Mono- and Dimeric Naphthalenones from the Marine-Derived Fungus Leptosphaerulina chartarum 3608

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Abstract: Five new naphthalenones, two enantiomers (−)-1 and (+)-1 leptothalenone A, (−)-4,8-dihydroxy-7-(2-hydroxy-ethyl)-6-methoxy-3,4-dihydro-2H-naphthalen-1-one (−)-2, (4S, 10R, 4′S)-leptothalenone B (5), (4R, 10S, 4′S)-leptothalenone B (6), and a new isocoumarine, 6-hydroxy-5,8-dimethoxy-3-methyl-1H-isochromen-1-one (4) were isolated from the marine-derived fungus Leptosphaerulina chartarum 3608. The structures of new compounds were elucidated by HR-ESIMS, NMR, and ECD analysis. All compounds were evaluated for cytotoxicity and anti-inflammatory activity. Compound 6 showed moderate anti-inflammatory activity by inhibiting the production of nitric oxide (NO) in lipopolysaccharide-stimulated RAW264.7 cells, with an IC50 value of 44.5 µM.

Keywords: Leptosphaerulina chartarum; naphthalenones; isocoumarine; anti-inflammatory

1. Introduction

Marine-derived micro-organisms are known as a rich source of natural products with novel structure and interesting bioactivities [1]. A large number of the new compounds from marine-derived fungi are discovered every year. Among these, naphthalenone derivatives are side products of the 1,8-dihydroxynaphthalene polyketide pathway [2], which are common in the secondary metabolites from fungi with diverse bioactivities, such as antimicrobial [3], antiviral [4], cytotoxic [5], and antimalarial activities [3]. Besides, naphthalenone derivatives have structural diversity not only in the planar structure but also in the absolute configuration. Abdel-Latef et al. reported a pair of epimers of (3R*,4S*)-3,4-dihydroxy-7-methyl-3,4-dihydro-1(2H)-naphthalenone and (3S*,4S*)-3,4-dihydroxy-7-methyl-3,4-dihydro-1(2H)-naphthalenone from the Algicolous marine fungus Acremonium sp. [6]. Wang et al. isolated a pair of naphthalenone enantiomers, corynene A and B, from sponge-derived fungus Corynespora cassiicola [7].

During our ongoing research on secondary metabolites from marine-derived fungus, five new naphthalenones, two enantiomers (−)-1 and (+)-1 leptothalenone A, (−)-4,8-dihydroxy-7-(2-hydroxy-ethyl)-6-methoxy-3,4-dihydro-2H-naphthalen-1-one ((−)-2), (4S, 10R, 4′S)-leptothalenone B (5),
(4R, 10S, 4'S)-leptothalenone B (6), and a new isocoumarine (4), along with two known compounds (+)-4,8-dihydroxy-7-(2-hydroxy-ethyl)-6-methoxy-3,4-dihydro-2H-naphthalen-1-one ((+)-2) and (+)-10-norparvulenone (3) (Figure 1) were isolated from *Leptosphaerulina chartarum* 3608 from a crinoid collected in Xuwen, Zhanjiang City, Guangdong Province, China. In this paper, we described the structure determination of the new compounds as well as the bioactivity assay of these isolated compounds from the CHCl₃ extract of *L. chartarum* 3608.

![Chemical structures of 1-6.](image)

2. Results and Discussion

Compound (±)-1 was obtained as a light brown powder and had a molecular formula C₁₃H₁₆O₅ according to the positive HR-ESIMS ion at m/z 275.0885 [M + Na]+ (calculated for C₁₃H₁₆O₅Na, 275.0890), indicating six degrees of unsaturation. The ¹H NMR data (Table 1) revealed the presence of one five-substituted aromatic proton [δ₇ 6.79 (1H, s, H-5)], one methoxyl [δ₇ 3.92 (3H, s, H-9)], one methyl [δ₇ 1.48 (3H, d, J = 6.8, H-11)], two methenes [δ₇ 2.62 (1H, m, H-2), δ₇ 2.79 (1H, m, H-2)], δ₇ 2.01 (1H, m, H-3), δ₇ 2.26 (1H, m, H-2)], two oxygenated methines [δ₇ 4.77 (1H, dd, J = 8.9, 3.9, H-4), δ₇ 5.31 (1H, s, H-11)]. The ¹³C NMR and DEPT spectra displayed a ketone carbonyl (δ₁₃ 204.6), six aromatic carbons (δ₁₃ 164.8, 162.7, 150.2, 119.1, 111.1, 102.2), an oxygenated methyl (δ₁₃ 56.4), two oxygenated methine (δ₁₃ 68.6 and 63.4), and two methylenes (δ₁₃ 36.1 and 32.7). Further analyses of the NMR data showed that compound 1 shared a good similarity with the known compound O-methylasparvenone [8], except for an extra hydroxyl group which was connected to C-10 based on the chemical shift values (δ₁₃/C 5.31 and 63.4, H-10 and C-10) and the ¹H-¹H COSY correlation of H-10 and H-11, as well as the HMBC correlation of H-11 with C-10 and C-7. Thus, the planar structure of 1 was successfully assigned as 9-hydroxyl-O-methylasparvenone (Figure 1).

