Abstract: Three new benzoferanoids, asperfuranoids A–C (1–3), two new phenylpropanoid derivatives (6 and 7), and nine known analogues (4, 5, and 8–14) were isolated from the liquid substrate fermentation cultures of the mangrove endophytic fungus Aspergillus sp. ZJ-68. The structures of the new compounds were determined by extensive spectroscopic data interpretation. The absolute configurations of 1–3 were assigned via the combination of Mosher’s method, and experimental and calculated electronic circular dichroism (ECD) data. Compounds 4 and 5 were a pair of enantiomers and their absolute configurations were established for the first time on the basis of their ECD spectra aided with ECD calculations. All isolated compounds (1–14) were evaluated for their enzyme inhibitory activity against α-glucosidase and antibacterial activities against four pathogenic bacteria (Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and Pseudomonas aeruginosa). Among them, compound 6 exhibited potent inhibitory activity against α-glucosidase in a standard in vitro assay, with an IC50 value of 12.4 µM, while compounds 8 and 11 showed activities against S. aureus, E. coli, and B. subtilis, with MIC values in the range of 4.15 to 12.5 µg/mL.

Keywords: benzoferanoids; phenylpropanoids; Aspergillus sp.; α-glucosidase; antibacterial activity
derivatives (6 and 7), together with nine known analogues (4, 5, and 8–14) (Figure 1). Compounds 4 and 5 were a pair of enantiomers and the determination of their absolute configurations is an important issue during structure determination. In this context, we report for the first time the absolute configurations of their central chirality elements. In the bioactivity assays, the α-glucosidase inhibitory activity and antibacterial activities against four pathogenic bacteria (S. aureus, E. coli, B. subtilis, and P. aeruginosa) of 1–14 were assessed. Herein, details of the isolation, structure elucidation, and biological activities of these compounds are described.

Figure 1. Structures of compounds 1–14.

2. Results

2.1. Structure Elucidation

The crude extract of Aspergillus sp. ZJ-68 was purified by repeatedly various chromatography to yield five new (1–3, 6, and 7) and nine known compounds (4, 5, and 8–14). The known compounds were identical to (−)-penicisochroman A (4) [13–15], (+)-penicisochroman A (5) [13–15], 2-(hydroxymethyl)-3-propylphenol (8) [16], peniciphenol (9) [13], penicibenxepinol (10) [17], (−)-brassicadiol (11) [14,18], (+)-pseudodeflectusin (12) [14,19], (−)-penicisochroman B (13) [13,14], and ustusorane A (14) [14,20] by the comparison of their spectral data (NMR and MS) as well as specific rotation data with those reported.

