# Novel Dibenzo[*b*,*e*]oxepinones from the Freshwater-Derived Fungus *Chaetomium* sp. YMF 1.02105

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**Key words**
- dibenzo[*b*,*e*]oxepinone
- *Chaetomium* sp.
- Chaetomiaceae
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- antimicrobial activity

## Abstract

Six new dibenzo[*b*,*e*]oxepinone metabolites, chaetones A–F (**1**–**6**), as well as three known compounds, 1-hydroxy-6-methyl-8-hydroxymethylxanthone (**7**), citreorosein (**8**), and emodin (**9**), were obtained from a freshwater-derived fungal strain *Chaetomium* sp. YMF 1.02105. Their structures were established on the basis of extensive spectroscopic data analysis and comparison with spectroscopic data reported. Compounds **1**–**6** are further additions to the small group of dibenzo[*b*,*e*]oxepinones represented by arugosins A–H. Compounds **1**–**7** were tested for their cytotoxic activities against A549, Raji, HepG2, MCF-7, and HL-60 cell lines. The results showed that compound **3** had significant cytotoxicity with $IC_{50}$ values of 1.2, 1.8, 1.9, 2.3, and 1.6 µg/mL, respectively, against the five cancer cell lines. All compounds showed modest antimicrobial activity against *Staphylococcus aureus* (ATCC 6538) in standard disk assays.

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## Introduction

Fungi are a well-known source of bioactive compounds, and the research for isolation of novel fungal metabolites that blossomed more than 40 years is still very active now [1]. At present, the search for new producers of biologically active compounds is diligently underway among fungi from unusual or specialized ecological niches, because the synthesis of new secondary metabolites and potential biologically active compounds that help them to survive and adapt to these existence conditions can be expected in these fungi with the greatest probability [2–4]. Due to different habitats from those of terrestrial fungi, the freshwater-derived fungi are of special interest. In fact, freshwater-derived fungi are being accepted as a potentially important source of novel bioactive secondary metabolites that might prove suitable for specific medicinal or agrochemical applications as many novel bioactive compounds have been gradually reported from freshwater-derived fungi *Annulatascus triseptatus* [5], *Dendrospora tenella* [6], *Decaisnella thyridioides* [7], *Helicodendron giganteum* [8], *Kirschsteiniothelia* sp. [9], *Massarina tunicate* [10–12], *Ophioceras venezuelense* [13], *Stachybotrys* sp. [14], and others.

Our research group has been interested in studies of bioactive compounds produced by freshwater-derived fungi. Previously, we have identified some unique biologically active metabolites from these fungi, such as astropaquinones [15], caryospomycins [16], colelomycerones [17], pseudohalonectrins [18], YMF 1029 A–E [19], and others. During our continuous characterization of structurally unique bioactive metabolites from freshwater-derived fungi, the organic solvent extract of the solid-substrate fermentation culture from *Chaetomium* sp. YMF 1.02105, which was isolated from the submerged wood collected from the River Bailong in Kunming, Yunnan Province, China, displayed antibacterial activity against *Staphylococcus aureus* (ATCC 6538) and inhibitory effects on the growth of two human tumor cell lines, A549 and MCF-7. Further investigation led to the discovery of six new dibenzo[*b*,*e*]oxepinone metabolites, chaetones A–F (**1**–**6**) along with three known compounds, 1-hydroxyl-6-methyl-8-hydroxymethylxanthone (**7**), citreorosein (**8**), and emodin (**9**) (**Fig. 1**) [20–22]. We herein report the isolation and structure elucidation of these compounds, as well as their cytotoxic and antimicrobial activities.

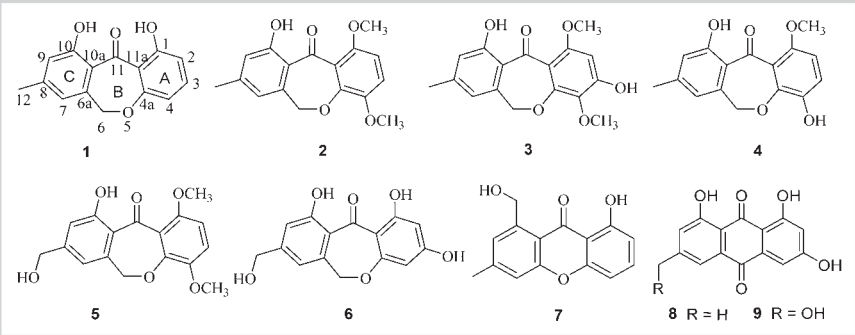


**Fig. 1** Chemical structures of compounds **1–9** isolated from *Chaetomium* sp. YMF 1.02105.

## Materials and Methods

### General experimental procedures

UV spectra were taken on a Shimadzu double-beam 210A spectrophometer (Shimadzu). Infrared (IR) spectra were obtained in KBr pellets with a Bio-Rad FTS-135 spectrophotometer (Bio-Rad). The nuclear magnetic resonance (NMR) spectra were recorded on DRX-500 NMR (Bruker) spectrometers, with TMS as an internal standard, and coupling constants were represented in Hertz. EI-MS data were taken on a VG Auto Spec 3000 mass spectrometer (VG). HR-MS (ESI-TOF) data were recorded with an API QSTAR Pulsar 1 spectrometer. Column chromatography was performed on silica gel G (200–300 mesh; Qingdao Marine Chemical Factory) and Sephadex LH-20 (AmershamPharmacia Biotech). Precoated silica gel plates (Qingdao Marine Chemical Factory) were used for TLC. Detection was done by spraying the plates with 5% $H_2SO_4$, followed by heating.

