3 s | |
| 2′ | 42.5 t | 3.36 (t) 6.2 | 42.4 t | 3.36 (t) 6.2 | 42.0 t | 3.39 (t) 6.2 |
| 3′ | 61.7 t | 4.39 (t) 8.8 | 61.5 t | 4.37 (t) 6.2 | 61.9 t | 4.39 (t) 6.2 |
| 3-OMe | 55.8 q | 3.81 s | | | 56.0 q | 3.80 s |
| 5-OMe | | | | | 56.3 q | 3.83 s |
| 6-OMe | 56.0 q | 3.84 s | 56.1 q | 3.81 s | | |
| 1-OH | | 12.77 s | | 12.72 s | | 12.71 s |
| 3-OH | | | | 11.89 s | | |
| 8-OH | | 11.63 s | | 11.60 s | | 11.69 s |

spectra displayed 15 carbon and 16 proton signals, respectively, and the data of $^{1}H$ NMR were correlated with those of $^{13}C$ NMR with the help of HSQC spectrum. These data corresponding to 1,2,3,6,8-pentasubstituted napthalene nucleus (C-1 ~ C-8, C-4a, C-8a; H-4, H-5, and H-7) (Watanabe et al., 2000), one 3-hydroxypropanoyl group (C-1′ ~ C-3′; $H_2$-2′, $H_2$-3′) (Hu et al., 2012), two methoxy groups (3-OM and 6-OM), and two phenolic hydroxy groups (1-OH and 8-OH). The 1,2,3,6,8-tetrasubstituted napthalene nucleus was supported by the HMBC correlations from H-4 to C-2, C-3, C-5,C-4a, and C-8a, from H-5 to C-4, C-6, C-7, C-4a, and C-8a, and from H-7 to C-5, C-6. C-8, and C-8a. Since the nucleus of compound was determined, the additional signals (3-hydroxypropanoyl, methoxy, phenolic hydroxy groups) were accounted for the remaining substituents. The location of 3-hydroxypropanoyl group was assigned to C-2 position on the basis of HMBC correlation of $H_2$-2′ ($\delta_H$ 3.36) with C-2 ($\delta_C$ 108.3). The HMBC correlations of the two methoxy protons ($\delta_H$ 3.81 and 3.84) with C-3 ($\delta_C$ 153.8) and C-6 ($\delta_C$ 164.4) concluded the linkage of two methoxy groups at C-3 and C-6, respectively. Finally, two hydroxy groups were assigned to C-1 and C-8 on the basis of HMBC correlations between the hydroxy proton ($\delta_H$ 12.77) and C-1 ($\delta_C$ 166.3), C-2 ($\delta_C$ 108.3), and C-8a ($\delta_C$ 104.6), as well as those between the other hydroxy proton ($\delta_H$ 11.63) and C-7 ($\delta_C$ 103.1), C-8 ($\delta_C$ 158.6), and C-8a ($\delta_C$ 104.6). In addition, the typical proton signals of H-4 ($\delta_H$ 6.43 s), H-5 ($\delta_H$ 6.59, d, $J$ = 2.2 Hz), and H-7 ($\delta_H$ 6.29, d, $J$ = 2.2 Hz) also supported the above substituents pattern. Thus, the structure of **1** was established as 3-hydroxy-1-(1,8- dihydroxy- 3,6-dimethoxynaphthalen-2-yl)propan-1-one (Fig. 2).

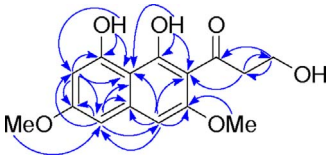


**Fig. 2.** The HMBC ( ) correlations of **1**.

Compound **2** was also obtained as yellow gum and it gave $[M + Na]^+$ peak at *m/z* 301.0681 in HRESIMS, consistent with a molecular formula of $C_{14}H_{14}O_6$. Its $^{1}H$ and $^{13}C$ NMR spectroscopic data were similar to those of **1**, which suggested that **2** were structurally related to **1**. The marked differences between them were the inexistence of a methoxy signal, and appearance of a phenolic hydroxy signal (3-OH) in **2**. This change indicated that the methoxy group in **1** was replaced by a phenolic hydroxy group in **2**. The HMBC correlations of phenolic hydroxy signal ($\delta_H$ 11.60) with C-2 ($\delta_C$ 106.8), C-3 ($\delta_C$ 156.2), and C-4 ($\delta_C$ 101.5) supported this phenolic hydroxy group located C-3. In addition, the other substituents positions also determined by the further analysis of its HMBC correlations. Thus, the structure of **2** was determined as 3-hydroxy-1-(1,3,8-trihydroxy-6-methoxynaphthalen-2-yl)propan-1-one.

Compound **3** was also assigned the molecular formula of $C_{15}H_{16}O_6$ as supported by the HRESIMS (*m/z* 315.0839 $[M + Na]^+$). Its $^{1}H$ and $^{13}C$ NMR spectroscopic data were also similar to those of compound **1**, except for the substituents position variation on naphthalene ring. The HMBC correlation of $H_2$-2′ with C-2 indicated that the location of 3-hydroxypropanoyl group at C-2. The HMBC correlations of the two methoxy protons ($\delta_H$ 3.80 and 3.83) with C-3 and C-5 revealed that two methoxy groups located at C-3 and C-5, respectively. Two hydroxy groups located C-1 and C-8 was determined by the HMBC correlations between the hydroxy proton ($\delta_H$ 12.71) and C-1, C-2, and C-8a, as well as those between the other hydroxy proton ($\delta_H$ 11.69) and C-7, C-8, and C-8a. In addition, the typical proton signals of H-4 ($\delta_H$ 7.09 s), H-6 ($\delta_H$ 6.76, d, $J$ = 8.2 Hz), and H-7 ($\delta_H$ 6.43, d, $J$ = 8.2 Hz) also supported the above substituents pattern. Accordingly, the structure of **3** was determined, and gives the system name of 3-hydroxy-1-(1,8- dihydroxy-3,5-dimethoxy naphthalen-2-yl) propan-1-one.

Compound **1–3** were screened for anti-MRSA activity against the growth of MRSA ZR11, and with vancomycin (MCI valve of 1 *mg*/mL) as positive control (Collyer et al., 2008). The results revealed that compounds **1-3** showed good anti-MRSA activity with MCI of 8, 4 and 4 *mg*/mL, respectively.