

## Histone Deacetylase Inhibitor Induced Lipase Inhibitors from Endophytic *Phomopsis* sp. 0391

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**Abstract:** Seven compounds including a new one named as 13-angeloyloxy-diplosporin (**1**) were isolated from the endophytic *Phomopsis* sp. 0391 cultivated in the presence of a histone deacetylase inhibitor. All of these isolates were evaluated for lipase suppressive activities and we firstly found that compounds cytosporone B (**5**) and dothiorelone A (**6**) displayed significant lipase inhibited activities compared to the positive control (Orlistat, IC<sub>50</sub> = 43 µg/mL) with the IC<sub>50</sub> values at 115 and 275 µg/mL, respectively.

**Keywords:** Phomopsis; endophytic fungi; chemical epigenetics approach; lipase inhibitor; NMR. © 2019 ACG Publications. All rights reserved.

### 1. Introduction

Filamentous fungi is a rich source of biologically active natural product metabolites with wide-ranging biological activity [1-5]. The genome sequencing studies showed that many fungi strains presence of many cryptic biosynthetic gene clusters encoding uncharacterized secondary metabolites [1]. While, such compounds are difficult to obtain under standard culture conditions because most of these biosynthetic gene clusters are silent and need to be active using different methods [6-7]. Chemical epigenetics approach is one of an effective technique for promoting the transcription of silent biosynthetic pathways involved in the fungi natural products discovery [8-11]. Asai and coauthors found that some endophytic fungi cultivated in the presence of 500 µM suberoyl bis-hydroxamic acid (SBHA) (a histone deacetylase inhibitor) could promote some fungi to produce some novel natural products such as spiroindicumides A and B, mollipilins A–E, and chaetophenols A–F,

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isolated from some different filamentous fungi [8]. In our research for finding bioactive natural products from endophytic fungi isolated from Traditional Chinese Medicine (TCM) [12-14], seven natural products including a new one have been isolated from the cultures of the fungus *P. sp.* 0391 endophytic to the stems of *Paris polyphylla* var. *yunnanensis* in the presence of a histone deacetylase inhibitor. (Figure 1). We found that the cultivation of *P. sp.* 0391 in the presence of 500  $\mu$ M SBHA significantly enhanced its secondary metabolite production, leading to the isolation of a new diplosporin derivative together with some other known ones. In this paper, we demonstrate that chemical epigenetic manipulation led to a substantial restructuring of secondary metabolite produced by an endophytic fungal isolate and the major compound's isolation, structure elucidation and lipase inhibited activities.

## 2. Materials and Methods

### 2.1. General Experimental Procedures

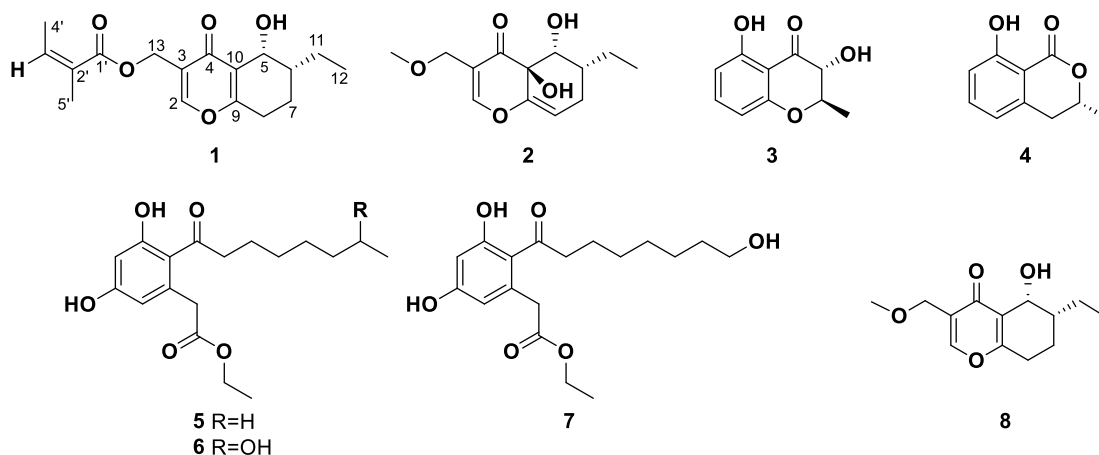
Optical rotations were measured with a JASCO P-1020 polarimeter. NMR spectra were recorded on Bruker AM-800 spectrometers. HRESIMS experiment was performed on a Bruker HCT/Esquire spectrometer and a Waters AutoSpec Premier P776 spectrometer. Column chromatography was performed with silica gel (200–300 mesh, Qingdao marine), RP-18 gel (40–63  $\mu$ m, YMC), and Sephadex LH-20 gel (40–70  $\mu$ m, GE Healthcare). Semipreparative HPLC was performed on a liquid chromatograph with a 9.4 mm  $\times$  25 cm column (Agilent, ZORBAX SB-C18). Fractions were monitored by TLC, and compounds were visualized by heating silica gel plates sprayed with 10% H<sub>2</sub>SO<sub>4</sub> in EtOH. All solvents including petroleum ether (60–90 °C) were distilled prior to use.

