





## Trichothecene Sesquiterpenes with Anti-osteosarcoma Cytotoxicity from the Fungus *Fusarium* sp. XPW68

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**Abstract:** Fusarinene A (**1**), a previously undescribed trichothecene sesquiterpene, and three known compounds, 4 $\beta$ -hydroxytrichotheca-9,12-diene (**2**), trichodermol (**3**), and trichodermin (**4**), were isolated from the extract of *Fusarium* sp. XPW68. Their structures and relative configurations were identified by detailed analysis of nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectrometry (HRESIMS) data. In the cytotoxic assay, compounds **1-4** exhibited selective or potent inhibition against five human osteosarcoma cell lines including 143B, MG-63, SaOS-2, SW1353, and U2OS, with IC<sub>50</sub> values ranging from 0.8 to 48.2  $\mu$ M.

**Keywords:** *Fusarium*; trichothecene sesquiterpene; structure elucidation; cytotoxic activity. © 2025 ACG Publications. All rights reserved.

### 1. Fungal Source

*Fusarium* sp. XPW68 was isolated from soil collected from Wenzhou, Zhejiang province in China. This strain was identified based on the internal transcribed spacer regions of rDNA, and the sequence showed 100% identity to that of known strain (KU841448.1) in the GenBank database with an accession number of PV242142. In addition, this fungus was deposited in The Third Affiliated Hospital of Wenzhou Medical University, China.

### 2. Previous Studies

Trichothecene sesquiterpenes, which usually feature a tetracyclic framework, have been isolated from a variety of fungi, such as *Fusarium* [1-4], *Trichoderma* [5,6], *Stachybotrys* [7], and *Trichothecium* [8]. A large number of trichothecenes displayed remarkable cytotoxicity toward eukaryotic organisms, and the structure-activity relationships exhibited that the epoxide moiety and rearrangement of the ring

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system acted as the crucial role in the cytotoxicity [9]. Thus, trichothecenes are promising to exploit new anticancer drugs.

### 3. Present Study

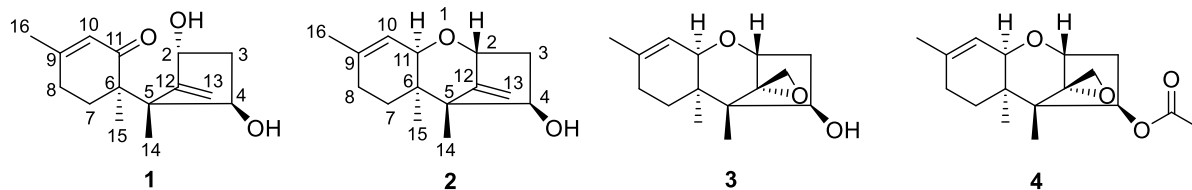
Large-scale fermentation was cultured in rice medium, prepared in 20 conical flasks (1000 mL) with each containing 100 g of rice and 180 mL of purified water. The fermentation was executed under static conditions at 26 °C for 15 days. To each flask, about 200 mL ethyl acetate (EtOAc) was added, and the extract was evaporated to dryness under vacuum to acquire 8.3 g of crude residue. The extract was subjected to RP-18 column chromatography (CC) with a series of solvent systems of MeOH–H<sub>2</sub>O (from 1:9 to 10:0), affording 10 fractions. Fraction 3 (MeOH–H<sub>2</sub>O, 3:7) was purified by silica gel CC with petroleum ether (PE)–EtOAc (from 5:1 to 0:1) and preparative thin layer chromatography (pTLC) (CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 20:1) to yield compound **1** (1.7 mg). Fraction 5 (MeOH–H<sub>2</sub>O, 1:1) was separated on silica gel CC with petroleum ether (PE)–EtOAc (from 5:1 to 1:1), Sephadex LH-2 (MeOH), and pTLC (PE–EtOAc, 1:1) to obtain compound **3** (18.4 mg). Fraction 6 (MeOH–H<sub>2</sub>O = 3:2) was purified using silica gel CC with petroleum ether (PE)–EtOAc (from 5:1 to 1:1) and pTLC (PE–EtOAc, 1:1) to give compounds **2** (15.5 mg) and **4** (21.6 mg).

