New Metabolites and Bioactive Chlorinated Benzophenone Derivatives Produced by a Marine-Derived Fungus *Pestalotiopsis heterocornis*

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Abstract: Four new compounds, including two isocoumarins, pestaloisocoumarins A and B (1, 2), one sesquiterpenoid degradation, isopolisin B (4), and one furan derivative, pestalotiol A (5), together with one known isocoumarin, gamahorin (3), and three chlorinated benzophenone derivatives, pestalachloride B (6), pestalachloride E (7) and a mixture of pestalalactone atropisomers (8a/8b), were isolated from a culture of the fungus *Pestalotiopsis heterocornis* associated with sponge *Phakellia fusca*. These new chemical structures were established using NMR and MS spectroscopic data, as well as single-crystal X-ray crystallographic analysis and CD Cotton effects. All of the isolated compounds were evaluated for their antimicrobial and cytotoxic activities. Isocoumarins 1–3, showed antibacterial activities against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* with MIC values ranging from 25 to 100 µg/mL and weak antifungal activities. Chlorinated benzophenone derivatives 6–8 exhibited antibacterial activities against *S. aureus* and *B. subtilis* with MIC values ranging from 3.0 to 50 µg/mL and cytotoxicities against four human cancer cell lines with IC50 values of 6.8–87.8 µM.

Keywords: antibacterial activity; antifungal activity; cytotoxicity; isocoumarin; *Pestalotiopsis heterocornis*

1. Introduction

Fungi of the genus *Pestalotiopsis* are widely distributed throughout the world and have proven to be a rich source of bioactive natural products [1]. Previous studies on the genus *Pestalotiopsis* have resulted in the isolation of a series of new natural products, including polyketides [2–5], terpenoids [6–8], alkaloids [9,10] and others [11,12], and many of these compounds exhibited various biological activities [2–12].

In recent decades, the genus *Pestalotiopsis* has been isolated as an endophyte from the tropical and subtropical rainforest plants [13]. However, only a few fungi of the genus *Pestalotiopsis* have been
reported from marine fauna [14]. Therefore, marine fungi of the genus Pestalotiopsis associated with the sponges could be expected to metabolize biologically interesting and chemically diverse compounds.

Following the above investigations, with the aim of discovering bioactive substances from marine-derived fungi, the secondary metabolites of a culture fermentation of Pestalotiopsis heterocornis, which was isolated from the sponge Phakellia fusca, were investigated. Four new compounds, including two isocoumarins, pestaloisocoumarin A (1) and pestaloisocoumarin B (2), one sesquiterpenoid degradation, isopelosin B (4), and one furan derivative, pestalachloride A (5), together with four known compounds, gamahorin (3) [15], pestalachloride B (6) [16], pestalachloride E (7) [17] and a mixture of pestalactone atropisomers (8a/8b) [18,19], were discovered (Figure 1). The structures of the new compounds were elucidated on the basis of spectroscopic data, circular dichroism (CD) Cotton effects and single-crystal X-ray crystallographic analysis. The cytotoxicities against four human cancer cell lines, antibacterial and antifungal activities against a panel of bacteria and fungi of these isolated compounds were evaluated in the present paper.

![Figure 1. Structures of Compounds 1–8.](image)

### 2. Results and Discussion

Compound 1 was obtained as a colorless transparent columnar crystal and possessed a molecular formula of C$_{12}$H$_{14}$O$_{5}$ as defined by the $^{13}$C nuclear magnetic resonance (NMR) and high-resolution electrospray ionization–mass spectrometry (HRESI–MS) data. The $^1$H NMR spectrum (Table 1) displayed signals for two methyls (δ$_H$ 2.24, s; δ$_H$ 1.36, d, J = 6.6 Hz), an oxygenated methine (δ$_H$ 4.90, q, J = 6.6 Hz) and an oxygenated methylene (δ$_H$ 3.81, d, J = 11.7 Hz; δ$_H$ 3.55, d, J = 11.7 Hz). In addition, two ortho-coupled aromatic protons at δ$_H$ 7.05 (d, J = 7.5 Hz) and δ$_H$ 7.47 (d, J = 7.5 Hz) were observed. The $^{13}$C NMR spectrum (Table 1) showed 12 carbons, including six aromatic carbons, one oxygenated quaternary carbon, one oxygenated methine, one hydroxymethyl, two methyls and one ester carbonyl carbon. These observations demonstrated that compound 1 was an isocoumarin derivative. Comparing the NMR data of 1 with those of acronemone C revealed their structural similarity [20], except for the different substituent on the benzene ring. Heteronuclear multiple-bond correlation spectroscopy HMBC correlations from H-9 (δ$_H$ 1.36) to C-3 (δ$_C$ 78.0) and C-4 (δ$_C$ 71.3), from H-10 (δ$_H$ 3.81 and 3.55) to C-3 (δ$_C$ 78.0), C-4 (δ$_C$ 71.3) and C-4a (δ$_C$ 139.2), from H-11 (δ$_H$ 2.24) to C-6 (δ$_C$ 136.9), C-7 (δ$_C$ 125.7) and C-8 (δ$_C$ 159.5) confirmed the planar structure of 1 as in Figure 1.