Compound 1 was of racemic nature because it lacked any CD (circular dichroism) maximum and optical rotation. Subsequent chiral HPLC purification of (±)-1 led to the separation of the two enantiomers, (−)-1 and (+)-1 (Figure 2a), which showed opposite optical rotations [(−)-1 ([α]₂⁰ D = −24.8, c 0.47, MeOH) and (+)-1 ([α]₂⁰ D = 27.9, c 0.30, MeOH)] and opposite Cotton effects in their CD spectra (Figure 2b). The experimental ECD spectra of (−)-1 exhibited negative Cotton effects (CEs) at 214, 310 nm, and positive CEs at 238, 282 nm, while (+)-1 exhibited positive CEs at 214, 310 nm, and negative CEs at 238, 282 nm. To determine the absolute configuration of (−)-1 and (+)-1, their theoretical ECD
spectra of four possible configurations [(4R,10R)-1, (4R,10S)-1, (4S,10R)-1, (4S,10S)-1] were calculated by a quantum chemical method at the [B3LYP/6-311+G(2d,p)] level and are shown in Figure 2b. The predicted ECD curve of (4R,10R)-1 was in accordance with the experimental ECD curve of (−)-1, and (4S,10S)-1 was also in agreement with the experimental ECD curve of (+)-1 (Figure 2b). This result suggested that the absolute configuration of (−)-1 was 4R, 10R and (+)-1 was 4S, 10S. Therefore, (±)-1 was named as (±)-leptothalenone A.

Table 1. 1H (400 MHz) NMR data of compounds 1, 2, and 4–6 (in CD3OD, J in Hz).

| No. | 1   | 2          | 4          | 5          | 6          |
|-----|-----|------------|------------|------------|------------|
| 2   |     | 2.62, m;   | 2.77, m;   | 2.60, m    | 2.60, m    |
|     |     | (17.7, 10.1, 4.6); | 2.77, m; | 2.60, m    | 2.60, m    |
| 3   |     | 2.01, m;   | 2.01, m;   | 2.25, m;   | 2.25, m;   |
|     |     | (17.7, 6.4, 4.6) | 2.24, m; | 2.00, m    | 2.00, m    |
| 4   | 4.77, dd (8.9, 3.9) | 4.76, dd (8.7, 3.9) | 6.50, d (1.0) | 4.73, ddd (13.2, 8.9, 3.9) | 4.72, ddd (12.1, 9.1, 3.8) |
| 5   | 6.79, s | 6.75, s | 6.60, d (1.0) | 6.66, s | 6.61, s |
| 7   |     | 6.50, s    | 6.50, s    | 3.83, s    | 3.79, s    |
| 9   | 3.92, s | 3.90, s | 2.20, d (1.0) | 3.83, s | 3.79, s |
| 10  | 5.31, q (6.8) | 2.86, m | 3.83, s | 5.17, q (6.8) | 5.17, q (6.8) |
| 11  | 1.48, d (6.8) | 3.56, t (7.5) | 3.72, s | 1.45, d (6.8) | 1.44, d (6.8) |
| 12  |     | 2.60, m    | 2.60, m    | 2.25, m;   | 2.25, m;   |
|     |     | (17.7, 10.1, 4.6) | 2.24, m; | 2.00, m    | 2.00, m    |
| 4'  |     | 4.73, ddd (13.2, 8.9, 3.9) | 4.72, ddd (12.1, 9.1, 3.8) | 6.63, s | 6.58, s |
| 5'  |     | 3.79, s    | 3.76, s    | 4.46, m    | 4.48, m    |

Figure 2. Chiral HPLC chromatogram (a); experimental and calculation ECD spectra (b) of (±)-1.