### Fungal material

*Chaetomium* sp. was isolated by Dr. Hong Zhu from a submerged woody substrate collected from the River Bailong in Kunming, Yunnan Province, China, in April 2006. The isolate was determined to be an unidentified species of *Chaetomium* by one of the authors (K.Z. Shen) on the basis of morphology and sequence analysis of the ITS region of the rDNA and assigned the accession number YMF 1.02105 in the culture collection at the Laboratory for Conservation and Utilization of Bio-Resources, Yunnan University, Yunnan Province, People's Republic of China. The fungal strain was subcultured on slants of potato dextrose agar (PDA) at 25 °C for 5 days. The agar plugs were used to inoculate 250 mL Erlenmeyer flasks, each containing 50 mL of the seed media (0.4% glucose, 1% malt extract, and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker (190 rpm) for 5 days. Preparation fermentation was carried out in ten 500-mL Erlenmeyer flasks, each containing 100 g of rice. Distilled $H_2O$ (100 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb/in$^2$. for 30 min. The flasks were cooled to room temperature and individually inoculated with 5.0 mL of the seed culture and incubated at 25 °C under static conditions for 50 days.

### Extraction and isolation

Following incubation, 300 mL of methanol was added to each flask. The suspension was filtered and the filtrate concentrated under vacuum at 50 °C. The aqueous residue (55.1 g) was partitioned into petroleum ether (bp 60–90 °C) and EtOAc layers. The EtOAc layer (13.4 g) was loaded onto a silica gel column [1800 g silica gel G (200–300 mesh), 8.0 cm i.d. × 180 cm] using petroleum ether-EtOAc-$CH_3OH$ gradient elution to afford six fractions (A–F) based on the TLC behavior. Fraction B (914 mg), obtained on elution with petroleum ether-EtOAc (65 : 35), was further subjected to passage over a Sephadex LH-20 column (3.5 cm i.d. × 180 cm) eluting with MeOH to yield subfractions B-1 and B-2. Subfraction B-1 (28 mg) was further purified by a silica gel column [5 g silica gel G (200–300 mesh), 0.8 cm i.d. × 25 cm] eluting with $CHCl_3$-$CH_3COCH_3$ (95 : 5) to yield **1** (8 mg). Subfraction B-2 (128 mg) was further separated into **2** (30 mg) and **3** (48 mg) by a silica gel column [10 g silica gel G (200–300 mesh), 1.0 cm i.d. × 35 cm] eluting with petroleum ether-$CH_3COCH_3$ (9 : 1), and a Sephadex LH-20 (1.6 cm i.d. × 120 cm) column with $CH_3OH$. Fraction C (800 mg), obtained on elution with petroleum ether-EtOAc (4 : 6), was further separated on a Sephadex LH-20 column (2.0 cm i.d. × 150 cm) eluting with MeOH, followed by a silica gel column [10 g silica gel G (200–300 mesh), 1.0 cm i.d. × 35 cm] with 80% petroleum ether-acetone (8 : 2) to afford **4** (20 mg) and **9** (8 mg). Fraction D (200 mg), obtained on elution with petroleum ether-EtOAc-MeOH (3 : 7 : 1), was further subjected to passage over a Sephadex LH-20 column (1.6 cm i.d. × 150 cm) eluting with MeOH to yield subfractions D-1 and D-2. Subfraction D-1 (28 mg) was further purified by preparative silica gel TLC (silica gel H, 20 × 20 × 0.1 cm film thickness) developed with petroleum ether-acetone (7 : 3) to yield **7** (15 mg). Subfraction D-2 (42 mg) gave **8** (5 mg) after further fractionation over a silica gel column [5 g silica gel G (200–300 mesh), 0.8 cm i.d. × 25 cm] eluted with 70% petroleum ether-EtOAc (7 : 3). Fraction E (3.8 g), obtained on elution with petroleum ether-EtOAc-MeOH (3 : 7 : 3), was further divided into subfractions E-1 to E-4 by passage over a silica gel column [100 g silica gel G (200–300 mesh), 3.0 cm i.d. × 100 cm] with a gradient elution of $CHCl_3$-$CH_3COCH_3$ (9 : 1 → 3 : 2). Subfraction E-2 (113 mg) was further separated over a Sephadex LH-20 gel column (1.6 cm i.d. × 150 cm) eluting with MeOH to give **5** (20 mg). Separation of subfraction E-3 (380 mg) over a silica gel column [20 g silica gel G (200–300 mesh), 1.6 cm i.d. × 40 cm], eluting with petroleum ether-acetone (1 : 1), afforded **6** (10 mg).

