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ARTICLE

# Isopentylated diphenyl ether derivatives from the fermentation products of an endophytic fungus *Phomopsis fukushii*

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## Abstract

Three new isopentylated diphenyl ethers, (**1–3**), together with two known isopentylated diphenyl ethers derivatives (**4** and **5**) were isolated from the fermentation products of an endophytic fungus *Phomopsis fukushii*. 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. The results showed that compounds **1–3** showed strong activity with diameter of inhibition zone (IZD) of 21.8 ± 2.4 mm, 16.8 ± 2.2 mm, and 15.6 ± 2.0 mm, respectively.

## Introduction

Natural products are among the most important resources of the clinically used antibacterial activity agents [1, 2]. Many compounds with antibacterial properties have been isolated from natural sources since actinomycin was discovered in 1940s. More than 60% of the currently known compounds with antibacterial activity are natural products or their derivatives [3]. Many diphenyl ethers from natural products show good bioactivity, and they play an important role in the control of microbial infection [4, 5].

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, some of which can produce a number of secondary metabolites with various biological activities including antimicrobial, antifungal, antimalarial, antivirus and antitumor compounds [6–8]. In our previous work, some new compounds with biological activities were obtained from the endophytic fungus of *Phomopsis* species [9–12]. 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.

The structures of compounds **1–5** are shown in Fig. 1a, and the 1H and 13C NMR data of the compounds **1–3** are listed in Table 1. By comparing with the literature, the known compounds were identified as 2-isopentenyldiorcinol (**4**) [13] and diorcinol E (**5**) [14].

## Results and discussion

Compound **1** was obtained as a pale-yellow gum. The molecular formula of $C_{21}H_{24}O_4$ was determined from the HRESIMS spectra showing the sodiated molecular ion at *m/z* 363.1578 $[M + Na]^+$ (calcd 363.1572). The UV absorptions at 206 and 282 nm showed an extended chromophore and a substituted aromatic ring. Its IR spectral data showed the presence of carbonyl groups (1695 $cm^{-1}$) and phenyl groups (1616, 1447, and 1334 $cm^{-1}$). The $^1H$ and $^{13}C$ NMR spectrum of **1** (Table 1) along with the analysis of the DEPT spectra displayed 21 carbon signals and 24 proton signals, respectively, corresponding to a

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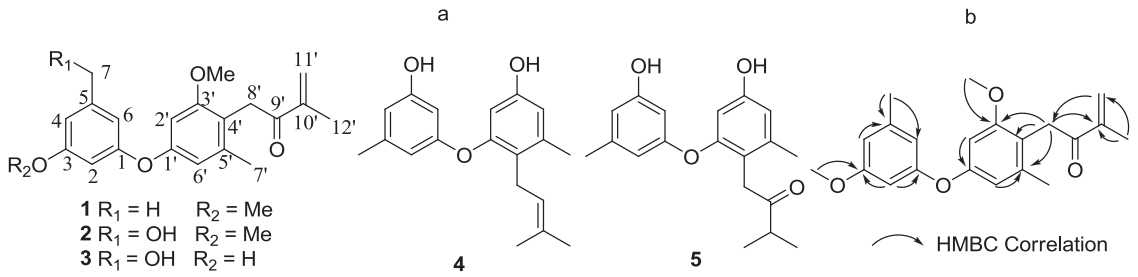