The relative configuration of 1 was defined on the basis of single-crystal X-ray diffraction analysis (Figure 2). The absolute configuration of 1 was determined by the electronic circular dichroism (ECD) spectra with quantum chemical calculations using the time dependent density functional theory (TD-DFT) method at the B3LYP/6-31 + G(d) level. The calculated ECD spectrum showed the same pattern as the experimental ECD spectrum of 1 (Figure 3). Thus, the absolute configuration of 1 was identified as 3R and 4S and named pestaloisocoumarin A.
Table 1. $^1$H nuclear magnetic resonance (NMR) (600 MHz) and $^{13}$C NMR (150 MHz) data for Compounds 1 and 2 in CD$_3$OD.

| Position | $\delta_C$ (Hz) | $\delta_H$ (Hz) | $\delta_C$ (Hz) | $\delta_H$ (Hz) |
|----------|----------------|----------------|----------------|----------------|
| 1        | 168.9, C       | 169.0, C       |                 |                |
| 3        | 78.0, CH       | 4.90, q (6.6)  | 82.5, CH        | 4.60, q (6.6)  |
| 4        | 71.3, C        | 67.9, C        |                 |                |
| 4a       | 139.2, C       | 145.3, C       |                 |                |
| 5        | 115.3, CH      | 7.05, d (7.5)  | 114.2, CH       | 7.16, d (7.7)  |
| 6        | 136.9, CH      | 7.47, d (7.5)  | 136.2, CH       | 7.64, d (7.7)  |
| 7        | 125.7, C       | 123.6, C       |                 |                |
| 8        | 159.5, C       | 159.4, C       |                 |                |
| 8a       | 106.1, C       | 106.4, C       |                 |                |
| 9        | 13.6, CH$_3$   | 1.36, d (6.6)  | 13.5, CH$_3$    | 1.43, d (6.6)  |
| 10       | 65.7, CH$_2$   | 3.81, d (11.7) | 3.55, d (11.7)  | 23.6, CH$_3$   | 1.57, s |
| 11       | 14.0, CH$_3$   | 2.24, s        | 60.3, CH$_2$    | 5.17, s        |
| AcO      |                |                | 171.1, C        | 19.2, CH$_3$   | 2.08, s |

Figure 2. Single crystal X-ray structure of 1.

Figure 3. Electronic circular dichroism (ECD) spectra of 1 and 2.
The HRESI–MS analysis of 2 showed a deprotonated ion at \( m/z \ 279.0865 \ [M - H]^- \). Analysis of the \(^1\text{H}\) and \(^{13}\text{C}\) NMR data (Table 1) indicated that 2 was very similar to gamahorin (3) [15], except that a doublet methyl in 3 was replaced by a singlet methyl in 2, and an oxygenated quaternary carbon was presented in 2 instead of a methine in 3. Furthermore, one more acetyl appeared in 2. HMBC experiments helped to determine the planar structure. HMBC correlations from H-9 (\( \delta_H 1.43 \)) to C-3 (\( \delta_C 82.5 \)) and C-4 (\( \delta_C 67.9 \)), from H-10 (\( \delta_H 1.57 \)) to C-3 (\( \delta_C 82.5 \)), C-4 (\( \delta_C 67.9 \)) and C-4a (\( \delta_C 145.3 \)), from H-11 (\( \delta_H 5.17 \)) to C-6 (\( \delta_C 136.2 \)), C-7 (\( \delta_C 123.6 \)), C-8 (\( \delta_C 159.4 \)) and C-13 (\( \delta_C 171.1 \)) confirmed that the acetyl was linked with C-11 and that C-4 was oxygenated. Nuclear overhauser effect (NOE) correlations between H-3 (\( \delta_H 4.60 \)) and H-10 (\( \delta_H 1.57 \)) suggested H-3 and the methyl at C-4 were \( \alpha \)-orientated and the methyl at C-3 and the hydroxyl at C-4 were \( \beta \)-orientated. The calculated ECD spectrum of 2 showed excellent agreement with experimental results, and the 3R and 4R configurations of 2 was confirmed (Figure 3). Thus, Compound 2 was determined and named pestaloisocoumarin B.