*Chaetone A* (**1**): bright yellow solid ($CH_3COCH_3$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 356.0 (3.55), 280.3 (3.68), 222.0 (4.12) nm; IR (film) $\nu_{max}$ 3525, 2949, 2850, 1628, 1597, 1583, 1486, 1448, and 1035 cm$^{-1}$; EIMS *m/z* (rel. int) 256 [M]$^+$ (100), 243 (39), 227 (54), 163 (34), 136 (48), 108 (31); HRMS (ESI-TOF) *m/z*: 279.0627 [M + Na]$^+$ (calcd. for $C_{15}H_{12}O_4Na$, 279.0633); NMR date are given in ▶ **Table 1**.

*Chaetone B* (**2**): bright yellow solid ($CH_3COCH_3$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 355.0 (3.56), 282.0 (3.72), 221.5 (4.02), 202.0 (4.20) nm; IR (film) $\nu_{max}$ 3425, 2959, 2921, 2850, 2839, 1631, 1597,

**Table 1** NMR data of compounds **1**–**6** in $CD_3COCD_3$ ($\delta$ ppm, $J$ Hz).

| No. | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | | 163.7 (s) | | 154.0 (s) | | 159.5 (s) | | 153.9 (s) | | 154.5 (s) | | 163.4 (s) |
| 1-$OCH_3$ | | | 3.85 (s) | 56.8 (q) | 3.79 (s) | 56.7 (q) | 3.88 (s) | 57.2 (q) | 3.79 (s) | 57.0 (q) | | |
| 1-OH | 13.50 (s) | | | | | | | | | | 12.20 (s) | |
| 2 | 6.78 (dd, 1.8, 9.0) | 112.6 (d) | 6.73 (d, 9.0) | 107.9 (d) | 6.44 (s) | 97.9 (s) | 6.90 (d, 8.9) | 110.5 (d) | 6.87 (d, 9.1) | 109.4 (d) | 6.33 (br. s) | 98.2 (d) |
| 3 | 7.46 (t, 9.0) | 137.4 (d) | 7.03 (d, 9.0) | 116.5 (d) | | 156.2 (s) | 7.06 (d, 8.9) | 120.7 (d) | 7.19 (d, 9.1) | 118.3 (d) | | 165.7 (s) |
| 3-OH | | | | | 10.30 (s) | | | | | | 8.58 (s) | |
| 4 | 6.85 (dd, 1.8, 9.0) | 106.6 (d) | | 144.4 (s) | | 132.8 (s) | | 142.5 (s) | | 145.6 (s) | 6.62 (br. s) | 94.1 (d) |
| 4-$OCH_3$ | | | 3.88 (s) | 56.9 (q) | 3.78 (s) | 61.8 (q) | | | 3.84 (s) | 57.2 (q) | | |
| 4-OH | | | | | | | 8.01 (br. s) | | | | | |
| 4a | | 155.8 (s) | | 149.8 (s) | | 156.3 (s) | | 147.8 (s) | | 150.5 (s) | | 157.7 (s) |
| 6 | 5.21 (s) | 77.5 (t) | 5.20 (s) | 77.3 (t) | 5.23 (s) | 77.9 (t) | 5.20 (s) | 77.8 (t) | 5.23 (s) | 77.6 (t) | 5.23 (s) | 77.8 (t) |
| 6a | | 142.1 (s) | | 141.1 (s) | | 141.9 (s) | | 142.4 (s) | | 143.0 (s) | | 143.5 (s) |
| 7 | 6.68 (d, 1.6) | 119.5 (d) | 6.50 (d, 1.5) | 118.3 (d) | 6.66 (d, 1.8) | 119.3 (d) | 6.64 (d, 1.6) | 119.3 (d) | 6.80 (d, 1.7) | 115.6 (d) | 6.83 (d, 1.8) | 116.1 (d) |
| 8 | | 147.3 (s) | | 146.7 (s) | | 147.1 (s) | | 147.6 (s) | | 152.0 (s) | | 152.2 (s) |
| 9 | 6.82 (d, 1.6) | 118.7 (d) | 6.80 (d, 1.5) | 118.2 (d) | 6.77 (d, 1.8) | 118.9 (d) | 6.84 (d, 1.6) | 118.5 (d) | 6.95 (d, 1.7) | 115.1 (d) | 6.99 (d, 1.8) | 115.4 (d) |
| 10 | | 165.3 (s) | | 164.4 (s) | | 165.9 (s) | | 165.1 (s) | | 164.8 (s) | | 165.0 (s) |
| 10-OH | 12.81 (s) | | 13.02 (s) | | 13.71 (s) | | 13.01 (s) | | 12.83 (s) | | 12.11 (s) | |
| 10a | | 118.2 (s) | | 117.7 (s) | | 118.1 (s) | | 118.3 (s) | | 118.6 (s) | | 119.0 (s) |
| 11 | | 194.8 (s) | | 194.1 (s) | | 193.8 (s) | | 195.4 (s) | | 197.1 (s) | | 196.8 (s) |
| 11a | | 113.4 (s) | | 123.7 (s) | | 115.2 (s) | | 124.5 (s) | | 124.8 (s) | | 101.9 (s) |
| 12 | 2.33 (s) | 21.6 (q) | 2.33 (s) | 21.7 (q) | 2.31 (s) | 21.5 (q) | 2.31 (s) | 21.5 (q) | 4.65 (br. s) | 63.6 (t) | 4.63 (br. s) | 63.3 (t) |
| 12-OH | | | | | | | | | 4.50 (br. s) | | 4.47 (br. s) | |