Compound (±)-2 was obtained as a light yellow powder. Its molecular formula was determined as C13H16O5 according to the positive HR-ESIMS ([M + Na]+ m/z 275.0885, calculated for C13H16O5Na and was the same as (±)-leptothalenone A. Through analysis of the NMR data of (±)-2 (Tables 1 and 2, Figure 3), the planar structure of (±)-2 was identified as the known compound 4,8-dihydroxy-7-(2-hydroxy-ethyl)-6-methoxy-3,4-dihydro-2H-naphthalen-1-one with positive optical rotation ([a]D20 = +28.0, c 0.1, MeOH) [9], whose configuration has never been determined. Using the
similar chiral HPLC purification method, (±)-2 was separated into two enantiomers, (−)-2 and (+)-2, which displayed opposite optical rotations [(−)-2 ([α]_D^{20} = −14.0, c 0.10, MeOH) and (+)-2 ([α]_D^{20} = 15.1, c 0.07, MeOH)] and opposite Cotton effects in their CD spectra. The calculated ECD spectra of (4R)-2 and (4S)-2 agreed well with the experimental ECD spectra of (−)-2 and (+)-2 (Figure 4). Thus, the configuration of known compound (+)-2 was assigned as 4R, while the enantiomer (−)-2 was a new compound with the configuration of 4S.

Table 2. 13C (100 MHz) NMR data of compounds 1, 2, and 4–6 (in CD3OD).

| No. | 1            | 2  | 4            | 5            | 6            |
|-----|--------------|----|--------------|--------------|--------------|
| 1   | 204.6, C     | 203.0, C | 162.1, C     | 204.4, C     | 204.4, C     |
| 2   | 36.1, CH2    | 34.5, CH2 | 36.1, CH2    | 36.1, CH2    | 36.1, CH2    |
| 3   | 32.7, CH2    | 31.4, CH2 | 156.3, C     | 32.7, CH2    | 32.8, CH2    |
| 4   | 68.6, CH     | 67.2, CH  | 98.9, CH     | 68.6, CH     | 68.7, CH     |
| 4a  | 150.2, C     | 147.7, C  | 135.3, C     | 151.3, C     | 151.2, C     |
| 5   | 102.2, CH    | 100.4, CH | 135.8, C     | 101.9, CH    | 101.8, CH    |
| 6   | 164.8, C     | 164.1, C  | 158.5, C     | 166.2, C     | 166.1, C     |
| 7   | 119.1, C     | 111.9, C  | 100.2, CH    | 116.7, C     | 116.7, C     |
| 8   | 162.7, C     | 162.0, C  | 161.0, C     | 164.3, C     | 164.3, C     |
| 8a  | 111.1, C     | 109.6, C  | 101.2, C     | 110.8, C     | 110.8, C     |
| 9   | 56.4, CH3    | 55.0, CH3 | 19.5, CH3    | 56.3, CH3    | 56.2, CH3    |
| 10  | 63.4, CH     | 25.3, CH2 | 56.4, CH3    | 70.3, CH     | 70.4, CH     |
| 11  | 22.2, CH3    | 60.1, CH2 | 61.6, CH3    | 20.0, CH3    | 20.1, CH3    |
| 1'  |             |           | 204.2, C     | 204.2, C     |              |
| 2'  |             |           | 36.1, CH2    | 36.1, CH2    |              |
| 3'  |             |           | 32.7, CH2    | 32.8, CH2    |              |
| 4'  |             |           | 68.6, CH     | 68.6, CH     |              |
| 4a' |             |           | 150.3, C     | 150.3, C     |              |
| 5'  |             |           | 101.3, CH    | 101.3, CH    |              |
| 6'  |             |           | 165.9, C     | 165.9, C     |              |
| 7'  |             |           | 113.0, C     | 113.1, C     |              |
| 8'  |             |           | 163.7, C     | 163.6, C     |              |
| 8a' |             |           | 110.7, C     | 110.7, C     |              |
| 9'  |             |           | 56.2, CH3    | 56.1, CH3    |              |
| 10' |             |           | 59.6, CH2    | 59.8, CH2    |              |

Compound 4 was obtained as a white crystal. The HR-ESIMS result (m/z 237.0812 [M + H]+) suggested the molecular formula of 4 was C_{12}H_{12}O_{3} with seven degrees of unsaturation. The 1D and 2D NMR data indicated that compound 4 shared the same isocoumarin skeleton as 6,8-dihydroxy-5-methoxy-3-methyl-1H-isochromen-1-one [10]. The only difference between them was that the hydroxyl group (8-OH) of the known compound was replaced by a methoxyl group (8-OCH3) of 4, which was further confirmed by the HMBC correlation of H-10 to C-5 and C-6. Hence, compound 4 was assigned as 6-hydroxy-5,8-dimethoxy-3-methyl-1H-isochromen-1-one.