Compound 4 was isolated as a colorless oil and gave the molecular formula C\(_{12}\)H\(_{18}\)O\(_3\) from HRESI–MS data. The \(^1\text{H}\) NMR and \(^{13}\text{C}\) NMR data (Table 2) of 4 were almost identical to those of the known compound polisin B [21], which is a 11,12,15-norbisabolane sesquiterpenoid. Analysis of the correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) and HMBC correlations exhibited that 4 was a isomer of polisin B. Except for carbons at C-1 (\( \Delta \delta_C +2.0 \)), C-2 (\( \Delta \delta_C -1.2 \)), C-4 (\( \Delta \delta_C +5.2 \)), C-5 (\( \Delta \delta_C +1.3 \)), C-6 (\( \Delta \delta_C +2.4 \)) and C-11 (\( \Delta \delta_C -2.2 \)), the carbons in the lactone ring of 4 presented the same \(^{13}\text{C}\) NMR data with those of polisin B, which indicated that 4 possesses the same relative configuration at C-4 and C-7 as polisin B. NOE correlations observed from H-12 (\( \delta_H 1.37 \)) to H-8\( \alpha \) (\( \delta_H 1.98 \)), H-3\( \alpha \) (\( \delta_H 1.81 \)) and H-6 (\( \delta_H 4.00 \)) from H-6 (\( \delta_H 4.00 \)) to H-3\( \alpha \) (\( \delta_H 1.81 \)), and from H-4 (\( \delta_H 2.06 \)) to H-8\( \beta \) (\( \delta_H 2.24 \)) indicated that H-4 and 6-OH were \( \beta \)-orientated and that H-6 and the methyl at C-7 were \( \alpha \)-orientated (Figure 4). Thus, the structure of 4 was assigned and named isopolisin B.

**Table 2.** \(^1\text{H}\) NMR (600 MHz) and \(^{13}\text{C}\) NMR (150 MHz) data for Compounds 4 and 5.

| Position | \( \delta_C \) type | \( \delta_H \) (\( J \) in Hz) | \( \delta_C \) type | \( \delta_H \) (\( J \) in Hz) | \( \delta_C \) type | \( \delta_H \) (\( J \) in Hz) |
|----------|----------------------|------------------|----------------------|------------------|----------------------|------------------|
| 1        | 134.4, C             |                  | 134.7, C             |                  | 16.7, CH\(_3\)     | 1.75, dd (6.4, 1.5) |
| 2        | 123.5, CH            | 5.57, brd (5.0)  | 124.2, CH            | 5.57, brd (4.4)  | 130.4, CH           | 5.78, dd (14.7, 6.6) |
| 3        | 26.1, CH\(_2\)      | 2.10, m 1.81, m  | 26.5, CH\(_2\)      | 2.12, m 1.82, m  | 126.3, CH           | 5.60, brdd (14.7, 7.2) |
| 4        | 37.2, CH             | 2.06, m          | 37.4, CH             | 2.03, m          | 82.9, CH            | 4.13, d (7.9)       |
| 5        | 32.3, CH\(_2\)      | 1.95, brd (13.0) | 1.48, td (13.0, 4.0) | 32.5, CH\(_2\)  | 1.97, brd (13.2)  | 1.49, td (13.2, 3.7) | 81.6, C           |
| 6        | 67.1, CH             | 4.00, brs         | 68.0, CH             | 4.07, brs        | 82.7, CH            | 3.75, d (4.5)       |
| 7        | 89.0, C              | 88.3, C           | 84.7, CH             | 3.58, td (6.6, 4.5) |                 |                  |
| 8        | 30.5, CH\(_2\)      | 2.24, ddd (13.0, 10.2, 8.8) | 31.2, CH\(_2\)  | 2.19, ddd (13.2, 10.1, 9.0) | 35.9, CH\(_2\)  | 1.65, m           |
| 9        | 28.4, CH\(_2\)      | 2.72, ddd (13.0, 10.2, 8.8) | 2.58, ddd (13.0, 10.0, 4.7) | 29.0, CH\(_2\)  | 2.65, ddd (18.2, 10.0, 9.0) | 18.9, CH\(_2\)  | 1.50, m 1.42, m |
| 10       | 178.1, C             | 176.7, C          | 13.0, CH\(_3\)      | 0.96, t (7.4)    |                 |                  |
| 11       | 19.6, CH\(_3\)      | 1.78, s           | 20.8, CH\(_3\)      | 1.80, s          | 62.6, CH\(_2\)     | 3.69, d (11.3) 3.51, d (11.3) |
| 12       | 21.5, CH\(_3\)      | 1.37, s           | 23.1, CH\(_3\)      | 1.37, s          |                 |                  |
| OH       |                      |                  |                      |                  | 3.65, brs         |                  |
Compound 5 was obtained as colorless oil with the molecular formula C_{11}H_{20}O_4 as determined by HRESI-MS. The ^1H NMR spectrum (Table 2) showed five methines, including two olefinic methines at δ_H 5.78, 5.60, three oxygenated methines at δ_H 4.13, 3.75, 3.58, three methylenes, including one oxygenated methylene at δ_H 3.69, 3.51 and two methyls at δ_H 1.75, 0.96. COSY correlations between H-1/H-2/H-3/H-4 and between H-6/H-7/H-8/H-9/H-10 gave two fragments I and II. HMBC correlations between H-11 (δ_H 3.69, 3.51) and C-5 (δ_C 81.6) deduced a Fragment III as in Figure 4. HMBC correlations between H-4 (δ_H 4.13) and C-5 (δ_C 81.6), C-8 (δ_C 35.9), between H-6 (δ_H 3.75) and C-5 (δ_C 81.6), between H-11 (δ_H 3.69, 3.51) and C-4 (δ_C 82.9), C-5 (δ_C 81.6), C-6 (δ_C 82.7) confirmed the linkage between Fragments I, II and III (Figure 4). NOE correlations from H-4 (δ_H 4.13) to H-7 (δ_H 3.58), H-11 (δ_H 3.69, 3.51), from H-6 (δ_H 3.75) to H-8 (δ_H 1.65) indicated that H-4, H-7 and the hydroxymethyl at C-5 were cis and H-6 and the propyl at C-7 were cis configurations (Figure 4). The configuration of the double bond of 5 was confirmed as E geometry based on the coupling constant values between H-2 and H-3 (J_{H2,H3} = 14.7 Hz). Therefore, the structure of 5 was elucidated and named pestalotiol A.