### 2.2. Fungal Material

The culture of *P. sp.* 0391 was isolated from the stems of *Paris polyphylla* var. *yunnanensis* collected from Kunming, Yunnan, People's Republic of China, in 2017. The isolate was identified based on sequence (GenBank Accession No. MK311351) analysis of the ITS region of the DNA. The fungal strain was cultured on slants of potato dextrose agar at 30 °C for 3 days. Agar plugs were cut into small pieces (about 0.5  $\times$  0.5  $\times$  0.5 cm<sup>3</sup>) under aseptic conditions, and 15 pieces were used to inoculate three Erlenmeyer flasks (250 mL), each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract); the final pH of the media was adjusted to 6.5, and the flasks were sterilized by autoclave. Three flasks of the inoculated media were incubated at 30 °C on a rotary shaker at 170 rpm for 5 days to prepare the seed culture. Fermentation was carried out in 50 Fernbach flasks (500 mL), each containing 200 mL of potato dextrose broth in the presence of 500  $\mu$ M SBHA incubated at 30 °C on a rotary shaker at 200 rpm for 7 day.

### 2.3. Fermentation, Isolation, and Purification

The fermented material was extracted with EtOAc (5  $\times$  10.0 L), and the organic solvent was evaporated to dryness under vacuum to afford a crude extract (5.6 g). The crude extract was purified by CC (column chromatography on SiO<sub>2</sub> with a petroleum ether–ethyl acetate gradient system 10:1, 5:1, 2:1, 1:1, 0:1) to yield five main fractions, *Fr.* 1–5. Compound **4** (3.8 mg) was mainly detected in *Fr.* 2 (petroleum ether–ethyl acetate, 5:1; 100 mg). *Fr.* 4 (petroleum ether–ethyl acetate, 1:1; 320 mg) was purified by semi-preparative HPLC (3 mL/min, detector UV 230 nm, MeCN–H<sub>2</sub>O, 40:60) to yield **2** (7.0 mg, 8.2 min), **3** (4.6 mg, 11.8 min), **1** (17.8 mg, 18.3 min), and **5** (68 mg, 22.3 min), respectively. *Fr.* 5 (600 mg) was purified by RP-18 gel CC with MeOH–H<sub>2</sub>O (30:70, 45:55, 60:40, 75:25, and 100:0) to yield *Fr.* 5.1–5.5. *Fr.* 5.3 (90 mg, MeOH–H<sub>2</sub>O, 60:40) was purified by semi-preparative HPLC (3 mL/min, detector UV 230 nm, MeCN–H<sub>2</sub>O, 40:60) to yield **6** (11.1 mg, 13.5 min) and **7** (18.3 mg, 13.9 min), respectively.