Compounds 5 and 6 were both isolated as a light brown amorphous powder from the same fraction using the RP-HPLC [MeOH-H$_2$O 60:40, t$_{R}$ (5) = 52 min, t$_{R}$ (6) = 56 min], implying they were epimers. The different optical rotation ([α]_D^{20} = −11.9 (MeOH, c 0.21) of 6, [α]_D^{20} = 11.4 (MeOH, c 0.21) of 6) indicated that 6 was a stereoisomer of 5. They had the same molecular formula C_{25}H_{25}O_{9}, which was determined by HR-ESIMS at m/z 495.1628 (calculated for C_{25}H_{25}O_{9}Na, 495.1626), corresponding to 12 degrees of unsaturation. The IR spectrum displayed absorption at 3383 cm$^{-1}$ indicative of the existence of hydroxyl group. The 1H and 13C NMR spectra of compounds 5 and 6 revealed the presence of 24 protons and 25 carbons (two ketone carbonyls, ten aromatic quaternary carbons, two aromatic methines, three methyls, five methylenes, three methine). There were 14 sp$^2$ carbon signals (two of which were carbonyls) that appeared pairwise in 13C NMR spectrum indicating that 5 and 6 were heterodimers of naphthalenones. Detailed analysis of the 1H-1H COSY and HMBC spectra (Figure 3), compounds 5 and 6 had the same planar structures as two monomeric units leptothalenone A (1) and 10-norpavulinone.
(3). The two monomeric units were connected through an ether bond (10-O-10′) based on the HMBC correlations from H-10′ to C-7′ and C-10. Therefore, the planar structures of 5 and 6 were defined as 1,5-dihydroxy-3-methoxy-8-oxo-5,6,7,8-tetrahydronaphthalen-2-yl(ethoxy)methyl)-4,8-dihydroxy-6-methoxy-3,4-dihydronaphthalen-1(2H)-one.

![Figure 3](image)

**Figure 3.** Key $^1$H-$^1$H COSY (red line) and HMBC (blue arrow) correlations of compounds 1, 2, 4 and 5.

The absolute configurations of 5 and 6 were established by comparing the experimental ECD data with the calculated values, in combination with biosynthetic considerations. Among the isolated monomeric naphthalenones, (+)-10-norparvulenone (3) was only obtained as an enantiomerically pure compound with $S$ configuration, supporting by the X-ray single-crystal diffraction (Figure S45). The biosynthetic pathway suggests that the configurations at C-4′ of 5 and 6 were the same as those of the monomeric 10-norparvulenone (3). The ECD of the remaining four configurations [(4R, 10S, 4′S) (4S, 10R, 4′S) (4R, 10R, 4′S) (4S, 10S, 4′S)] was calculated by a quantum chemical method at the [B3LYP/6-311+g(2d,p)] level. The theoretical ECD curve of (4S, 10R, 4′S) agreed well with the experimental ECD curve of (−)-5, and (4R, 10S, 4′S) was also in agreement with the experimental ECD curve of (+)-6 (Figure 4).

The known compound, (+)-10-norparvulenone (3) was identified by NMR, MS and optical rotation data analysis and comparison of spectroscopic data with the literature [9]. The optical rotation of 10-norparvulenone was $[\alpha]_{D}^{20} = -27.0$ (MeOH, c 0.21), and that of 3 was $[\alpha]_{D}^{20} = +16.6$ (MeOH, c 0.21),
which indicated their absolute configurations were opposite. The absolute configuration of 3 was 4S supported by X-ray single-crystal diffraction using anomalous scattering of Cu Kα radiation with Flack parameter = 0.00(6) (Figure S45). Thus, compound 3 was named as (+)-10-norparvulenone.

All the compounds were tested for their inhibition activity against LPS-activated NO production in RAW264.7 cells using the Griess assay. Compound 6 displayed moderate inhibitory effects on the production of NO with an IC50 value of 44.5 ± 1.1 µM, compared to the positive control indomethacin (IC50 = 37.5 ± 1.6 µM), while the other compounds showed no significant anti-inflammatory activity (IC50 > 100 µM). In addition, some of naphthalenones have been found to have cytotoxic activity according to previous studies [2,5,11,12]. Thus, all the isolated compounds were evaluated for their cytotoxicity against A549 (lung cancer), HeLa (cervical cancer), and MCF-7 (breast cancer) human cancer cell lines using MTT assay and displayed no cytotoxicity against all three cell lines at 50 µM.

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were measured on an MCP 200 polarimeter by using an Na lamp (Shimadzu). UV spectra were obtained on a Blue Star A spectrophotometer. A Fourier transformation infra-red spectrometer coupled with infra-red microscope EQUINOX 55 (Bruker, Rheinstetten, Germany) was used to record the IR spectra. NMR spectra were obtained on a Bruker Avance 400 MHz spectrometer with tetramethylsilane as the internal standard. HR-ESIMS data were obtained on a LTQ-Orbitrap LC-MS spectrometer (Thermo Corporation, Waltham, MA, USA). ESIMS spectra were obtained on an ACQUITY QDA (Waters Corporation, Milford, MA, USA). HPLC was carried out on an Essentia LC-16 with an SPD-16 Detector (Shimadzu, Shanghai, China). Column chromatography was carried out on silica gel (100–200 mesh, 200–300 mesh, Qing dao Marine Chemical Factory, Qingdao, China) and Sephadex LH-20 (GE Healthcare, Little Chalfont, UK).