Compounds 3, 6–8 were identified as gamahorin (3) [15], pestalachloride B (6) [16], pestalachloride E (7) [17] and a mixture of pestalactone atropisomers (8a/8b) [18,19] by comparison of the ^1H NMR, ^13C NMR and mass spectroscopy MS data with those reported.

All of the isolated compounds were evaluated for their cytotoxic activities against four human cancer cell lines via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diiphenyltetrazolium bromide assay (MTT) assay (Table 3) and antimicrobial activities against three bacteria and three fungi using a micro broth dilution method (Table 4). Chlorinated benzophenone derivatives 6, 7 and a mixture of 8a/8b exhibited moderate cytotoxicities against four human cancer cell lines with half maximal inhibitory concentration (IC_{50}) values 6.8–87.8 µM; while Compounds 1–5 did not show an obvious inhibition effect against any test cancer cell lines at 100 µM. Isocoumarins 1–3 and chlorinated benzophenone derivatives 6–8 showed antibacterial activities against Gram-positive bacteria Staphylococcus aureus and Bacillus subtilis with minimum inhibitory concentration (MIC) values ranging from 3 to 100 µg/mL. Among them, isocoumarins 1–3 also exhibited weak antifungal activities against three test fungi or part of them with MIC values 100 µg/mL. Compounds 6–8 were inactive against three test fungi at 100 µg/mL, and Compounds 4, 5 did not show antimicrobial activity against any test microorganism at 100 µg/mL.

![Figure 4](image)

**Figure 4.** ^1H-^1H COSY (correlation spectroscopy) HMBC (heteronuclear multiple-bond correlation spectroscopy) correlations of 5 and NOE (nuclear overhauser effect) correlations of 4 and 5. Fragment I: -CH-CH=CH-CH_3; Fragment II: -CH(OH)CHCH_2CH_2CH_3; Fragment III: The moiety in the blue dotted line box.

**Table 3.** Cytotoxic activities of Compounds 6–8 (half maximal inhibitory concentration, IC_{50} in µM).

| Compound | BGC-823 | H460 | PC-3 | SMMC-7721 |
|----------|---------|------|------|-----------|
| 6        | 6.8     | 23.6 | 28.1 | 7.9       |
| 7        | 48.0    | 87.8 | 55.1 | 40.2      |
| 8a/8b    | 53.8    | 48.2 | 66.1 | 41.5      |
| Adriamycin | 1.5   | 1.0  | 1.8  | 2.2       |

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Table 4. Antimicrobial activities of Compounds 1–3, 6–8 (minimum inhibitory concentration, MIC µg/mL).

| MIC          | 1 | 2 | 3 | 6 | 7 | 8a/8b | Control |
|--------------|---|---|---|---|---|-------|---------|
| Bacillus subtilis | 50 | 25 | 100 | 3 | 50 | 50 | 0.25 \(^a\) |
| Staphylococcus aureus | 25 | 25 | 100 | 3 | 25 | 50 | 0.13 \(^a\) |
| Escherichia coli | - | - | - | - | - | - | 0.13 \(^a\) |
| Candida albicans | 100 | - | - | - | - | - | 1.0 \(^b\) |
| Candida parapsilosis | 100 | - | 100 | - | - | - | 2.0 \(^b\) |
| Cryptococcus neoformans | 100 | 100 | 100 | - | - | - | 2.0 \(^b\) |

\(^a\) Ciprofloxacin; \(^b\) Amphotericin B.