*Fusarinene A (1)*: Colorless oil;  $[\alpha]_D^{20} = +11.6$  ( $c = 0.03$ , MeOH); <sup>1</sup>H (500 MHz) and <sup>13</sup>C (125 MHz) NMR data, see Table 1; HRESIMS:  $m/z$  273.1466 [ $M + Na$ ]<sup>+</sup> (calcd. for C<sub>15</sub>H<sub>22</sub>NaO<sub>3</sub>, 273.1467).

*Cytotoxic Assay*: Compound **1** was evaluated for cytotoxicity against five human osteosarcoma cell lines (143B, MG-63, SaOS-2, SW1353, and U2OS) by means of the MTT assay [10]. These cells were cultivated for 24 h in 96-well plates, and tested compounds and the positive control with gradient concentrations (0.05, 0.1, 1.0, 10.0, 20.0, 50.0 μM) were added into wells. After incubating for 96 h, MTT solution was pipetted into each well and the cells were incubated for 4 h. The UV/VIS absorbance was recorded with a microplate spectrophotometer at 570 nm. 5-Fluorouracil and DMSO were used as the positive and negative controls, respectively.

Compound **1** was isolated as colorless oil, and the molecular formula was deduced to be C<sub>15</sub>H<sub>22</sub>O<sub>3</sub> based on its HRESIMS data. The <sup>1</sup>H NMR spectrum (Table 1) exhibited one olefinic proton signal at  $\delta_H$  5.76, (s, H-10), a pair of vinyl proton signals at  $\delta_H$  5.24 (s, H-13a) and 5.11 (s, H-13b), two oxygenated methine signals at  $\delta_H$  4.32 (d,  $J = 4.3$  Hz, H-2) and 4.67 (dd,  $J = 10.6$  and  $6.8$  Hz, H-4), and three methyl signals at  $\delta_H$  1.21 (s, H<sub>3</sub>-14), 1.29 (s, H<sub>3</sub>-15), and 1.93 (s, H<sub>3</sub>-16). The <sup>13</sup>C NMR and DEPT spectra showed 15 carbon signals, assigned to three methyls at  $\delta_C$  18.2 (C-15), 20.8 (C-14), and 23.9 (C-16), four methylenes (including one olefinic carbon) at  $\delta_C$  28.9 (C-8), 31.6 (C-7), 40.7 (C-3), and 113.5 (C-13), three methines (including two oxygenated carbons and one olefinic carbon) at  $\delta_C$  74.7 (C-2), 76.6 (C-4), and 126.9 (C-10), and five quaternary carbons (including two olefinic carbons and one carbonyl carbon) at  $\delta_C$  49.4 (C-6), 52.2 (C-5), 161.0 (C-12), 161.6 (C-9), and 207.1 (C-11). Comparing NMR data of **1** with those of 3,6α-dimethyl-2β-(1β-methyl-2-methylenecyclopentyl)cyclohex-2-enone [11] indicated their similarity, differing in the presence of two oxygenated methines ( $\delta_C$  74.7, C-2;  $\delta_C$  76.6, C-4) and the lack of two methylenes. The HMBC cross-peaks from H<sub>2</sub>-13 to C-2, C-5, and C-12 and from H<sub>3</sub>-14 to C-4, C-5, C-6, and C-12 as well as the <sup>1</sup>H-<sup>1</sup>H COSY correlations between H<sub>2</sub>-3 with H-2/H-4 indicated that two oxygenated methines replaced two methylenes at C-2 and C-4 (Figure 2). The <sup>1</sup>H-<sup>1</sup>H COSY correlation between H<sub>2</sub>-7 and H<sub>2</sub>-8 and the HMBC cross-peaks from H<sub>3</sub>-15 to C-5, C-6, C-7, and C-11 and from H<sub>3</sub>-16 to C-8, C-9, and C-10 generated the planar structure of **1**. The relative configuration of **1** was determined by the coupling constants and NOESY correlations. The small coupling constant between H-2 and H-3b and the large coupling constant between H-3b and H-4 (Table 1) suggested the opposite orientations of H-2 and H-4, which was confirmed by the NOESY correlations between H-2 and H-3b and between H-4 and H-3a, while H-2 and Me-14 were cofacial, which was supported by the NOESY correlation between H<sub>3</sub>-14 and H-3b (Figure 2). The relative configuration of C-6 was deduced to be *R*\* based on biosynthetic considerations.