**Figure 1.** Chemical structures of compounds **1-8**

**2.4. Spectral Data of 13-angeloyloxy-diplosporin (1):** White amorphous powder,  $[\alpha]_D^{25} = +50.0$  (c 0.1, CH<sub>3</sub>OH); HRESIMS:  $m/z$  307.1534 [M + H]<sup>+</sup> (calcd 307.1545 for C<sub>17</sub>H<sub>23</sub>O<sub>5</sub>); for <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1 and Figures S3-S16.

### 2.5. Determination of Pancreatic Lipase Inhibition

The pancreatic lipase inhibition activity assay was conducted as previously [15]. P-nitrophenyl laurate was used as substrate. Porcine pancreatic lipase (Kayon-Shanghai, China) solution (150 mg/mL) was first centrifuged at 10,000g at 4 °C for 5 min to obtain the supernatant enzyme. The substrate solution consisted of p-nitrophenyl laurate dissolved in 5 mM (pH 5.0) sodium acetate (1:100 m/v), as well as Triton X-100 (1:100 v/v). The substrate solution was placed in boiling water for 2 min to fully dissolve the reaction substrate. The sample is dissolved in 50% DMSO solution.

The control consisted of 400 μl of Tris buffer, 350 μl of prepared reaction substrate solution, 100 μl of 50% DMSO solution, and 150 μl of enzyme. The treatment samples consisted of 400 μL of Tris buffer, 350 μL of prepared reaction substrate solution, 100 μL of sample solution, and 150 μL of enzyme. A blank was needed for each group. The last step involved the incubation of the entire reaction mixture at 37 °C for 2 h followed by centrifugation at 10,000g for 3 min. The absorbance (OD) of the reaction mixture was measured at 400 nm by using a SpectraMax M5 microplate reader and the inhibition of pancreatic lipase activity (%) was determined as follows:  $(OD_{\text{control}} - OD_{\text{sample}}) / OD_{\text{control}} \times 100\%$ . The pancreatic lipase inhibitory activity was expressed as microgram of Orlistat equivalents (μg Orlistat/g extract).

## 3. Results and Discussion

### 3.1. Structure Elucidation

Compound **1**, obtained as a white, amorphous powder, gave the molecular formula of C<sub>17</sub>H<sub>22</sub>O<sub>5</sub> from its HRESIMS (calcd for [M + H]<sup>+</sup> 307.1545, found 307.1534), indicating seven degrees of unsaturation.

The <sup>13</sup>C NMR and DEPT spectra of **1** displayed 17 carbon signals corresponding to three methyls, four methylenes (of which one was oxygenated), four methines (including one oxygenated and two olefinic carbon), and six quaternary carbons (including one keto carbonyl group, one ester carbonyl carbon, and four olefinic carbons) (Figure S3 and S4). This was consistent with a skeleton of a diplosporin polyketide. The carbon resonances at  $\delta_C$  169.0, 128.8, 139.7, 16.0, and 20.7 revealed the presence of one angeloyloxy group, which is quite similar to the data of angeloyloxy group at 9-angeloyloxythymol at  $\delta_C$  168.9, 126.9, 138.9, 15.8, and 20.5 [16]. Comparison of its NMR data (Table 1) with those of 13-O-methyl-diplosporin (**8**) [17] revealed similarities except for the lack of the signal

