Bibenzyl Derivatives From Leaves of *Dendrobium officinale*

Gang Ren¹, Wen-Zan Deng¹, Yan-Fei Xie², Chun-Hua Wu³, Wen-Yan Li¹, Chuan-Yun Xiao¹, and Yun-Long Chen⁴

Abstract

Nine compounds were isolated from leaves of *Dendrobium officinale*, including 1 new bibenzyl derivative, denofficin (I), and 8 known structurally related compounds, dendrocandin B (2), dendrocandin U (3), 3,4-dihydroxy-5,4′-dimethoxy bibenzyl (4), moscatilin (5), 4,4′-dihydroxy-3,5-dimethoxy bibenzyl (6), (5)-3,4,α-trihydroxy-5,4′-dimethoxy bibenzyl (7), gigantol (8), densiflorol A (9). The structures of these compounds were identified by spectroscopic methods. All isolated compounds were screened for their cytotoxicity against human cervical cancer cell line HeLa cells. Of them, compounds 1, 3, 5, 6, and 8 were found to have the capabilities of proliferation inhibition against HeLa cells with half-maximal inhibitory concentration values ranging from 8.0 to 92.4 μM.

Keywords

Tiepishihu, *Dendrobium officinale*, bibenzyl derivatives, denofficin, cytotoxicity

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*Dendrobium officinale*, an herb plant endemic to China, belongs to a member of *Dendrobium* plants (Orchidaceae) and mainly distributed in eastern and southeastern China. The dried or fresh stems of this plant have been used as the precious traditional Chinese medicine for antipyretic, eyes-benefiting, and tonic purposes for thousands of years. In the Chinese Pharmacopoeia (2010 Edition), *D. officinale* has been recorded officially as the original material of “Tiepishihu”.¹ In the process of making “Tiepishihu”, the leaves of *D. officinale* are usually removed, which leads to a huge waste of biological resources. Although multiple phytochemical studies focusing on stems of *D. officinale* have displayed the occurrence of polysaccharides, essential oils, alkaloids, and bibenzyls, etc,² the chemical constituents of its leaves still remain unknown. As a part of the comprehensive development and utilization of leaf resources of *D. officinale*, the phytochemical investigation was performed. Herein, we described the isolation, structural elucidation of one new bibenzyl derivative, denofficin (I), as well as 8 structurally related known compounds, dendrocandin B (2), dendrocandin U (3), 3,4-dihydroxy-5,4′-dimethoxy bibenzyl (4), moscatilin (5), 4,4′-dihydroxy-3,5-dimethoxy bibenzyl (6), (5)-3,4,α-trihydroxy-5,4′-dimethoxy bibenzyl (7), gigantol (8), densiflorol A (9). Besides, their cytotoxic potential for human cervical cancer cell line HeLa cells was evaluated.

Denofficin (I), a whitish amorphous powder, has a positive reaction with ferric chloride reagent, proving the presence of the phenolic moiety. The molecular formula was determined to be C₃₆H₃₇O₁₀ by the high-resolution electrospray ionisation mass spectrometry (HR-ESI-MS) (supplemental Figure S1) which afforded the quasi-molecular ion peak at m/z 629.2388 ([M – H]⁻, calcd. for C₃₆H₃₇O₁₀, 629.2392). The infrared spectrum (supplemental Figure S2) of 1 displayed the absorptions for hydroxyl group (3519 and 3445 cm⁻¹), methoxy group (2936 and 2852 cm⁻¹), carbonyl group (1733 cm⁻¹), and aromatic ring (1606, 1516, and 1454 cm⁻¹). The ultraviolet absorption (Figure S3) maxima at λmax 273 nm suggested the presence of a bibenzyl skeleton.³⁵ The ¹H and ¹³C NMR data (Table 1) of 1 is highly similar to those of the known bibenzyl derivative, dendrocandin B (2, Figure 1).³ The main difference is the extra