**Fig. 1** **a** Structures of compounds **1–5**. **b** Key HMBC correlations of 1

1,3,5-trisubstituted benzene ring (C-1 ~C-6; H-2, H-4, and H-6), a 1,3,4,5-tetrasubstituted benzene ring (C-1′ ~C-6′; H-2 and H-6), two methyl groups (C-7 and C-7′; $H_3$-7 and $H_3$-7′), two methoxy groups ($\delta_C$ 55.9 q and 56.3 q; $\delta_H$ 3.81 s and 3.86 s), and one 3-methyl-2-oxobut-3-enyl group (C-8′~C-12′; $H_2$-8′, $H_2$-11′, and $H_3$-12′) [15]. Further analysis of the $^{13}C$ NMR data revealed that compound **1** contains four $sp^2$ oxidized aromatic quaternary carbons (C-1, C-3, C-1′, and C-3′). In addition to two methoxy groups on benzene rings, two benzene rings should be connected through ether bonds by an oxygen atom to support the existence of four oxidations of aromatic quaternary carbon. Accordingly, compound **1** should be a diphenyl ether derivative, and this deduction had also been confirmed by the comparison of the NMR data with those of known compounds [16]. Since the nucleus of compound was determined, the additional carbons (two methyl groups, two methoxy goups, and one 3-methyl-2-oxobut-3-enyl group) were accounted for the remaining substituents. The correlations of H2-8′ (δH 4.28 s) with C-3′ (δC 162.9 s), C-4′ (δC 124.6 s), and C-5′ (δC 140.7 s) in HMBC spectra indicated that 3-methyl- 2-oxobut-3-enyl group was located to the C-4′ position (See Fig. 1b). Two methoxy group located at C-3 and C-3′ were supported by the HMBC correlation of two methoxy protons ($\delta_H$ 3.81 s and 3.86) with C-3 and C-3′, respectively. Finally, two methyl groups were assigned to C-5 and C-5′ on the basis of HMBC correlations from $H_3$-7 to C-4, C-5, and C-6, from H-4 and H-6 to C-7, as well as those from $H_3$-7′ to C-4′, C-5′, and C-6′, from H-6′ to C-7′, respectively (see Fig. 1b). In addition, the typical proton signals of H-2 ($\delta_H$ 6.44 s), H-4

**Table 1** NMR spectroscopic data of compounds **1–3** ($^1H$: 500 MHz; $^{13}C$: 125 MHz) in $CDCl_3$

| No | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) |
| 1 | 155.8 qC | | 156.0 qC | | 157.4 qC | |
| 2 | 104.0 CH | 6.44 s | 102.4 CH | 6.46 s | 104.1 CH | 6.42 s |
| 3 | 160.6 qC | | 161.2 qC | | 158.3 qC | |
| 4 | 110.1 CH | 6.52 s | 108.6 CH | 6.57 s | 110.1 CH | 6.54 s |
| 5 | 141.3 qC | | 143.4 qC | | 143.9 qC | |
| 6 | 111.9 CH | 6.47 s | 110.7 CH | 6.50 s | 111.7 CH | 6.47 s |
| 7 | 22.6 CH3 | 2.35 s | 67.4 CH3 | 4.69 s | 67.6 CH3 | 4.70 s |
| 1′ | 166.6 qC | | 166.8 qC | | 166.9 qC | |
| 2′ | 100.2 CH | 6.28 d (2.4) | 100.5 CH | 6.28 d (2.4) | 100.6 CH | 6.26 d (2.4) |
| 3′ | 162.9 qC | | 162.4 qC | | 162.6 qC | |
| 4′ | 124.6 qC | | 124.8 qC | | 124.9 qC | |
| 5′ | 140.7 qC | | 140.9 qC | | 140.5 qC | |
| 6′ | 110.7 CH | 6.33 d (2.4) | 110.8 CH | 6.33 d (2.4) | 110.9 CH | 6.32 d (2.4) |
| 7′ | 23.1 CH3 | 2.27 s | 23.2 CH3 | 2.28 s | 23.1 CH3 | 2.30 s |
| 8′ | 38.8 CH2 | 4.28 s | 38.6 CH2 | 4.24 s | 38.2 CH2 | 4.22 s |
| 9′ | 201.3 qC | | 201.5 qC | | 201.3 qC | |
| 10′ | 144.5 qC | | 144.3 qC | | 144.5 qC | |
| 11′ | 123.3 CH2 | 5.83, 6.13 s | 123.1 CH2 | 5.85, 6.14 s | 123.2 CH2 | 5.85, 6.13 s |
| 12′ | 16.8 CH3 | | 16.6 CH3 | | 16.4 CH3 | |
| –OMe-3 | 55.9 CH3 | 3.81 s | 56.0 CH3 | 3.80 s | | |
| –OMe-3′ | 56.3 CH3 | 3.86 s | 56.4 CH3 | 3.86 s | 56.2 CH3 | 3.85 s |
| Ar–OH | | | | | | 10.38 s |

($\delta_H$ 6.52 s), H-6 ($\delta_H$ 6.47 s), H-2′ [$\delta_H$ 6.28 (d) 2.4], and H-6′ [$\delta_H$ 6.33 (d) 2.4] also supported the above substituents pattern on diphenyl ether nucleus. Therefore, the structure of **1** was established as 1-(4-(3-methoxy-5- methylphenoxy)-2-methoxy-6-methylphenyl)-3-methylbut-3-en-2-one.