of a methoxy group in **8** instead of an angeloyloxy group ( $\delta_C$  169.0, 128.8, 139.7, 16.0, and 20.7) in **1** at C-13, which was supported by the HMBC correlations from H<sub>2</sub>-13 ( $\delta_H$  4.97, s, 2H) to C-2, C-3, C-4, and C-1', from H-3' ( $\delta_H$  6.13, m, 1H) to C-1', C-4', and C-5', and from H<sub>3</sub>-4' ( $\delta_H$  1.94, ddd,  $J = 7.3, 3.0, 1.4$  Hz, 3H) and H<sub>3</sub>-5' ( $\delta_H$  1.86, dd,  $J = 3.0, 1.5$  Hz, 3H) to C-1', C-2', and C-3'. In addition, the HMBC spectrum of **1** showed correlations from one of H-2 ( $\delta_H$  8.13, s, 1H), to C-3, C-4, C-9, and C-13, from H-5 ( $\delta_H$  4.82, d,  $J = 2.0$  Hz, 1H), to C-4, C-6, C-7, C-9, and C-10, and from H<sub>2</sub>-8 ( $\delta_H$  2.66, m, 2H) to C-5, C-6, C-7, C-9, and C-10. Furthermore, H<sub>2</sub>-7 ( $\delta_H$  1.73, m, 2H) showed HMBC correlations to C-5, C-6, C-8, and C-9. Other correlations were H-6 ( $\delta_H$  1.38, m, overlap, 1H) with C-5, C-7, C-11, and C-12, H-11a ( $\delta_H$  1.40, m, overlap, 1H) and H-11b ( $\delta_H$  1.63, m, 1H) with C-5, C-6, C-7, and C-12, and H<sub>3</sub>-12 ( $\delta_H$  1.02, t,  $J = 7.3$  Hz, 3H) with C-6 and C-11. These observed HMBC correlations, coupled with a spin system (CHCH(CH<sub>3</sub>CH<sub>2</sub>)CH<sub>2</sub>CH<sub>2</sub>, H-5/H-6/(H<sub>2</sub>-11/H<sub>3</sub>-12)/H<sub>2</sub>-7/H<sub>2</sub>-8) determined by <sup>1</sup>H-<sup>1</sup>H COSY and HSQC data, established the gross structure of **1**. The HRESIMS (Figure S1 and Figure S2) data at  $m/z = 207.1017$  (C<sub>12</sub>H<sub>15</sub>O<sub>3</sub>, M-angeloyloxy group-H<sub>2</sub>O+H), 289.1429 (C<sub>17</sub>H<sub>21</sub>O<sub>4</sub>, M - H<sub>2</sub>O + H), 307.1534 (C<sub>17</sub>H<sub>23</sub>O<sub>5</sub>, M + H), and 329.2353 (C<sub>17</sub>H<sub>22</sub>O<sub>5</sub>Na, M + Na) also supported the structure of **1**. In the ROESY spectrum (Figure S16) of **1**, correlations of H-5 with H-6 showed that H-5 and H-6 in **1** had  $\beta$ -configurations. Therefore, **1** is named as 13-angeloyloxy-diplosporin.

The structures of **2-7** were elucidated by comparison of their 1D NMR data with literatures and named as 3-methoxymethyl-agistatine D (**2**) [17], (-)-gynuraone (**3**) [18], mellein (**4**) [19], cytosporone B (**5**) [20], dothiorelone A (**6**) [21], and dothiorelone C (**7**) [21].

**Table 1.** NMR data of compound **1** (<sup>1</sup>H NMR: 800 MHz, <sup>13</sup>C NMR: 200 MHz,  $\delta$  in ppm,  $J$  in Hz, recorded in methanol-*d*<sub>4</sub>)

Positin	$\delta_C$ (ppm)	$\delta_H$ (ppm)	HMBC	<sup>1</sup> H- <sup>1</sup> H COSY	ROESY
2	156.7 (CH)	8.13 s	3, 4, 9, 13	-	H <sub>2</sub> -13
3	124.4 (C)	-	-	-	-
4	178.8 (C)	-	-	-	-
5	62.2 (CH)	4.82 (d, $J = 2.0$ )	4, 6, 7, 9, 10	H-6	H-6, H-11b, H <sub>3</sub> -12
6	41.9 (CH)	1.38 overlap	5, 7, 11, 12	H-5, H <sub>2</sub> -7	H-5,
7	22.6 (CH <sub>2</sub> )	1.73 m	5, 6, 8, 9	H-6, H <sub>2</sub> -8	H <sub>2</sub> -8, H <sub>3</sub> -12
8	28.7 (CH <sub>2</sub> )	2.66 m	5, 6, 7, 9, 10	H <sub>2</sub> -7	H <sub>2</sub> -7
9	169.0 (C)	-	-	-	-
10	125.5 (C)	-	-	-	-
11	25.3 (CH <sub>2</sub> )	1.40a overlap 1.63b m	5, 6, 7, 12 5, 6, 7, 12	H-11b, H <sub>3</sub> -12 H-11a, H <sub>3</sub> -12	H-5, H <sub>3</sub> -12
12	11.9 (CH <sub>3</sub> )	1.02 (t, $J = 7.3$ )	6, 11	H-11a, H-11b	H-5, H <sub>2</sub> -7, H-11b
13	58.8 (CH <sub>2</sub> )	4.97 s	1', 2, 3, 4	-	H-2
1'	169.0 (C)	-	-	-	-
2'	128.8 (C)	-	-	-	-
3'	139.7 (CH)	6.13 m	1', 4', 5'	H <sub>3</sub> -4'	H <sub>3</sub> -4', H <sub>3</sub> -5'
4'	16.0 (CH <sub>3</sub> )	1.94 (ddd, $J = 7.3, 3.0, 1.4$ )	1', 2', 3'	H-3'	H-3'
5'	20.7 (CH <sub>3</sub> )	1.86 (dd, $J = 3.0, 1.5$ )	1', 2', 3'	-	H-3'