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appearance of a set of signals assigned for \(p\)-hydroxyphenyl propionyl group (\(\delta_H 7.04 \text{ [2H, d, 8.3 Hz]}, 6.70 \text{ [2H, d, 8.3 Hz]}, 2.83 \text{ [2H, m], and 2.58 [2H, m]; } \delta_C 132.4, 129.4, 115.4, 154.1, 29.9, 35.9 \text{ and 172.4]) in 1. This indicated that 1 was the \(p\)-hydroxyphenyl propionyl acylation derivative of dendrocandin B, which was further supported by the HR-ESI-MS data. The \(p\)-hydroxyphenyl propionyl acylation took place at the hydroxyl of C-9\(^{\prime}\), as suggested by the heteronuclear multiple

| Position | \(\delta_H \) (m, \( J \) in Hz) | \(\delta_C \) | Position | \(\delta_H \) (m, \( J \) in Hz) | \(\delta_C \) |
|----------|-------------------------------|-------------|----------|-------------------------------|-------------|
| 1        | 134.6                         |             | 1        | 134.5                         |             |
| 2        | 6.49 (1 H, d, 1.6)             | 109.4       | 2′, 6′   | 7.04 (2 H, d, 8.3)            | 129.4       |
| 3        | 143.9                         |             | 3′, 5′   | 6.83 (2 H, d, 8.3)            | 113.8       |
| 4        | 130.8                         |             | 4′       | 157.9                         |             |
| 5        | 148.5                         |             | a        | 2.81 (2 H, m)                 | 37.9        |
| 6        | 6.32 (1 H, d, 1.6)             | 105.2       | a′       | 2.85 (2 H, m)                 | 36.9        |
| 1′       | 133.7                         |             | 1″       | 126.9                         |             |
| 2″, 6″   | 7.10 (2 H, d, 8.5)            | 129.4       | 2″, 6″   | 6.55 (2 H, s)                 | 104.2       |
| 3″, 5″   | 6.83 (2 H, d, 8.3)            | 113.8       | 3″, 5″   | 147.3                         |             |
| 4″       | 135.5                         |             | 4″       | 135.5                         |             |
| 7″       | 4.77 (1 H, d, 7.9)            | 76.6        | 7″       | 4.96 (1 H, d, 8.5)            | 76.4        |
| 8″       | 4.20 (1 H, m)                 | 75.5        | 8″       | 3.98 (1 H, m, 8.0, 3.0, 3.0)  | 78.2        |
| 9″\(^{\alpha}\) | 4.34 (1 H, dd, 12.3, 3.1)   | 63.1        | 9″\(^{\beta}\) | 4.02 (1 H, dd, 12.3, 4.6)   | 3.90 (1 H, m) |
| 1″′      | 132.4                         |             | 1″′      | 132.4                         |             |
| 2″′, 6″′ | 7.04 (2 H, d, 8.3)            | 129.4       | 2″′, 6″′ | 6.55 (2 H, s)                 | 104.2       |
| 3″′, 5″′ | 6.83 (2 H, d, 8.3)            | 113.8       | 3″′, 5″′ | 147.3                         |             |
| 4″′      | 135.5                         |             | 4″′      | 135.5                         |             |
| 7″′      | 2.83 (2 H, m)                 | 29.9        | 7″′      | 2.83 (2 H, m)                 | 29.9        |
| 8″′      | 2.58 (2 H, m)                 | 35.9        | 8″′      | 2.58 (2 H, m)                 | 35.9        |
| 9″′      | 172.4                         |             | 9″′      | 172.4                         |             |
| MeO-5    | 3.84 (3 H, s)                 | 56.2        | MeO-5    | 3.85 (3 H, s)                 | 56.0        |
| MeO-4′   | 3.79 (3 H, s)                 | 55.3        | MeO-4′   | 3.79 (3 H, s)                 | 55.3        |
| MeO-3″′, 5″′ | 3.87 (6 H, s) | 56.4 | MeO-3″′, 5″′ | 3.92 (6 H, s) | 56.4 |

\(^a\)Bruker Avance 600 spectrometer; chemical shifts (ppm) referred to CDCl\(_3\) (\(\delta_H 7.26; \delta_C 77.16\)).
The cytotoxicity of all isolated compounds against human cervical cancer cell line HeLa cells was evaluated by a colorimetric cell counting kit-8 (CCK-8) assay described previously.12 The cytotoxicity of all isolated compounds against human cervical cancer cell line HeLa cells was evaluated by a colorimetric cell counting kit-8 (CCK-8) assay described previously.12

Figure 2. The key heteronuclear multiple bond correlations (H→C) of 1.