1580, 1486, 1455, 1438, 1376, 1337, 1320, 1278, 1260, 1211, 1177, 1095, 1077, 1058, 1035, 974, 958, 908, 839, 813, 766, 742, 718, 674, 616, 584, 571 $cm^{-1}$; EIMS $m/z$ (rel. int) 300 $[M]^+$ (62), 285 (9), 269 (100), 254 (24), 241 (18), 229 (31), 214 (7), 169 (5); HRMS (ESI-TOF) $m/z$: 323.0902 $[M + Na]^+$ (calcd. for $C_{17}H_{16}O_5Na$, 323.0895); NMR date are given in **Table 1**.

*Chaetone C* (**3**): bright yellow solid ($CH_3COCH_3$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 359.5 (3.53), 279.0 (3.68), 211.0 (4.19) nm; IR (film) $\nu_{max}$ 3423, 2922, 2851, 1626, 1572, 1533, 1490, 1447, 1376, 1364, 1316, 1265, 1197, 1173, 1123, 1099, 1031, 976, 949, 912, 842, 751, 572 $cm^{-1}$; EIMS $m/z$ (rel. int) 316 $[M]^+$ (85), 301 (22), 285 (100), 273 (20), 270 (39), 255 (27), 245 (37), 229 (11), 213 (12), 185 (17), 161 (25); HRMS (ESI-TOF) $m/z$: 339.0829 $[M + Na]^+$ (calcd. for $C_{17}H_{16}O_6Na$, 339.0844); NMR date are given in **Table 1**.

*Chaetone D* (**4**): bright yellow solid ($CH_3COCH_3$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 355.0 (3.49), 280.0 (3.68), 222.0 (3.91) nm; IR (film) $\nu_{max}$ 3419, 2954, 2924, 2852, 2837, 1631, 1599, 1581, 1480, 1447, 1428, 1395, 1360, 1327, 1276, 1228, 1190, 1177, 1092, 1035, 1022, 1012, 980, 961, 916, 862, 828, 801, 747, 725, 711, 672, 601, 573 $cm^{-1}$; EIMS $m/z$ (rel. int) 286 $[M]^+$ (70), 271 (8), 255 (100), 227 (27), 215 (49), 187 (11), 149 (30); HRMS (ESI-TOF) $m/z$: 287.0951 $[M + H]^+$ and 309.0741 $[M + Na]^+$ (calcd. for $C_{16}H_{14}O_5$ 287.0921 and $C_{16}H_{14}O_5Na$, 309.0738, respectively); NMR date are given in **Table 1**.

*Chaetone E* (**5**): orange yellow solid ($CH_3COCH_3$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 354.0 (3.43), 278.0 (3.62), 202.0 (4.18) nm; IR (film) $\nu_{max}$ 3525, 2926, 2853, 1698, 1666, 1631, 1575, 1485, 1460, 1434, 1402, 1327, 1274, 1259, 1207, 1181, 1031, 1143, 1090, 1067, 968, 912, 840, 818, 786, 548 $cm^{-1}$; EIMS $m/z$ (rel. int) 316 $[M]^+$ (57), 285 (100), 270 (20), 245 (25); HRMS (ESI-TOF) $m/z$: 339.0839 $[M + Na]^+$ (calcd. for $C_{17}H_{16}O_6Na$, 339.0844); NMR date are given in **Table 1**.