### 3.2. Pancreatic Lipase Inhibition Activity

All isolated compounds were tested for lipase inhibited activity, compounds cytosporone B (**5**) and dothiorelone A (**6**) strongly inhibited lipase activities compared to the positive control (Orlistat, IC<sub>50</sub> = 43  $\mu$ g/mL) with the IC<sub>50</sub> values at 115 and 275  $\mu$ g/mL, respectively.

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## Supporting Information

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## References

- [1] T. Hautbergue, E. L. Jamin, L. Debrauwer, O. Puel and I. P. Oswald (2018). From genomics to metabolomics, moving toward an integrated strategy for the discovery of fungal secondary metabolites, *Nat. Prod. Rep.* **35**, 147-173.
- [2] H. Jiang, W. J. Man, A. J. Hou, L. Yang, X. D. Xing, M. L. Yan, X. Y. Guo and L. Yang (2018). The chemical constituents from the active fractions of *Eleutherine bulbosa* with their antimicrobial activity, *Nat. Prod. Res.* 1-7. <https://doi.org/10.1080/14786419.2018.1530229>
- [3] R. N. Kharwar, A. Mishra, S. K. Gond, A. Stierle and D. Stierle (2011). Anticancer compounds derived from fungal endophytes: their importance and future challenges, *Nat. Prod. Rep.* **28**, 1208-1228.
- [4] S. Krishnamurthy, K. N. Murthy and S. Thirumale (2018). Characterization of Ankaflavin from *Penicillium aculeatum* and its cytotoxic properties, *Nat. Prod. Res.* 1-6. <https://doi.org/10.1080/14786419.2018.1522633>
- [5] Zulqarnain, Z. Iqbal, R. Cox, J. Anwar, N. Ahmad, K. Khan, M. Iqbal, N. Manzoor and S. U. Khattak (2018). Antifungal activity of compounds isolated from *Aspergillus niger* and their molecular docking studies with tomatinase, *Nat. Prod. Res.* 1-5. <https://doi.org/10.1080/14786419.2018.1548447>
- [6] G. L. Challis (2008). Genome mining for novel natural product discovery, *J. Med. Chem.* **51**, 2618-2628.
- [7] J. Yaegashi, B. R. Oakley and C. C. Wang (2014). Recent advances in genome mining of secondary metabolite biosynthetic gene clusters and the development of heterologous expression systems in *Aspergillus nidulans*, *J. Ind. Microbiol. Biotechnol.* **41**, 433-442.
- [8] T. Asai, T. Taniguchi, T. Yamamoto, K. Monde and Y. Oshima (2013). Structures of Spiroindicumides A and B, unprecedented carbon skeletal spiro lactones, and determination of the absolute configuration by vibrational circular dichroism exciton approach, *Org. Lett.* **15**, 4320-4323.
- [9] R. H. Cichewicz (2010). Epigenome manipulation as a pathway to new natural product scaffolds and their congeners, *Nat. Prod. Rep.* **27**, 11-22.