3.2. Fungal Material

The fungal strain 3608 was isolated from a crinoid collected in Xuwen, Zhanjiang City, Guangdong Province, China, in August 2014. It was identified as *L. chartarum* by ITS sequence, and the sequence data have been submitted to and deposited in the GenBank database under accession number MF980969. The fungal strain has been preserved at the school of marine science, Sun Yat-Sen University.

3.3. Extraction, Isolation, and Characterization

The fungus *L. chartarum* 3608 was cultured at room temperature (25–30 °C) for one month in 1000 mL Erlenmeyer flasks containing rice medium, composed of 60 mL rice, 80 mL H2O, and 3% sea salt. After 30 days of cultivation, the fermented rice substrate of 170 flasks was extracted three times with MeOH to yield the organic extract (70 g). The organic extract was portioned into three phases by successive extraction with n-hexane (43 g), CHCl3 (8 g), EtOAc (17 g). The CHCl3 phase was subjected to a silica gel column (6 × 12 cm, 100–200 mesh) and was eluted with PE-EtOAc (v/v, 80:20, 70:30, 60:40, 50:50, 40:60, 20:80 and 0:100, 400 × 5 mL each gradient) to yield seven fractions (A–F). Fr. D was separated by silica gel CC (3.5 × 12 cm, 200–300 mesh), eluted with PE-EtOAc (v/v 70:30, 60:40, 65:45, 50:50, 55:45 and 30:70) and combined with the same fractions based on the TLC results to yield Fr.D.1–Fr.D.5. Fr.D.1 was further subjected to silica gel CC (2 × 12 cm, 200–300 mesh) by isocratic elution by PE-EtOAc (65:35) to yield compound 4 (6 mg). After recrystallization, the racemates of 1 and 2 were obtained from Fr.D.2 and Fr.D.3, respectively. The racemate of 1 was separated by chiral PR-HPLC (30% CH3CN-H2O, flow rate 1 mL/min, Ultimate Amy-SR column 10 × 250 mm, 5 µm) to yield (−)-1 (5 mg), (+)-1 (5 mg). The racemate of 2 was also separated by chiral PR-HPLC (30% CH3CN-H2O, flow rate 1 mL/min, Ultimate Amy-SR column 10 × 250 mm, 5 µm) to yield (−)-2 (7 mg), and (+)-2 (7 mg). Fr.E was subjected to silica gel CC (3.5 × 12 cm, 200–300 mesh) and eluted with PE-EtOAc (40:60) to yield 3 (300 mg). Fr.F was subjected to
RP-C18 (3.5 × 12 cm), eluted with 60% MeOH-H₂O, and then purified by PR-HPLC (60% MeOH-H₂O, flow rate 1 mL/min, Ultimate XB-C18 column 10 × 250 mm, 5 µm) to yield 5 (3 mg) and 6 (2 mg), whose retention times were 52 min and 56 min, respectively.

3.3.1. (−)-Leptothenalene A ((−)-1)

Light brown powder; [α]$_D^{20}$ = −24.8 (MeOH, c 0.47); UV (MeOH) λ$_{max}$ (log ε) 222 (4.00), 286 (3.92) nm; CD (MeOH) λ$_{max}$ (Δε) 214 (−26.9), 238 (+2.74), 282 (+11.6), 310 (−6.24) nm; IR (neat) ν$_{max}$ 3381, 2923, 1626, 1416, 1286, 1219, 1157, 1080, 843 cm$^{-1}$; $^1$H NMR (400 MHz, MeOD) and $^{13}$C NMR (100 MHz, MeOD) data, see Tables 1 and 2; HR-ESIMS m/z 275.0885 [M + Na]$^+$ (calculated for C$_{13}$H$_{16}$O$_{3}$Na, 275.0890).

3.3.2. (+)-Leptothenalene A ((+)-1)

Light brown powder; [α]$_D^{20}$ = 27.9 (MeOH, c 0.30); UV (MeOH) λ$_{max}$ (log ε) 222 (3.91), 286 (3.79) nm; CD (MeOH) λ$_{max}$ (Δε) 214 (+21.8), 238 (−1.75), 282 (−5.20), 310 (+4.54) nm; IR (neat) ν$_{max}$ 3381, 2923, 1626, 1416, 1286, 1219, 1157, 1080, 843 cm$^{-1}$; $^1$H NMR (400 MHz, MeOD) and $^{13}$C NMR (100 MHz, MeOD) data, see Tables 1 and 2; HR-ESIMS m/z 275.0885 [M + Na]$^+$ (calculated for C$_{13}$H$_{16}$O$_{3}$Na, 275.0890).