*Chaetone F* (**6**): orange yellow solid ($CH_3COCH_3$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 356.0 (3.48), 279.3 (3.68), 202.0 (4.18) nm; IR (film) $\nu_{max}$ 3518, 2921, 2851, 1687, 1623, 1485, 1459, 1431, 1304, 1255,

| Compd. | $IC_{50}$ (μg/mL) | | | | |
|---|---|---|---|---|---|
| | A549 | Raji | HepG2 | MCF-7 | HL-60 |
| **1** | 89.2 | 78.7 | 95.6 | 75.6 | 84.1 |
| **2** | 50.7 | 44.5 | 36.8 | 63.2 | 76.1 |
| **3** | 1.2 | 1.8 | 1.9 | 2.3 | 1.6 |
| **4** | 33.5 | 40.2 | 28.4 | 53.1 | 56.8 |
| **5** | 14.5 | 16.7 | 13.2 | 25.7 | 26.4 |
| **6** | 8.1 | 7.8 | 6.7 | 5.9 | 9.1 |
| **7** | > 100 | > 100 | > 100 | > 100 | > 100 |
| **DDP**[b] | 2.8 | 2.1 | 2.6 | 2.4 | 2.1 |

[a] Each data point was the average value of two experimental measurements [b] DDP, an antitumor agent, was used as a positive control

**Table 2** $IC_{50}$ values of the tested compounds toward different tumor cell lines[a].

1213, 1162, and 1028 $cm^{-1}$; EIMS *m/z* (rel. int) 288 $[M]^+$ (26), 272 (35), 257 (100), 242 (5), 217(11); HRMS (ESI-TOF) *m/z*: 311.0523 $[M + Na]^+$ (calcd. for $C_{15}H_{12}O_6Na$, 311.0531); NMR date are given in ▶ **Table 1**.

### Bioassay

The purity (> 95%) of the tested compounds for the evaluation of the cytotoxic and antimicrobial activities was determined by HPLC.

The cytotoxic activities *in vitro* of compounds **1**–**7** against A549, Raji, HepG2, MCF-7, and HL-60 tumor cell lines were determined by the MTT assay as reported previously [23]. *Cis*-platinum (DDP; Sigma) was used as a positive control. The results are shown in ▶ **Table 2**.

Antimicrobial assays were conducted using the agar diffusion method according to a literature procedure [24]. The target microbes included *Staphylococcus aureus* (ATCC6538), *Enterococcus faecalis* (ATCC 19433), *Streptococcus mutans* (ATCC 25175), *Aspergillus fumigatus* (ATCC 10894), *Candida albicans* (ATCC 10231), and *Geotrichum candidum* (AS2.498). Ciprofloxacine (for injection, purchased from Yichang Humanwell Pharmaceutical Co. Ltd.) was used as a positive control. Test compounds were absorbed onto individual paper disks (6 mm diameter) at 50 μg/disk.

## Results and Discussion

▼

Chaetone A (**1**) was isolated as a bright yellow solid. Its molecular formula, $C_{15}H_{12}O_4$, was determined on the basis of the positive HRESIMS at *m/z* 279.0627 $[M + Na]^+$, (calcd. 279.0633), and this was supported by the $^1H$, $^{13}C$, and DEPT NMR data. The IR spectrum showed the presence of OH (3525 $cm^{-1}$) and conjugated carbonyl (1628 $cm^{-1}$) groups. The UV spectrum showed an absorption band at $\lambda_{max}$ 356.0, 280.3, and 222.0 nm, which was consistent with the absorptions of a dibenzophenone chromophore as previously reported [20, 25]. The $^1H$ NMR spectra (▶ **Table 1**) showed the signals for an aryl methyl group ($\delta_H$ 2.33, s, $CH_3$-12), an oxygenated methylene ($\delta_H$ 5.21, s, $CH_2$-6), and two hydrogen-bonded phenolic OH protons ($\delta_H$ 13.50 and 12.81, OH-1 and OH-10, respectively). These data also showed the presence of five aromatic protons, and their coupling constants as well as COSY couplings indicated that **1** had a 1,2,3-trisubstituted aromatic ring [$\delta_H$ 6.78 (dd, *J* = 1.8, 9.0 Hz, H-2), 7.46 (t, *J* = 9.0 Hz, H-3), 6.85 (dd, *J* = 1.8, 9.0 Hz, H-4)] and a 1,2,3,5-tetrasubstituted aromatic ring [$\delta_H$ 6.68 (d, *J* = 1.6 Hz, H-7) and 6.82 (d, *J* = 1.6 Hz, H-9)]. The $^{13}C$ NMR and DEPT experiments (▶ **Table 1**) revealed the presence of an aryl methyl group ($\delta_C$ 21.6), an aryl ketone carbonyl ($\delta_C$ 194.8), an oxygenated methylene carbon ($\delta_C$ 77.5), and 12 aromatic carbon resonances. All of these data are very similar to those of arugosin F [20], except that the $^1H/^{13}C$ signals for the hemiacetal moiety of arugosin F had been substituted for signals of an oxomethylene group in the spectra of **1**, which was consistent with the molecular formula. Supporting evidence for this assignment was obtained from the HMBC correlations from OH-1 to C-1, C-2 and C-11a, H-2 to C-11a, C-4 and C-11, H-3 to C-1, C-2, C-4, and C-4a, H-4 to C-2, C-3, and C-11a, $H_2$-6 to C-4a, C-6a, C-7, C-10a, and C-11, H-7 to C-6a, $CH_3$-12, C-9, and C-10a, H-9 to C-10, $CH_3$-12, C-10a, and C-7, $CH_3$-12 to C-7, C-8, and C-9, and finally OH-10 to C-10, C-9, and C-10a (▶ **Fig. 2**). In addition, the NOESY correlations between protons $CH_3$-12 and H-9 and H-7, OH-10 and H-9, and OH-1 and H-2 also indicated the positions of the methyl and hydroxyl groups in **1**. On the basis of the above evidence, the structure of chaetone A (**1**) was elucidated as 1,10-dihydroxy-8-methyldibenzo[*b,e*]oxepin-11 (6H)-one.