Table 2. Cytotoxicity of Bibenzyls 1-9 From D. officinale Against HeLa Cells.

| Compounds | IC_{50} (µM) | Compounds | IC_{50} (µM) |
|-----------|--------------|-----------|--------------|
| 1         | 20.2 ± 1.3   | 6         | 8.0 ± 1.5    |
| 2         | 91.1 ± 11.2  | 7         | >100         |
| 3         | 41.5 ± 2.4   | 8         | 92.4 ± 6.4   |
| 4         | >100         | 9         | >100         |
| 5         | 16.8 ± 2.6   | Cisplatin* | 5.3 ± 0.7    |

IC_{50} half-maximal inhibitory concentration.

*aPositive control.

Experimental

General Procedure

IR spectrum was executed on Shimadzu Iraffinity-1 fourier-transform infrared spectrometer with potassium bromide disc (Shimadzu Co., Kyoto, Japan). Optical rotation was determined on a JASCO P-1020 polarimeter (JASCO International Corp., Ltd, Tokyo, Japan) at room temperature. NMR spectra were recorded on a Bruker Advance 600 spectrometer (Bruker Biospin, Rheinstetten, Germany) using CDCl3 as a solvent. HR-ESI-MS analyses were implemented on an AB SCIEX Triple TOF 5600+ mass spectrometer (AB SCIEX Co., Framingham, MA, USA). Column chromatography (CC) was performed on HP-20 (75-150 µm, Mitsubishi Chemical Co., Tokyo, Japan), octadecylsilyl (ODS) gel (75-150 µm, YMC Co., Kyoto, Japan), MCI GEL CHP20P (75-150 µm, Mitsubishi Chemical Co., Tokyo, Japan), and Sephadex LH-20 (25-100 µm, GE Healthcare Bio-Sciences, Amersham, Sweden). Precoated thin layer chromatography plates with silica gel GF254 (10-40 µm; Yantai Jiang You silic- cone Development Co., Ltd., Yantai, China) were used to
detect the purity of the isolates achieved by coating with 10% sulfuric acid (H$_2$SO$_4$) in ethanol (EtOH), followed by heating. Preparative high-performance liquid chromatography (PHPLC) was executed on a LC3000 liquid chromatograph (Beijing Tong Heng Innovation Technology Co., Ltd, Beijing, China) armed with an ODS column (5 µm, 250 mm × 30 mm i.d., Sepax Technologies, Inc.).

Plant Materials

The leaves of *D. officinale* were collected from the planting base of Yueqing Yanfeixue Shihu Co. Ltd., Yueqing city, Zhejiang province, China, in December 2014, and identified by Dr Xu Cheng, associate researcher of College of Life Sciences, Zhejiang University. The voucher specimen (TCM20140151) was deposited in the Herbarium of the Department of Pharmacognosy, Research Center of Natural Resources of Chinese Medicinal Materials and Ethnic Medicine, Jiangxi University of Traditional Chinese Medicine.