3.3.3. (−)-4,8-Dihydroxy-7-(2-hydroxy-ethyl)-6-methoxy-3,4-dihydro-2H-naphthalen-1-one ((−)-2)

White powder; [α]$_D^{20}$ = −14.0 (MeOH, c 0.10); UV (MeOH) λ$_{max}$ (log ε) 224 (4.26), 287 (3.17) nm; CD (MeOH) λ$_{max}$ (Δε) 213 (−13.1), 239 (1.40), 279 (3.75) nm; IR (neat) ν$_{max}$ 3374, 2945, 2954, 1622, 1417, 1292, 1211, 1138, 1092, 831 cm$^{-1}$; $^1$H NMR (400 MHz, MeOD) and $^{13}$C NMR (100 MHz, MeOD), see Tables 1 and 2; HR-ESIMS m/z 275.0885 [M + Na]$^+$ (calculated for C$_{13}$H$_{16}$O$_{3}$Na, 275.0890).

3.3.4. (+)-4,8-Dihydroxy-7-(2-hydroxy-ethyl)-6-methoxy-3,4-dihydro-2H-naphthalen-1-one ((+)-2)

White powder; [α]$_D^{20}$ = 15.1 (MeOH, c 0.07); UV (MeOH) λ$_{max}$ (log ε) 224 (4.26), 287 (3.17) nm; CD (MeOH) λ$_{max}$ (Δε) 214 (+12.3), 230 (−1.42), 282 (−4.47); IR (neat) ν$_{max}$ 3374, 2945, 2954, 1622, 1417, 1292, 1211, 1138, 1092, 831 cm$^{-1}$; $^1$H NMR (400 MHz, MeOD) δ 2.59 (dd, J = 17.7, 10.1, 4.6 Hz, H-2a); 2.77 (dd, J = 17.7, 6.4, 4.6 Hz, H-2b); 2.01 (m, H-3a); 2.24 (m, H-3b); 4.76 (dd, J = 8.7, 3.9 Hz, H-4); 6.75 (s, H-5); 2.86 (m, H-10); 3.56 (t, J = 7.5 Hz, H-11); 3.90 (s, H-9); $^{13}$C NMR (100 MHz, MeOD) δ 203.0 (C, C-1); 34.5 (CH$_2$, C-2); 31.4 (CH$_2$, C-3); 67.2 (CH$_2$, C-4a); 147.7 (CH$_2$, C-4a); 100.4 (CH, C-5); 164.1 (C, C-6); 111.9 (C, C-7); 162.0 (C, C-8); 109.6 (C, C-8a); 25.3 (CH$_2$, C-10); 60.1 (CH$_2$, C-11); 55.0 (CH$_3$, C-9); HR-ESIMS m/z 275.0885 [M + Na]$^+$ (calculated for C$_{13}$H$_{16}$O$_{3}$Na, 275.0890).

3.3.5. (+)-10-Norparvulenone (3)

Yellow powder; [α]$_D^{20}$ = 16.6 (MeOH c 0.14); UV (MeOH) λ$_{max}$ (log ε) 224(4.32), 285 (4.18); 320 (3.72) nm; CD (MeOH) λ$_{max}$ (Δε) 216 (+18.4), 240 (−3.14), 274 (−6.20), 305 (4.75) nm; IR (neat) ν$_{max}$ 3379, 2939, 1616, 1416, 1290, 1132, 993, 829 cm$^{-1}$; $^1$H NMR (400 MHz, MeOD) δ 2.61 (dd, J = 12.9, 8.2, 4.6 Hz, H-2a); 2.76 (dd, J = 15.6, 8.5, 4.6 Hz, H-2b); 2.01 (m, H-3a); 2.25 (m, H-3b); 4.77 (dd, J = 9.0, 3.8 Hz, H-4); 6.77 (s, H-5); 4.63 (s, H-9); 3.92 (s, 6-OCH$_3$); $^{13}$C NMR (100 MHz, MeOD) δ 204.6 (C, C-1); 36.3 (CH$_2$, C-2); 33.0 (CH$_2$, C-3); 68.9 (CH, C-4); 151.4 (CH, C-4a); 102.1 (CH, C-5); 166.1 (C, C-6); 116.0 (C, C-7); 164.0 (C, C-8); 111.4 (C, C-8a); 53.1 (CH$_2$, C-9); 56.7 (CH$_3$, 6-OCH$_3$); ESIMS m/z 236.9 [M − H]$^−$.