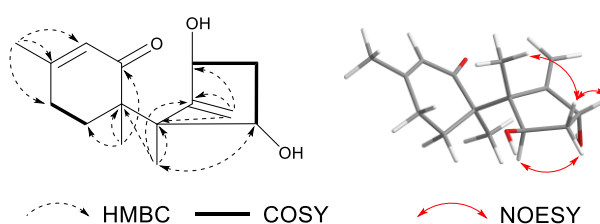
The structures of the three known compounds were identified as 4 $\beta$ -hydroxytrichotheca-9,12-diene (**2**) [12,13], trichodermol (**3**) [14], and trichodermin (**4**) [6] by analysis of the NMR data with those reported in the literature (Figure 1).



**Figure 1.** Chemical structures of compounds **1-4**

**Table 1.**  $^1\text{H}$  (500 MHz) and  $^{13}\text{C}$  (125 MHz) NMR data of compound **1** ( $\delta$  in ppm) in  $\text{CDCl}_3$

Position	$\delta_{\text{H}}$	$\delta_{\text{C}}$
2	4.32, d (4.3)	74.7, CH
3a	2.16, dd (13.5, 6.6)	40.7, $\text{CH}_2$
3b	1.70, ddd (13.5, 10.9, 5.1)	
4	4.67, dd (10.6, 6.8)	76.6, CH
5		52.2, C
6		49.4, C
7a	2.20, m	31.6, $\text{CH}_2$
7b	1.87, ddd (13.6, 5.2, 1.7)	
8a	2.43, m	28.9, $\text{CH}_2$
8b	2.24, m	
9		161.6, C
10	5.76, s	126.9, CH
11		207.1, C
12		161.0, C
13a	5.24, s	113.5, $\text{CH}_2$
13b	5.11, s	
14	1.21, s	20.8, $\text{CH}_3$
15	1.29, s	18.2, $\text{CH}_3$
16	1.93, s	23.9, $\text{CH}_3$



**Figure 2.** Key  $^1\text{H}$ - $^1\text{H}$  COSY, HMBC, and NOESY correlations of **1**

Compounds **1-4** were assayed for cytotoxicity against five human osteosarcoma cell lines including 143B, MG-63, SaOS-2, SW1353, and U2OS. The results (Table 2) showed that **1-4** could selectively inhibit the growth of all the tested cells, while compounds **3** and **4** showed significant cytotoxic activities against the five human osteosarcoma cell lines with  $\text{IC}_{50}$  values below 10.0  $\mu\text{M}$ . Furthermore, the structure-activity relationship analysis indicated that the epoxy group plays a crucial role in cytotoxicity, as demonstrated by the significantly higher cytotoxicity of compounds **3** and **4** compared to compounds **1** and **2**. In addition, the acetoxy group at C-4 may also contribute, to some extent, to the cytotoxicity.

**Table 2.** Cytotoxic activities for compounds **1-4** (IC<sub>50</sub>, µM)

Compounds	143B	MG-63	SaOS-2	SW1353	U2OS
<b>1</b>	>50.0	33.9	>50.0	45.8	>50.0
<b>2</b>	37.5	43.7	>50.0	>50.0	48.2
<b>3</b>	6.1	4.9	5.4	9.7	6.6
<b>4</b>	3.3	2.1	0.8	8.4	4.2
5-Fluorouracil	0.4	0.7	0.6	1.1	0.8

In conclusion, our investigation of the soil-derived fungus *Fusarium* sp. XPW68 led to the isolation of one new and four known trichothecene sesquiterpenes including fusarinene A (**1**), 4β-hydroxytrichotheca-9,12-diene (**2**), trichodermol (**3**), and trichodermin (**4**). These trichothecene sesquiterpenes significantly expanded the natural product library of *Fusarium*. Additionally, all compounds were evaluated for human osteosarcoma cell lines inhibitory activities, and potent inhibition activities against those cell lines of **3** and **4** and the structure-activity relationship analysis could provide promising lead compounds for anti-osteosarcoma therapy.

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## Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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