Chaetone B (**2**) was isolated as a bright yellow solid and showed similar UV absorptions to those of **1**. The molecular formula of **2** was determined to be $C_{17}H_{16}O_5$ by HRESIMS analysis (*m/z*, 323.0902 $[M + Na]^+$, calcd. 323.0895). The comparison of the spectroscopic data of **2** with those of **1** (▶ **Table 1**) showed similarities except that the trisubstituted phenyl ring C in **1** was replaced by the 1,2,3,4-tetrasubstituted phenyl group in **2**, which contained two *ortho*-coupled protons at $\delta_H$ 6.73 (d, *J* = 9.0 Hz, H-2) and 7.03 (d, *J* = 9.0 Hz, H-3) and two methoxy groups ($\delta_H/\delta_C$ 3.85/56.8 and 3.88/56.9). Furthermore, the positions of two methoxy substitutions in the 1,2,3,4-tetrasubstituted phenyl moiety at C-1 and C-4 were readily elucidated by the HMBC correlations of $H_2$-6 with C-4a, C-6a, C-7, C-10a, and C-11, $OCH_3$-1 with C-1 and C-2, H-2 with C-1, C-4, C-4a, C-11a, and C-11, H-3 with C-1, C-4, C-4a, and C-11a, and $OCH_3$-4 with C-3 and C-4 as well as the NOESY cross-peaks of $OCH_3$-1 ($\delta_H$ 3.85, s) with H-2 ($\delta_H$ 6.73, d), and H-3 ($\delta_H$ 7.03, d) with O**C**$H_3$-4 ($\delta_H$ 3.88, s) (▶ **Fig. 2**). On the basis of the spectroscopic data, chaetone B (**2**) was elucidated as 10-hydroxyl-1,4-dimethoxy-8-methyldibenzo[*b,e*]oxepin-11(6H)-one.

Chaetone C (**3**) was isolated as a bright yellow solid. The molecular weight of **3** was 16 mass units larger than that of chaetone B (**2**), suggesting the presence of an additional hydroxyl group. The $^1H$ NMR and $^{13}C$ NMR data of **3** (▶ **Table 1**) clearly indicated that this hydroxyl group was attached at C-3. Supporting evidence for this assignment was obtained from the chemical shifts of C-1 ($\delta_C$ 159.5, C), C-2 ($\delta_C$ 97.9, CH), C-3 ($\delta_C$ 156.2, C), C-4 ($\delta_C$ 132.8, C), C-4a ($\delta_C$ 156.3, C), and 4-$OCH_3$ ($\delta_C$ 61.8) in **3** [C-1 ($\delta_C$ 154.0, C), C-2 ($\delta_C$ 107.9, CH), C-3 ($\delta_C$ 116.5, CH), C-4 ($\delta_C$ 144.4, C), C-4a ($\delta_C$ 149.8, C), and 4-$OCH_3$ ($\delta_C$ 56.9) in **2**, respectively]. Moreover, the presence of a hydroxyl group at C-3 was corroborated by the HMBC

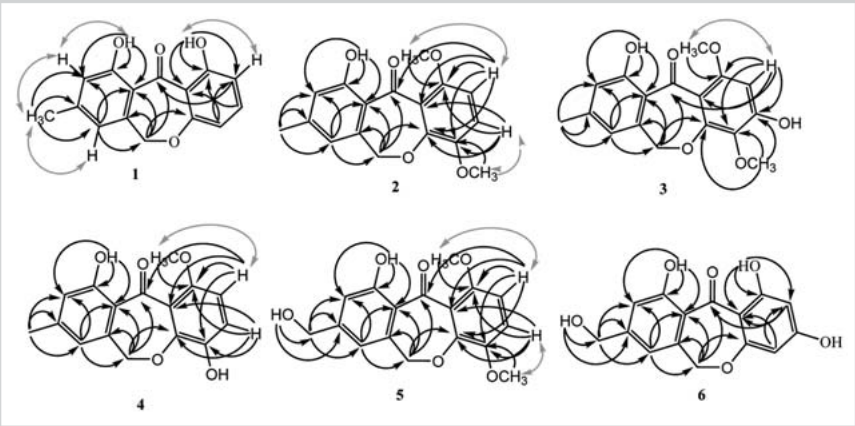


**Fig. 2** Key HMBC and NOESY correlations for compounds **1**–**6**.

correlations from $H_2$-6 to C-4a, C-6a, C-7, C-10a and C-11, $OCH_3$-1 to C-1, H-2 to C-1, C-3, C-4, C-11 and C-11a, and OC**H**$_3$-4 to C-3, C-4, and C-4a as well as the NOESY correlation between $OCH_3$-1 ($\delta_H$ 3.79, s) and H-2 ($\delta_H$ 6.44, s) (**Fig. 2**), but none between $OCH_3$-4 and H-2. Analysis of 2D NMR data led to the assignment of proton and carbon resonances of **3** (**Table 1**). On the basis of the spectroscopic data, chaetone C (**3**) was elucidated as 3,10-dihydroxy-1,4-dimethoxy-8-methyldibenzo[*b,e*]oxepin-11(6H)-one.