3.3.6. 6-Hydroxy-5,8-dimethoxy-3-methyl-1H-isochromen-1-one (4)

White powder; UV (MeOH) λ$_{max}$ (log ε) 244 (2.76), 335 (2.07) nm; IR (neat) ν$_{max}$ 3192, 2943, 2858, 1689, 1593, 1446, 1367, 1277, 1221, 1047, 980, 829, 746 cm$^{-1}$; $^1$H NMR (400 MHz, MeOD) and $^{13}$C NMR (100 MHz, MeOD) data, see Tables 1 and 2; HR-ESIMS m/z 237.0812 [M + H]$^+$ (calculated for C$_{12}$H$_{13}$O$_5$, 237.0758).
3.3.7. (4S, 10R, 4’S)-Leptothalenone B (5)

Light brown amorphous powder; $[\alpha]_{D}^{20} = -11.9$ (MeOH, $c$ 0.21); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 222 (3.35), 286 (3.20), 326 (2.74) nm; CD (MeOH) $\lambda_{\text{max}}$ (Δε) 227 (11.5), 286 (−16.3), 315 (6.10) nm; IR (neat) $\nu_{\text{max}}$ 3383, 2933, 1614, 1291, 1061, 831 cm$^{-1}$; $^1$H NMR (400 MHz, MeOD) and $^{13}$C NMR (100 MHz, MeOD) data, see Tables 1 and 2; HR-ESIMS $m/z$ 495.1628 [M + Na$^+$] (calculated for C$_{25}$H$_{28}$O$_9$Na, 495.1626).

3.3.8. (4R, 10S, 4’S)-Leptothalenone B (6)

Light brown amorphous powder; $[\alpha]_{D}^{20} = 11.4$ (MeOH, $c$ 0.21); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 222 (3.35), 286 (3.20), 326 (2.74) nm; CD (MeOH) $\lambda_{\text{max}}$ (Δε) 227 (11.5), 286 (−16.3), 315 (6.10) nm; IR (neat) $\nu_{\text{max}}$ 3383, 2933, 1614, 1291, 1061, 831 cm$^{-1}$; $^1$H NMR (400 MHz, MeOD) and $^{13}$C NMR (100 MHz, MeOD) data, see Tables 1 and 2; HR-ESIMS $m/z$ 495.1628 [M + Na$^+$] (calculated for C$_{25}$H$_{28}$O$_9$Na, 495.1626).

3.4. Cytotoxic Assay

All compounds were evaluated for their cytotoxicity against three human cancer cell lines, human lung adenocarcinoma (A549), human cervical carcinoma (HeLa), and the human breast adenocarcinoma cell line (MCF-7). The three tumor cell lines were generously provided by the cell bank of the Chinese Academy of Sciences (Shanghai, People’s Republic of China). The cytotoxic activities of the tested compounds were assayed according to the MTT method by using 96 well plates [13]. In brief, the cells were cultured in MEM medium, supplemented with 10% fetal bovine serum in a humidified atmosphere with 5% CO$_2$ at 37 °C. Then, 198 µL adherent cells at the density of 4 × 10$^4$ cell/mL were seeded into each well of the 96-well cell culture plates and incubated in 5% CO$_2$ at 37 °C for 12 h to form a monolayer on the flat bottoms. Then, 2 µL test compounds dissolved in DMSO were added at concentrations of 50 µM. The plate was then incubated in 5% CO$_2$ at 37 °C. After 24 h, the supernatant per well was removed and subsequently added to 90 µL fresh medium and 10 µL MTT was added into each well and incubated for 4 h. The supernatant per well was carefully removed, and 110 µL DMSO was added. The plate was then vortex shaken for 15 min to dissolve blue formazan crystals. The optical density (OD) of each well was measured on a microplate reader (Multiskan GO, Thermo Scientific) at a wavelength of 490 nm. Inhibition rate (%) = (OD$_{\text{control}}$ − OD$_{\text{treated}}$)/OD$_{\text{control}}$ × 100%.

3.5. Anti-Inflammation Bioassays

The anti-inflammatory activity of the pure compounds was evaluated based on the reported procedures [14].

4. Conclusions

In summary, seven naphthalenones and one isocoumarine, including six new compounds, were isolated from the marine-derived fungus *Leptosphaerulina chartarum* 3608. The structures of the new compounds were established by analysis of HR-ESIMS and NMR spectroscopic data, and the absolute configurations were further determined by comparison of the experimental and calculated ECD spectra. Among all isolated compounds, only compound 6 showed moderate anti-inflammatory activity with IC$_{50}$ = 44.5 ± 1.1 µM. All secondary metabolites 1–6 exhibited no cytotoxicity against A549, HeLa, and MCF-7 cancer cell lines at 50 µM.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/1660-3397/16/5/173/s1.