3. Experimental Section

3.1. General Experimental Procedures

Optical rotations were determined using an AntonPaar MCP200 automatic polarimeter (Anton Paar Ltd., Graz, Austria). Ultraviolet spectra were measured with a BeckmanCoulter DU 730 nucleic acid/protein analyzer (Beckman Coulter, Inc., Brea, CA, USA). Infra-red (IR) spectra were recorded with a Bruker Tensor 27 FTIR spectrometer (film) (Bruker Optics, Ettlingen, Germany). 1D and 2D NMR spectra were collected on a Bruker AV-600 spectrometer (Bruker, Rheinstetten, Germany), \(\delta\) in ppm rel. to tetramethylsilane (TMS), \(J\) in Hz. ESIMS were recorded on an Agilent 1290-6420 Triple Quadrupole LC–MS spectrometer (Agilent Technologies, Santa Clara, CA, USA). HRESI–MS were performed using a BrukerMicroTOF-Q mass spectrometer (Bruker, Daltonics, Billerica, MA, USA). Silica gel (100–200 mesh, 200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), Sephadex LH-20 (GE Healthcare Bio-sciences AB, Uppsala, Sweden) and YMC*GEL ODS-A (S-50 \(\mu\)m, 12 nm) (YMC Co., Ltd., Kyoto, Japan) were used for column chromatography. Semipreparative high performance liquid chromatography (HPLC) was performed using an Octadecylsilyl silica (ODS) column (250 \(\times\) 10 mm, 5 \(\mu\)m, YMC-ODS-A). CD spectra were measured on a Biologic MOS-450 spectra polarimeter (Biologic Science, Claix, France). X-ray crystallographic analysis was carried out on a Bruker SMART APEX-II diffractometer (Bruker Biospin Group, Karlsruhe, Germany). MTT and antimicrobial assays were analyzed using a microplate reader (BioTek Synergy H1, BioTek Instruments, Inc., Vermont, VT, USA).

3.2. Fungal Material

The fungal strain, \(P\). heterocornis, was isolated from the sponge \(P\). fusca, which was collected from the Xisha Islands of China in 2012. The strain was identified by Xiuping Lin, and a voucher specimen (No. XWS03F09) was deposited in the CAS Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China.

3.3. Fermentation, Extraction and Isolation

The fungal strain \(P\). heterocornis was cultivated in 1000 mL conical flasks containing solid rice medium (each flask contained 200 g of rice, 5 g of artificial sea salt; 200 mL of distilled water, boiled in an autoclave for 20 min at 121 \(^\circ\)C, at 28 \(^\circ\)C without shaking for 36 days. The total of rice culture was extracted with EtOAc three times. The combined EtOAc extract was evaporated to dryness under reduced pressure to afford 182.5 g of crude extract.

The extract was subjected to silica gel column chromatography (CC) (\(\text{CH}_2\text{Cl}_2/\text{MeOH} v/v, 50:1–0:100\)) to yield 6 fractions (Frs. 1–6). Fraction 3 was subjected to Sephadex LH-20 chromatography (MeOH) to produce three subfractions (Frs. 3.1–3.3). Fr. 3.1 was isolated by CC on silica gel eluted with \(\text{CH}_2\text{Cl}_2/\text{acetone} (15:1)\) to afford five subfractions (Frs. 3.1.1–3.1.5). Fr. 3.1.2 was further separated by ODS CC, eluting with MeOH/water (85%) and then purified by preparative TLC (\(\text{CH}_2\text{Cl}_2/\text{MeOH}, 20:1\)) to yield Compound 4 (8.5 mg). Fr. 3.1.4 was further separated by ODS CC, eluting with MeOH/water (70%) to give Compound 5 (7.5 mg). Fraction 4 was separated using
sila gel column chromatography eluting with CH₂Cl₂/acetone (10:1) to yield five subfractions (Frs. 4.1–4.5). Fr. 4.5 was subjected to repeated column chromatography (Sephadex LH-20 and ODS) and further purified by semipreparative HPLC (65% MeOH/H₂O) to give Compounds 6 (7.0 mg), 7 (5.0 mg) and a mixture of 8a/8b (2.0 mg), respectively. Fraction 6 was fractionated on Sephadex LH-20 eluted with MeOH to produce 3 fractions (Frs. 6.1–6.3). Fr. 6.2 was separated by silica gel column chromatography, eluting with CH₂Cl₂/acetone (4:1) to yield four subfractions (Frs. 6.2.1–6.2.4). Fr. 6.2.3 was further purified by HPLC (45% MeOH/H₂O) to give Compounds 1 (4.5 mg), 2 (8.0 mg) and 3 (8.0 mg), respectively.