Extraction and Isolation

The air-dried and powdered leaves of *D. officinale* (11.0 kg) were extracted with 95% EtOH 3 times (100 L for each extraction) at room temperature. The filtrate was evaporated in vacuo to produce a residue (485.6 g), which was fractionated by a HP-20 macroporous resin column chromatography (CC) (15 × 45 cm) eluted with a gradient of EtOH/water (H$_2$O) (0% → 95%) to give 10 fractions (frs. H1–H10). Fr. H5 (5.8 g) was subjected to MCI CHP-20P resin CC (4 × 30 cm) eluted with a gradient of EtOH/water (H$_2$O) (30% → 95%) to yield 10 subfractions (Frs. H5M1–H5M10). Fr. H5M4 (1.4 g) was further separated by Sephadex LH-20 gel CC (2 × 200 cm) eluted with 100% methanol to afford 4 subfractions (Frs. H5M4L1–H5M4L4). Fr. H5M4L3 (63.4 mg) was then purified by PHPLC (ODS, 5 µm, 2 × 25 cm) eluting with 45% methanol to obtain compound 6 (5.4 mg, $t_R$ 25 minutes). Fr. H5M5 (0.8 g) was further fractioned by CC (3 × 25 cm) on silica gel (300 mesh) eluted with petroleum/ethyl acetate (11:1, 10:1, 9:1, 8:1, v/v) to give 7 subfractions (Frs. H5M5S1–H5M5S7). Fr. H5M5S1 (0.3 g) was purified by Sephadex LH-20 gel CC (2 × 200 cm) eluted with 100% methanol, followed by PHPLC (ODS, 5 µm, 2 × 25 cm) eluted with 30% acetonitrile to give 7 (2.6 mg, $t_R$ 21 minutes). In a similar manner, 5 (2.3 mg, $t_R$ 24 minutes) was obtained from Fr. H5M5S1. Fr. H6 (9.3 g) was subjected to MCI CHP-20P resin CC (4 × 30 cm) eluted with a gradient of EtOH/H$_2$O (40% → 90%) to produce 6 subfractions (Frs. H6M1–H6M6). Fr. H6M5 (0.7 g) was separated by CC (2 × 200 cm) over Sephadex LH-20 gel eluted with 100% methanol to afford 3 subfractions (Frs. H6M5L1–H6M5L5). Fr. H6M5L4 (51.9 mg) was purified by PHPLC (ODS, 5 µm, 2 × 25 cm) eluted with 70% acetonitrile to obtain 9 (2.1 mg, $t_R$ 28 minutes). In a similar manner, 2 (40.7 mg, $t_R$ 31 minutes), 7 (5.1 mg, $t_R$ 20 minutes) and 8 (3.5 mg, $t_R$ 36 minutes) were obtained from Fr. H6M3 (2.7 g), respectively. Fr. H7 (23.6 g) was subjected to CC over MCI CHP-20P resin (4 × 30 cm) eluted with a gradient of EtOH/H$_2$O (20% → 90%) to yield 8 fractions (Frs. H7M1–H7M8). Fr. H7M6 (3.3 g) was separated by CC (2 × 200 cm) over Sephadex LH-20 gel eluted with 90% methanol to afford 10 subfractions (Frs. H7M6L1–H7M6L10). H7M6L6 (143.2 mg) was purified by PHPLC eluted with 50% acetonitrile to obtain 1 (5.8 mg, $t_R$ 32 minutes) and 3 (2.2 mg, $t_R$ 26 minutes), respectively.

Denofficin (1)

Whitish amorphous powder.

[α]$_D^{25}$: −5.8 (c 0.25, MeOH).

IR (KBr): 3519, 3445, 2936, 2852, 1733, 1606, 1545, 1346, 1232, 1116 cm$^{-1}$.

UV (MeOH) $\lambda_{max}$ (log $e$) 273 (3.80) nm.

$^1$H and $^{13}$C NMR (CDCl$_3$): Table 1.

HR-ESI-MS: $m/z$ [M − H]$^-$ calcd. for C$_{36}$H$_{37}$O$_{10}$ 629.2392; found: 629.2388.

Evaluation for in Vitro Cytotoxicity Against HeLa Cells

One day after exponentially growing HeLa cells were seeded at 5 × 10$^3$ cells/well in a 96-well plate, the culture medium was changed to the experimental medium supplemented with compounds tested at a series of concentrations. After incubation for 24 hours, 10 µL of CCK-8 was added and incubated for an additional 3 hours, and then optical density (OD) value was measured by spectrophotometer under 450 nm. Cell inhibitory rate was calculated as follows: cell inhibitory rate = (OD$_{control}$ − OD$_{experiment}$) / (OD$_{control}$ − OD$_{blank}$) × 100%.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.
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