Chaetone D (**4**), obtained as a bright yellow solid, gave similar $^1$H and $^{13}$C NMR spectroscopic data (**Table 1**) to those of **2**, except for the absence of a signal due to the methoxy group on position C-4 and the presence of an exchangeable proton signal at $\delta_H$ 8.01 (br. s). Analysis of the $^1$H and $^{13}$C NMR spectroscopic data of **4** revealed signals of 14 protons and 16 carbon atoms. The molecular formula of **4** was determined as $C_{16}H_{14}O_5$ by HRMS (ESI-TOF) analysis (*m/z*, 287.0951 $[M + H]^+$ and 309.0741 $[M + Na]^+$, calcd. 287.0921 and 309.0738, respectively). This suggested that for **4**, a hydroxyl group replaced the methoxyl group in the molecule of **2**. The NOESY correlation between the 1-OC**H**$_3$ protons ($\delta_H$ 3.88, s) with H-2 ($\delta_H$ 6.90, d, $J = 8.9$ Hz), but none between 1-OC**H**$_3$ protons with H-3 ($\delta_H$ 7.06, d, $J = 8.9$ Hz), confirmed the position of the hydroxyl group at C-4 in **4** (**Fig. 2**). The IR and UV spectra of **4** were also similar to those of **2**. On the basis of the above spectroscopic data, chaetone D was elucidated as 4,10-dihydroxy-1-methoxy-8-methyldibenzo[*b,e*]oxepin-11(6*H*)-one.

Chaetone E (**5**) possessed the same molecular formula ($C_{17}H_{16}O_6$) as that of chaetone C (**3**) and also showed similar UV to those of **3**, suggesting **5** was an isomer of **3**. Analysis of the $^1$H, $^{13}$C and HMQC NMR data for **5** (**Table 1**) revealed the presence of structural features similar to those found in **2**, except that the aryl methyl group ($\delta_H/\delta_C$ 2.33/21.7) in **2** was replaced by the signals for a hydroxymethyl unit [$\delta_H/\delta_C$ 4.65 (br.s, $H_2$-12)/63.6] and an exchangeable proton [$\delta_H$ 4.50 (br.s, O**H**-12)] in **5**, and this observation was supported by HMBC correlations from the new oxygenated methylene protons at $\delta_H$ 4.65 ($H_2$-12) to C-7 ($\delta_C$ 115.6), C-8 ($\delta_C$ 152.0), and C-9 ($\delta_C$ 115.1), and the exchangeable proton at $\delta_H$ 4.50 (O**H**-12) to C-12 ($\delta_C$ 63.6) and C-8 (**Fig. 2**). On the basis of the above spectroscopic data, chaetone E was elucidated as 10-hydroxyl-8-(hydroxymethyl)-1,4-dimethoxydibenzo[*b,e*]oxepin-11(6*H*)-one.

Chaetone F (**6**) was isolated as a bright yellow solid, displaying a UV spectrum that had a close similarity to those of **1**–**5**. The molecular formula of **6** was determined to be $C_{15}H_{12}O_6$ by HRESIMS analysis (*m/z*, 311.0523 $[M + Na]^+$, calcd. 311.0531), which indicated 10 degrees of unsaturation. On the basis of comparison of the $^1$H NMR and $^{13}$C NMR data of **6** with those of **5** (**Table 1**), the 1,2,3,5-tetrasubstituted benzyl system was found to remain in **6**, but another 1,2,3,4-tetrasubstituted benzyl moiety in **5** was replaced by the new 1,2,3,5-tetrasubstituted phenyl moiety [6.62 (br.s, H-4) and 6.33 (br.s, H-2)] in **6**. Furthermore, the substitution patterns of the new phenyl ring were deduced by the HMBC correlations from $H_2$-6 to C-4a, C-6a, C-7, C-10a, and C-11, OH-1 to C-1, C-11a, and C-2, H-2 to C-1, C-3, C-4, and C-11a, and H-4 to C-4a, C-11a, C-3, and C-2, as well as the chemical shift values of C-1 ($\delta_C$ 163.4, C) and C-3 ($\delta_C$ 165.7, C) (**Fig. 2**). On the basis of the spectroscopic data, chaetone F (**6**) was elucidated as 1,3,10-trihydroxy-8-(hydroxymethyl)dibenzo[*b,e*]oxepin-11(6H)-one.