Pestaloisocoumarin A (1): colorless transparent columnar crystal; [α]ᵢ°D +13.3 (c 0.60, MeOH); UV (MeOH) λmax (log ε) 249 (4.51), 321 (4.37) nm; IR (film) νmax 3301, 2922, 2852, 1727, 1667, 1617, 1459, 1421, 1247, 1123, 1063 cm⁻¹; CD λmax (Δε) 243 (−4.45), 265 (46.16), 312 (17.72); ¹H NMR and ¹³C NMR data, see Table 1; HRESI–MS m/z 237.0786 [M – H]⁻ (calcd. for C₁₂H₁₃O₅S, 237.0763).

Pestaloisocoumarin B (2): white amorphous solid; [α]ᵢ°D −16.0 (c 0.50, MeOH); UV (MeOH) λmax (log ε) 246 (4.32), 321 (4.27) nm; IR (film) νmax 3404, 2991, 2920, 2852, 1736, 1669, 1623, 1341, 1382, 1246, 1158, 1048 cm⁻¹; CD λmax (Δε) 249 (−12.63), 270 (4.11), 308 (4.17); ¹H NMR and ¹³C NMR data, see Table 1; HRESI–MS m/z 279.0865 [M – H]⁻ (calcd. for C₁₄H₁₅O₅, 279.0869).

Isopolisin B (4): colorless oil; [α]ᵢ°D −19.0 (c 0.84, MeOH); IR (film) νmax 3414.6, 2922.3, 2855.4, 1763.0, 1672.4, 1605.4, 1543.5, 1451.9, 1383.0, 1288.0, 1259.1, 1203.6, 1148.3, 965.5 cm⁻¹; ¹H NMR and ¹³C NMR data, see Table 2; HRESI–MS m/z 289.1520 [M + Na]⁺ (calcd. for C₁₂H₁₄O₃Na, 289.1527). 3.4. X-ray Crystallography of 1

Colorless transparent columnar crystals of 1 were obtained from a 10:1 (v/v) mixture of MeOH and H₂O. Crystal data of 1, C₁₂H₁₄O₅S, MW = 238.23, monoclinic, crystal size 0.10 × 0.10 × 0.08 mm, space group P2₁, unit cell dimensions a = 7.5403 (2) Å, b = 12.2371 (4) Å, c = 12.5115 (4) Å, α = 90°, β = 99.153 (2)°, γ = 90°, volume = 1139.75 (6) Å³, Z = 4 e calcld. = 1.388 g/cm³, θ range = 3.58–68.28°, Mo Kα radiation, wavelength = 1.54178 Å, temperature = 173 K, F (000) = 504, reflections collected 11,422, unique 4032 [R(int) = 0.0325], completeness to θ = 68.28°, 99.7%, the final refinement gave R₁ = 0.0455 and wR₂ = 0.1250 (w = 1/σ²(F²), S = 1.054, maximum transmission 0.7531, minimum transmission 0.7323, absolute structure parameter = 0.1 (2).

Bruker SMART APEX-II data collection, the structures were solved by direct methods using SHELXS-97 and refined by means of full-matrix least squares on F².

3.5. Computational Work

The stable conformational analysis was carried out with SYBYL software (Tripos, San Francisco, CA, USA) using the MMFF94S (Merck Molecular Force Field 94S) force field with an energy cutoff of 10 kcal/mol. The stable conformers were used for geometry optimization at the B3 LYP/6-31G(d) level with a CPCM (conductor-like conductor polarizable continuum model) solvent model for methanol in the Gaussian 09 software. The ECD spectra of stable conformers were then calculated based on the TDDFT method at the B3 LYP/6-31 + G(d) level in methanol [22]. The final ECD curves were generated based on the rotatory strengths with a half-band of 0.3 eV by SpecDis [23] and calculated from the spectra of individual conformers according to their contribution to the Boltzmann weighting. The theoretical spectra have been corrected based on the UV correction.
3.6. Cytotoxicity Assay

The cytotoxicities of 1–8 were evaluated against four human carcinoma cell lines, including a human gastric carcinoma cell line (BGC-823), a human large-cell lung carcinoma cell line (H460), a human prostate cancer cell line (PC-3) and a human hepatocellular carcinoma cell line (SMMC-7721) in an MTT assay as previously reported [24]. The IC$_{50}$ value was defined as a 50% reduction of absorbance from the control assay. Adriamycin (Sigma Inc., St Louis, MO, USA) was assayed as a positive control.

3.7. Antimicrobial Assay

A micro broth dilution assay as previously reported [25] was used to evaluate the MICs of 1–8 against three bacteria (Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922) and three fungi (Candida albicans MYA-2867, Candida parapsilosis ATCC 22019 and Cryptococcus neoformans ATCC 208821). The MIC was defined as the lowest concentration of the antimicrobial agent that completely inhibited visual growth of an organism. Ciprofloxacin and amphotericin B (Sigma Inc.) were used as positive controls against bacteria and fungi, respectively.