1-(4-(3-(hydroxymethyl)-5-methoxyphenoxy)-2-methoxy-6-methylphenyl)-3-methylbut-3-en-2-one (**2**) was also isolated as pale-yellow gum and it gave an $[M + Na]^+$ peak at *m/z* 379.1514 (calcd 379.1521), consistent with a molecular formula of $C_{21}H_{24}O_5$. The data of $^1H$ NMR were assigned to $^{13}C$ NMR with the help of HSQC spetrum (Table 1). Its $^1H$ and $^{13}C$ NMR spectroscopic data were similar to those of **1**, which suggested that compound **2** was structurally related to **1**. The marked differences between them were due to the inexistence of a methyl group, and the appearance of a hydroxymethyl group ($\delta_H$ 4.69 s; $\delta_C$ 67.4 t) in compound **2**. These changes indicated that a methyl group in **1** was replaced by a hydroxymethyl group in compound **2**. In addition, the obvious chemical shift differences of the upfield shift of C-5 from $\delta$ 141.3 ppm to $\delta$ 143.4 ppm suggested the substituent group should be varied at C-5. The HMBC correlations of $H_2$-7 with C-4, C-5, and C-6 also indicated the hydroxymethyl group located C-5. The other substituents positions were also confirmed by the further analysis of its HMBC correlations. The structure of **2** is therefore determined.

Compound **3** was also assigned a molecular formula of $C_{20}H_{22}O_5$ as supported by the HRESIMS [*m/z* 365.1361 $[M + Na]^+$ (calcd 365.1365 for $C_{20}H_{22}NaO_5$)], corresponding to 12 degrees of unsaturation. Its $^1H$ and $^{13}C$ NMR spectroscopic data were also similar to those of compound **2**, except for the presence of a phenolic hydroxyl group signal ($\delta_H$ 10.38 s), and the absence of a methoxy group. The substituent group variation at C-3 was supported by the obvious chemical shift differences of the downfield shift of C-3 from $\delta_C$ 161.2 ppm to $\delta_C$ 158.3 ppm, and the HMBC correlations between phenolic hydroxyl proton ($\delta_H$ 10.38) and C-2, C-3, and C-4. Accordingly, the structure of **3** was determined, and gave the system name of 1-(4-(3-hydroxy-5-(hydroxymethyl)phenoxy)- 2-methoxy-6-methylphenyl)-3-methylbut-3-en-2-one.

Compounds **1–3** were screened for anti-MRSA activity according to arbitrary criterion with diameters of inhibition zone (IZD) as follow: very weak inhibition (with IZD of 6–8 mm), weak inhibition (with IZD of 8–12 mm), good inhibition (with IZD of 12–16 mm), and strong inhibition (with IZD >16 mm) activities respectively. The tetracycline (The IZD ≥ 16 mm) had been used as positive control. The IZD was 32 mm and the negative control to zero. The results revealed that compounds **1–3** showed strong inhibitions with IZD of $21.8 \pm 2.4$ mm, $16.8 \pm 2.2$ mm, and $15.6 \pm 2.0$ mm, respectively. The IZD data are close to those of positive control.

## Materials and methods

### General experimental procedures

UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained in KBr disc on a Bio-Rad Win inferred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. $^1H$, $^{13}C$, and 2D NMR spectra were recorded on Bruker DRX-500 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200–300 mesh), or on silica gel H (10–40 μm), Qingdao Marine Chemical Inc., China). Preparative HPLC was carried out on an Agilent 1100 HPLC equipped with ZORBAX-$C_{18}$ (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

### Fungal material

The culture of *P. fukushii* was isolated from the rhizome of *Paris polyphylla var. yunnanensis* collected from Kunming, Yunnan, People's Republic of China, in 2014. The strain was identified by one of authors (Gang Du) based on the analysis of the ITS sequence (Genbank Accession number KP068615. It was cultivated at room temperature for 7 days on potato dextrose agar at 28 °C. Agar plugs were inoculated into 250 mL Erlenmeyer flasks each containing 100 mL potato dextrose broth and cultured at 28 °C on a rotary shaker at 180 rpm for five days. Large scale fermentation was carried out in 200 Fernbach flasks (500 mL) each containing 100 g of rice and 120 mL of distilled $H_2O$. Each flask was inoculated with 5.0 mL of cultured broth and incubated at 25 °C for 45 days.