- [10] X. Wang, J. G. Sena Filho, A. R. Hoover, J. B. King, T. K. Ellis, D. R. Powell and R. H. Cichewicz (2010) Chemical epigenetics alters the secondary metabolite composition of guttate excreted by an atlantic-forest-soil-derived *Penicillium citreonigrum*, *J. Nat. Prod.* **73**, 942-948.
- [11] J. X. Zhu, L. J. Ding and S. He (2018). Discovery of a new biphenyl derivative by epigenetic manipulation of marine-derived fungus *Aspergillus versicolor*, *Nat. Prod. Res.* 1-5.
- [12] W. G. Wang, A. Li, B. C. Yan, S. B. Niu, J. W. Tang, X. N. Li, X. Du, G. L. Challis, Y. S. Che, H. D. Sun and J. X. Pu (2016). LC-MS-guided isolation of Penicilfuranone A: a new antifibrotic furancarboxylic acid from the plant endophytic fungus *Penicillium* sp. sh18, *J. Nat. Prod.* **79**, 149-155.
- [13] W. G. Wang, L. Q. Du, S. S. Sheng, A. Li, Y. P. Li, G. G. Cheng, G. P. Li, G. L. Sun, Q. F. Hu and Y. Matsuda (2019). Genome mining for fungal polyketide-diterpenoid hybrids: discovery of key terpene cyclases and multifunctional P450s for structural diversification, *Org. Chem. Front.* **6**, 571-578.
- [14] B. C. Yan, W. G. Wang, D. B. Hu, X. Sun, L. M. Kong, X. N. Li, X. Du, S. H. Luo, Y. Liu, Y. Li, H. D. Sun and J. X. Pu (2016). Phomopchalasins A and B, two cytochalasans with polycyclic-fused skeletons from the endophytic fungus *Phomopsis* sp. shj2, *Org. Lett.* **18**, 1108-1111.
- [15] C. T. Zhang, Y. L. Ma, F. D. Gao, Y. X. Zhao, S. B. Cai and M. J. Pang (2018). The free, esterified, and insoluble-bound phenolic profiles of *Rhus chinensis* mill. fruits and their pancreatic lipase inhibitory activities with molecular docking analysis, *J. Funct. Foods* **40**, 729-735.
- [16] Y. L. Wang, J. M. Li, H. Wang, D. Q. Jin, H. Q. Chen, J. Xu, Y. Ohizumi and Y. Q. Guo (2014). Thymol derivatives from *Eupatorium fortunei* and their inhibitory activities on LPS-induced NO production, *Phytochemistry Lett.* **7**, 190-193.
- [17] A. Ibrahim, D. Sørensen, H. A. Jenkins, B. E. McCarry and M. W. Sumarah (2014). New diplosporin and agistatine derivatives produced by the fungal endophyte *Xylaria* sp. isolated from *Vitis labrusca*, *Phytochemistry Lett.* **9**, 179-183.
- [18] W. Y. Lin, Y. H. Kuo, Y. L. Chang, C. M. Teng, E. C. Wang, T. Ishikawa and I. S. Chen (2003). Anti-platelet aggregation and chemical constituents from the rhizome of *Gynura japonica*, *Planta Med.* **69**, 757-764.
- [19] B. Kalinova, J. Kindl, P. Jiros, P. Zacek, S. Vasickova, M. Budesinsky and I. Valterova (2009). Composition and electrophysiological activity of constituents identified in male wing gland secretion of the bumblebee parasite *Aphomia sociella*, *J. Nat. Prod.* **72**, 8-13
- [20] J. Beau, N. Mahid, W. N. Burda, L. Harrington, L. N. Shaw, T. Mutka, D. E. Kyle, B. Barisic, A. V. Olphen and B. J. Baker (2012). Epigenetic tailoring for the production of anti-infective cytosporones from the marine fungus *Leucostoma persoonii*, *Mar. Drugs* **10**, 762-774.
- [21] Q. W. Tan, P. H. Fang, J. C. Ni, F. L. Gao and Q. J. Chen (2017). Metabolites produced by an endophytic *Phomopsis* sp. and their anti-TMV activity, *Molecules* **22**, 2073.

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