4. Conclusions

In this study, eight compounds, including four new metabolites, were isolated from the marine-derived fungus _P. heterocornis_. The structures of the isolated compounds were elucidated by the detailed analysis of spectroscopic data, as well as single-crystal X-ray crystallographic analysis and CD Cotton effects. All compounds were evaluated for their antibacterial and cytotoxic activities. Isocoumarins 1–3 showed antimicrobial activities against both Gram-positive bacteria and fungi. Chlorinated benzophenone derivatives 6–8 showed cytotoxicities and antibacterial activities against Gram positive bacteria.

**Supplementary Materials:** The materials (1D and 2D NMR, HRESI–MS spectra of Compounds 1–2, 4, 5) are available at www.mdpi.com/1660-3397/15/3/69/s1.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

**References**

1. Yang, X.Y.; Zhang, J.Z.; Luo, D.Q. The taxonomy, biology and chemistry of the fungal _Pestalotiopsis_ genus. Nat. Prod. Rep. 2012, 29, 622–641. [CrossRef] [PubMed]
2. Akone, S.H.; Amrani, M.E.; Lin, W.H.; Lai, D.W.; Proksch, P. Cytosporins F-K, new epoxyquinols from the endophytic fungus _Pestalotiopsis theae_. Tetrahedron Lett. 2013, 54, 6751–6754. [CrossRef]
3. Wang, J.F.; Wei, X.Y.; Lu, X.; Xu, F.Q.; Wan, J.T.; Lin, X.P.; Zhou, X.F.; Liao, S.R.; Yang, B.; Tu, Z.C.; et al. Eight new polyketide metabolites from the fungus _Pestalotiopsis vaccinii_ endogenous with the mangrove plant _Kandelia candel_ (L.) Druce. Tetrahedron 2014, 70, 9695–9701. [CrossRef]
4. Li, J.; Xie, J.; Yang, Y.H.; Li, X.L.; Zeng, Y.; Zhao, P.J. Pestalpolys A-D, cytotoxic polyketides from _Pestalotiopsis_ sp. c013. Planta Med. 2015, 81, 1285–1289. [CrossRef] [PubMed]
5. Hwang, I.H.; Swenson, D.C.; Gloer, J.B.; Wicklow, D.T. Disseminins and spiciferone analogues: Polyketide-derived metabolites from a fungiculous isolate of _Pestalotiopsis disseminae_. J. Nat. Prod. 2016, 79, 523–530. [CrossRef] [PubMed]
6. Liu, L.; Li, Y.; Li, L.; Cao, Y.; Guo, L.D.; Liu, G.; Che, Y.S. Spiroketals of Pestalotiopsis fici provide evidence for a biosynthetic hypothesis involving diversified diels-alder reaction cascades. *J. Org. Chem.* **2013**, *78*, 2992–3000. [CrossRef] [PubMed]

7. Hemberger, Y.; Xu, J.; Wray, V.; Proksch, P.; Wu, J.; Bringmann, G. Pestalotiopsens A and B: Stereochemically challenging flexible sesquiterpene-cyclopaldic acid hybrids from *Pestalotiopsis* sp. *Chem. Eur. J.* **2013**, *19*, 15556–15564. [CrossRef] [PubMed]

8. Wu, B.; Wu, X.D.; Sun, M.; Li, M.H. Two novel tyrosinase inhibitory sesquiterpenes induced by CuCl$_2$ from a marine-derived fungus *Pestalotiopsis* sp. Z233. *Mar. Drugs* **2013**, *11*, 2713–2721. [CrossRef] [PubMed]

9. Luo, D.Q.; Chen, Y.P.; Zhang, J.; Shi, B.Z.; Yang, Z.Q.; Chen, C. A new glycinine derivative and a new indole alkaloid from the fermentation broth of the plant endophytic fungus *Pestalotiopsis podocarpi* isolated from the Chinese podocarpaceae plant *Podocarpus macrophyllus*. *Heli. Chim. Acta* **2013**, *96*, 309–311. [CrossRef]

10. Jia, Y.L.; Wei, M.Y.; Chen, H.Y.; Guan, F.F.; Wang, C.Y.; Shao, C.L. (+)- and (−)- Pestaloxazine A, a pair of antiviral enantiomeric alkaloid dimers with a symmetric spiroadizinane-piperazinedione skeleton from *Pestalotiopsis* sp. *Org. Lett.* **2015**, *17*, 4216–4219. [CrossRef] [PubMed]

11. Yang, X.L.; Zhang, S.; Hu, Q.B.; Luo, D.Q.; Zhan, Y. Phthalide derivatives with antifungal activities against the plant pathogens isolated from the liquid culture of *Pestalotiopsis photiniae*. *J. Antibirot.* **2011**, *64*, 723–727. [CrossRef] [PubMed]