### Anti-MRSA agar disc diffusion assay

The MRSA strain ZR11 was clinically isolated from infectious samples of critically ill patients in the Clinical Laboratory of the First People's Hospital of Yunnan Province, and confirmed by standard cefoxitin disk diffusion test following CLSI standard procedures [17]. The anti-MRSA activity of the compounds was evaluated via the disc diffusion method. The ZR11 strain was inoculated in Müeller Hinton Broth and were incubated at 37 °C for 24 h. The turbidity of bacterial suspension was adjusted to 0.5 McFarland standard which equals to $1.5 \times 10^8$ colny-forming units (CFU)/mL. Sterile filter paper discs (6 mm) were impregnated with 20 μl (50 μg) of each compound and placed on inoculated Müeller Hinton agar containing bacterial suspension which adjusted to 0.5 McFarland standard. The commercially available discs containing 30 μg Vancomycin were used as positive control whereas discs without samples (5% DMSO) acted as negative control. The inhibition zones including the diameter of the disc (mm) were

measured and compared after incubation at 37 °C for 24 h. The tests were carried out in triplicate for each sample.

## Extraction and isolation

The fermented substrate was extracted four times with 70% aqueous acetone (4 × 10 L) at room temperature and filtered. The crude extract (297.0 g) was applied to silica gel (200–300 mesh) column chromatography, eluting with a chloroform–acetone gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions (A–F). The further separation of fraction B (9:1, 12.2 g) by silica gel column chromatography, eluted with petroleum ether–acetic ether and preparative HPLC with 62% aqueous MeOH as mobile phase, flow rate 20 mL/min) afford **1** (15.5 mg) and **2** (17.3 mg). The further separation of fraction C (8:2, 22.6 g) by silica gel column chromatography, eluted with petroleum ether–acetic ether and preparative HPLC with 55% aqueous MeOH as mobile phase, flow rate 20 mL/min) afford **3** (12.2 mg), **4** (19.7 mg), and **5** (16.3 mg).

## Compounds characterization

### 1-(4-(3-methoxy-5-methylphenoxy)-2-methoxy-6-methylphenyl)-3-methylbut-3-en-2-one (1)

$C_{21}H_{24}O_4$, obtained as pale-yellow gum; UV (MeOH), $\lambda_{max}$ (log $\varepsilon$) 282 (3.64), 206 (4.55) nm; IR (KBr) $\nu_{max}$ 2934, 2839, 1695, 1616, 1447, 1334, 1160, 1059, 863 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data ($CDCl_3$, 500 and 125 MHz, respectively), Table 1; ESIMS *m/z* 363; HRESIMS (positive ion mode) *m/z* 363.1578 $[M + Na]^+$ (calcd 363.1572 for $C_{21}H_{24}NaO_4$).

### 1-(4-(3-(hydroxymethyl)-5-methoxyphenoxy)-2-methoxy-6-methylphenyl)-3-methylbut-3-en-2-one (2)

$C_{21}H_{24}O_5$, obtained as pale-yellow gum; UV (MeOH), $\lambda_{max}$ (log $\varepsilon$) 285 (3.60), 206 (4.58) nm; IR (KBr) $\nu_{max}$ 3387, 2932, 2844, 1698, 1614, 1455, 1352, 1165, 1047, 884 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data ($CDCl_3$, 500 and 125 MHz, respectively), Table 1; ESIMS *m/z* 379; HRESIMS (positive ion mode) *m/z* 379.1514 $[M + Na]^+$ (calcd 379.1521 for $C_{21}H_{24}NaO_5$).

### 1-(4-(3-hydroxy-5-(hydroxymethyl)phenoxy)-2-methoxy-6-methylphenyl)-3-methylbut-3-en-2-one (3)

$C_{20}H_{22}O_5$, obtained as pale-yellow gum; UV (MeOH), $\lambda_{max}$ (log $\varepsilon$) 280 (3.55), 206 (4.52) nm; IR (KBr) $\nu_{max}$ 3420, 2936, 2855, 1699, 1615, 1462, 1347, 1158, 1042, 870 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data ($C_5D_5N$, 500 and 125 MHz, respectively), Table 1; ESIMS *m/z* 365; HRESIMS (positive ion mode) *m/z* 365.1361 $[M + Na]^+$ (calcd 365.1365 for $C_{20}H_{22}NaO_5$).

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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