**HR-ESIMS, NMR, CD spectra of the new compounds as well as other supporting data.**

**Author Contributions:** Panpan Zhang, Lan Liu and Senhua Chen conceived and designed the experiments; Panpan Zhang performed the experiments; Chunxiu Jia, Jiajia Lang, Jing Li and Guangyuan Luo participated in the experimental process. Panpan Zhang, Sujun Yan and Senhua Chen analyzed the data.

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References

1. Blunt, J.W.; Carroll, A.R.; Copp, B.R.; Davis, R.A.; Keyzers, R.A.; Prinsep, M.R. Marine natural products. Nat. Prod. Rep. 2018, 35, 53–68. [CrossRef] [PubMed]

2. Barnes, E.C.; Jumpathong, J.; Lumyong, S.; Voigt, K.; Hertweck, C. Dalendonin, an Unprecedented Binaphthyl Derivative, and Diverse Polyketide Congeners from a Fungal Orchid Endophyte. Chem. Eur. J. 2016, 1, 4551–4555. [CrossRef] [PubMed]

3. Prabpai, S.; Wiyakrutta, S.; Sriubolmas, N. Phytochemistry Letters Antimycobacterial dihydronaphthalene from the endophytic fungus Nodulisporium sp of Antidesma ghaesembilla. Phytochem. Lett. 2015, 13, 375–378. [CrossRef]

4. Mentel, R.; Lindequist, U.; Jansen, R.; Drugs, M.; Sea, B. Balticsols A–F, New Naphthalenone Derivatives with Antiviral Activity, from an Ascomycetous Fungus. Chem. Biodivers. 2009, 6, 127–137.

5. Li, Y.X.; Himaya, S.W.A.; Dewapriya, P.; Kim, H.J.; Kim, S.K. Anti-proliferative effects of isosclerone isolated from marine fungus Aspergillus fumigatus in MCF-7 human breast cancer cells. Process Biochem. 2015, 49, 2292–2298. [CrossRef]

6. Abdel-lateff, A.; Ko, G.M.; Fisch, K.M.; Ho, U.; Jones, P.G.; Wright, A.D. New Antioxidant Hydroquinone Derivatives from the Algicolous Marine Fungus Acremonium sp. J. Nat. Prod. 2002, 65, 1605–1611. [CrossRef] [PubMed]

7. Zhao, D.; Shao, C.; Wang, C.; Wang, M.; Yang, L.; Wang, C. Naphthalenones and Depsidones from a Sponge-Derived Strain of the Fungus. Molecules 2016, 21, 4–9.

8. Bös, M.; Canesso, R.; Inoue-Ohga, N.; Nakano, A.; Takehana, Y.; Sleight, A.J. O-Methylasparvenone, A Nitrogen-free Serotonin Antagonist. Bioorg. Med. Chem. 1997, 5, 2165–2171. [CrossRef]

9. Hao, G.; Zhang, Q.H.; Jiang, M.M.; Tang, J.S.; Miao, C.D.; Kui, H.; Michio, N.; Wang, N.L.; Yao, X.S. Polyketides from a marine sponge-derived fungus Mycelia sterilia and proton-proton long-range coupling. Magn. Reson. Chem. 2008, 46, 1148–1152. [CrossRef] [PubMed]

10. Chen, S.; Liu, Y.; Liu, Z.; Cai, R.; Lu, Y.; Huang, X.; She, Z. Isocoumarins and benzofurans from the mangrove endophytic fungus Talaromyces amestolkiae possess α-glucosidase inhibitory and antibacterial activities. RSC Adv. 2016, 6, 26412–26420. [CrossRef]

11. Shah, R.; Stadler, M.; Anke, H.; Sterner, O. Naphthalenone and phthalide metabolites from Lachnum papyraceum. J. Nat. Prod. 1997, 60, 804–805.

12. Chen, G.; Pi, X.M.; Yu, C.Y. A new naphthalenone isolated from the green walnut husks of Juglans mandshurica Maxim. Nat. Prod. Res. 2015, 29, 174–179. [CrossRef] [PubMed]

13. Wang, J.; Xu, Q.; Zheng, M.; Ren, H.; Lei, T.; Wu, P.; Zhou, Z.; Wei, X.; Tan, J. Bioactive 30-Noroleanane Triterpenes from the Pericarps of Akebia trifoliate. Molecules 2014, 19, 4301–4312. [CrossRef] [PubMed]

14. Zhang, P.; Li, Y.; Jia, C.; Lang, J.; Niaz, S.-I.; Li, J.; Yuan, J.; Yu, J.; Chen, S.; Liu, L. Antiviral and anti-inflammatory meroterpenoids: Stachybonoids A–F from the crinoid-derived fungus Stachybotrys chartarum 952. RSC Adv. 2017, 7, 49910–49916. [CrossRef]