12. Li, J.; Wu, X.F.; Ding, G.; Feng, Y.; Jiang, X.J.; Guo, L.D.; Che, Y.S. α-Pyrones and pyranes from the plant pathogenic fungus *Pestalotiopsis scirpina*. *Eur. J. Org. Chem.* **2012**, *12*, 2445–2452. [CrossRef]

13. Keith, L.M.; Velasquez, M.E.; Zee, F.T. Identification and characterization of *Pestalotiopsis* spp. causing scab disease of guava, *Psidium guajava*, in Hawaii. *Plant Dis.* **2006**, *90*, 16–23. [CrossRef]

14. Wei, M.Y.; Li, D.; Shao, C.L.; Deng, D.S.; Wang, C.Y. (±)-Pestalachloride D, an antibacterial racemate of chlorinated benzophenone derivative from a soft coral-derived fungus *Pestalotiopsis* sp. *Mar. Drugs* **2013**, *11*, 1050–1060. [CrossRef] [PubMed]

15. Koshino, H.; Yoshihara, T.; Okuno, M.; Sakamura, S.; Tajimi, A.; Shimanuki, T. Gamahonolides A, B, and gamahorin, novel antifungal compounds from stromata of *Epichloe typhina* on *Phleum pratense*. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 1096–1099. [CrossRef] [PubMed]

16. Li, E.; Jiang, L.H.; Guo, L.D.; Zhang, H.; Che, Y.S. Pestalachlorides A-C, antifungal metabolites from the plant endophytic fungus *Pestalotiopsis adusta*. *Bioorg. Med. Chem. 2008*, *16*, 7894–7899. [CrossRef] [PubMed]

17. Xing, Q.; Gan, L.S.; Mou, X.F.; Wang, W.; Wang, C.Y.; Wei, M.Y.; Shao, C.L. Isolation, resolution and biological evaluation of pestalachlorides E and F containing both point and axial chirality. *RSC Adv.* **2016**, *6*, 22653–22658. [CrossRef]

18. Shao, C.L.; Wang, C.Y.; Xing, Q.; Wang, K.L. Manufacture of Chlorinated Benzophenone Compounds with *Pestalotiopsis* for Use as Marine Antifouling Agents. CN Patent 104,163,805, 26 November 2014.

19. Shao, C.L.; Wang, C.Y.; Xing, Q.; Wang, K.L. Manufacture of Chlorinated Benzophenone Compounds with *Pestalotiopsis* for Use as Marine Antifouling Agents. CN Patent 104,163,805, 26 November 2014.

20. Slavov, N.; Cvengros, J.; Neudorfl, J.M.; Schmalz, H.G. Total synthesis of the marine antibiotic pestalactone and its surprisingly facile conversion into pestalactalone and pestachloralactone A. *Angew. Chem. Int. Ed.* **2010**, *49*, 7588–7591. [CrossRef] [PubMed]

21. Rukachaisirikul, V.; Rodglin, A.; Sukpondma, Y.; Phongpaichit, S.; Buatong, J.; Sakayaroj, J. Phthalide and isocoumarin derivatives produced by an *Acremonium* sp. isolated from a mangrove *Rhizophora apiculata*. *J. Nat. Prod.* **2012**, *75*, 853–858. [PubMed]

22. Rukachaisirikul, V.; Rodglin, A.; Sukpondma, Y.; Phongpaichit, S.; Buatong, J.; Sakayaroj, J. Phthalide and isocoumarin derivatives produced by an *Acremonium* sp. isolated from a mangrove *Rhizophora apiculata*. *J. Nat. Prod.* **2012**, *75*, 853–858. [PubMed]

23. Bruhn, T.; Schaumloeffel, A.; Hemberger, Y.; Bringmann, G. SpecDis: Quantifying the comparison of calculated and experimental electronic circular dichroism spectra. *Chirality* **2013**, *25*, 243–249. [CrossRef] [PubMed]
24. Zheng, D.; Han, L.; Li, Y.Q.; Li, J.; Rong, H.; Leng, Q.; Jiang, Y.; Zhao, L.X.; Huang, X.S. Isostreptazolin and sannaphenol, two new metabolites from Streptomyces sannanensis. *Molecules* 2012, 17, 836–842. [CrossRef] [PubMed]

25. Ding, N.; Jiang, Y.; Han, L.; Chen, X.; Ma, J.; Qu, X.; Mu, Y.; Liu, J.; Li, L.; Jiang, C.; et al. Bafilomycins and odoriferous sesquiterpenoids from Streptomyces albogangus isolated from Elephas maximus Feces. *J. Nat. Prod.* 2016, 79, 799–805. [CrossRef] [PubMed]

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