The other three compounds were identified as 1-hydroxy-6-methyl-8-hydroxymethylxanthone (**7**), citreorosein (**8**), and emodin (**9**) shown in **Fig. 1**, respectively, by comparison of their spectroscopic data with published values [20–22].

The isolated compounds (**1**–**7**) were evaluated for their effects on five tumor cell lines, A549, Raji, HepG2, MCF-7, and HL-60, using the MTT assay. As shown in **Table 2**, compounds **1**–**6** displayed *in vitro* inhibitory activities against the five tumor cell lines to various degrees. Among them, compound **3** showed the most potent cytotoxicity against all evaluated cell lines with $IC_{50}$ values of 1.2, 1.8, 1.9, 2.3, and 1.6 µg/mL, respectively, which were even stronger than DDP, while compound **6** exhibited moderate growth inhibition with $IC_{50}$ values of 8.1, 7.8, 6.7, 5.9, and 9.1 µg/mL, respectively. However, the xanthone-type dibenzophenone **7** was inactive (**Table 2**). On the basis of the structures of compounds **1**–**6**, the appearance of hydroxyl and methoxyl groups in the above-mentioned compounds markedly increased the activities, suggesting that the hydrophobicity of the dibenzo[*b,e*]oxepin core in these compounds may be important to cytotoxic activities for arugosin-type dibenzophenones.

In addition, compounds **2**–**6** showed activities against *Staphylococcus aureus* (ATCC 6538) when tested at 50 µg/disk in standard disk assays, affording inhibitory zones ranging from 11 to 15 mm (ciprofloxacin: 20 mm zone of inhibition at 50 µg/disk). However, none of these compounds exhibited antibacterial activity against *Enterococcus faecalis* (ATCC 19433) and *Streptococcus mutans* (ATCC 25175) or antifungal activity against *Aspergillus fumigatus* (ATCC 10894), *Candida albicans* (ATCC 10231), and *Geotrichum candidum* (AS2.498) at 50 µg/disk. These bioactivity data could provide valuable information for future synthetic and pharmacologic studies to identify cytotoxic/antimicrobial compounds that are more potent and selective against cancer cells/pathogens.

Chaetones A–F (**1**–**6**) are new members of the dibenzo[*b,e*]oxepinone class of metabolites, and they are structurally related to the arugosins with arugosin F as the closest precedent [20]. However, **1**–**6** differ significantly from arugosin F and the other arugosins by the presence of an oxygenated methylene unit rather than a hemiacetal methine moiety. It has been suggested that the known arugosins result from the biological oxidation of anthraquinone/anthrone precursors [26,27] and biosynthetic studies of similar compounds support this hypothesis [28,29]. Additionally, biosynthetic studies suggested that the bi- and tricyclic arugosins and compounds from the xanthone family are biogenetically related. Thus, judging from the fact of the co-occurrence of the dibenzo[*b,e*]oxepinone metabolites **1**–**6**, xanthone 1-hydroxy-6-methyl-8-hydroxymethylxanthone (**7**), as well as anthraquinones, citreorosein (**8**) and emodin (**9**), the biosynthesis of **1**–**6** probably proceeds in a manner similar to that of known arugosins.

To our knowledge, the dibenzo[*b,e*]oxepinones are rare in the dibenzophenone class of compounds, and only twelve dibenzo[*b,e*]oxepinone metabolites have so far been reported from fungi in nature, which include arugosins A–E from *Aspergillus variecolor* [26], *A. rugulosus* [30], and *A. silvaticus* [31], arugosin F from *Ascodesmis sphaerospora* [20], arugosins G and H from *Emericella nidulans* var. *acristata* [32], arugosin I from *Penicilium* sp. [25], massarinin A from *Massarina tunicate* [12], pestalone from *Pestalotia* sp. [33], and leptosphaerin D from *Leptosphaeria* sp. [34]. These metabolites were commonly reported to possess antibacterial/antifungal/antitumor/cytotoxic activities. Among the known natural dibenzo[*b,e*]oxepinones, pestalone is probably the most remarkable representative. Besides a moderate *in vitro* cytotoxicity against various tumor cell lines (mean $GI_{50}$=6.0 µM), pestalone was reported to exhibit highly potent antibiotic activity against methicillin-resistant *Staphylococcus aureus* (MIC = 37 ng/mL) and vancomycin-resistant *Enterococcus faecium* (MIC = 78 ng/mL). Consequently, pestalone is considered as a particularly promising molecule with antibiotic properties [35–37]. Thus, our finding that the freshwater-derived fungus YMF 1.02105 could produce dibenzo[*b,e*]oxepinones whose structures, close to that of pestalone, could be of significant biomedical importance. Further studies are required to examine the dibenzo[*b,e*]oxepinones biosynthetic capacities of this fungus and to obtain sufficient quantities of **1**–**6** and their analogues in order to fully evaluate their biological activities.

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## Conflict of Interest

The authors declare no conflict of interest.

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