Asperfuranoid A (1) was obtained as a pale yellow oil with a molecular formula C_{15}H_{22}O_{4}, as inferred from the HRESIMS [M + Na]^+ ion at m/z 289.14070 (calcd for C_{15}H_{22}NaO_{4}, 289.14103) and NMR data, suggesting five degrees of unsaturation. Examination of the ^1H NMR data (Table 1) of 1 revealed unambiguously the presence of a 1,2,3,4-tetrasubstituted benzene [δ_H 7.04 and 6.87 (each 1H, d, J = 7.6 Hz)], one oxygenated methylene [δ_H 4.74 and 4.63 (each 1H, d, J = 7.6 Hz)], two oxygenated methines [δ_H 4.74 (m) and 4.57 (t, J = 9.0 Hz)], two methylenes [δ_H 3.19 (dd, J = 15.7, 8.5 Hz), 3.08 (dd, J = 15.7, 9.0 Hz), 1.87 (m), and 1.78 (m)], and three aliphatic methyils [δ_H 1.36 (s), 1.14 (s), and 0.94 (t, J = 7.4 Hz)]. The ^13C NMR data (Table 1) of 1 disclosed 15 carbon resonances that were identified by HSQC (Supplementary material, Figure S6) as five nonprotonated carbons (four olefinic carbons and one sp^3 oxygen-bearing carbon), four methine carbons (two olefinic carbons and two sp^3 oxygen-bearing carbons), three sp^3 methylene carbons, and three methyl carbons. The molecular formula of 1 required five degrees of unsaturation, but only six olefinic carbons resonating at δ_C 158.6 (C, C-9b), 126.6 (C, C-3a), 124.7 (CH, C-4), 118.5 (CH, C-5), 142.5 (C, C-5a), and 119.8 (C, C-9a) were detected, indicating the bicyclic nature of 1. The planar structure of 1 was further
established by the 2D NMR data (Figure 2). A hydrogenated benzofuran ring was deduced by the COSY correlations (H-2/H2-3 and H-4/H-5) and the HMBC correlations from H-2 to C-9b (δC 158.6) and C-3a (δC 126.6), from H-3 to C-3a, C-4 (δC 124.7), and C-9b (δC 158.6), and from H-4 to C-5a (δC 142.5) and C-9b. The locations of two hydroxy groups at C-6 and C-11 were suggested by the chemical shifts of C-6 (δC 72.6, δH 4.74) and C-11 (δC 71.7). The additional COSY correlations of H-6/H2-7/H3-10 and the HMBC correlations from H-6 (δH 4.74) to C-5 (δC 118.5), C-5a, and C-9a, and from H2-7 to C-5a allowed the establishment of a propan-1-ol group, which was linked to C-5a of the benzofuran moiety. The presence of a hydroxyisopropyl group which was assigned at C-2 was deduced from the HMBC correlations from H 3-12 and H 3-13 to C-11 (δC 72.6). The additional COSY correlations of H-6/H2-7/H3-10 and the HMBC correlations from H-6 (δH 4.74) to C-5 (δC 118.5), C-5a, and C-9a, and from H2-7 to C-5a allowed the establishment of a propan-1-ol group, which was linked to C-5a of the benzofuran moiety. The presence of a hydroxyisopropyl group which was assigned at C-2 was deduced from the HMBC correlations from H-2 to C-9b and H2-7 to C-11 (δC 71.7) and C-2 (δC 89.6). Finally, the planar structure of 1 was established by connecting the hydroxymethyl group to C-9a as evidenced by the HMBC correlations from H2-9 to C-5a, C-9a, and C-9b (Figure 2).

| No. | δC, Type | δH, mult (J/Hz) | δC, Type | δH, mult (J/Hz) | δC, Type | δH, mult (J/Hz) |
|-----|----------|-----------------|----------|-----------------|----------|-----------------|
| 2   | 89.6, CH  | 4.57, t (9.0)   | 89.6, CH | 4.60, t (8.9)   | 89.7, CH | 4.37, s         |
| 3   | 30.7, CH2 | 3.10, dd (5.8, 15.6) | 30.7, CH2 | 3.21, dd (9.5, 15.8) | 199.9, C  | 7.47, s         |
| 3a  | 126.6, C |                  | 127.5, C |                  | 142.3, C |                  |
| 4   | 124.7, CH | 7.04, d (7.6)   | 124.6, CH | 7.06, d (7.6)   | 124.2, CH | 7.47, d (7.9)   |
| 5   | 118.5, CH | 4.87, d (7.6)   | 120.0, CH | 6.84, d (7.6)   | 117.6, CH | 6.63, d (7.9)   |
| 5a  | 142.5, C |                  | 141.0, C |                  | 142.6, C |                  |
| 6   | 72.6, CH | 4.74, m         | 72.6, CH | 5.41, m         | 101.7, CH | 5.69, s         |
| 7   | 30.2, CH2 | 1.87, m         | 139.3, CH | 6.13, dddd (4.6, 10.5, 17.5) | 160.7, C | 5.28, d (13.2) |
| 9   | 55.9, CH2 | 4.74, d (12.0)  | 56.2, CH2 | 4.81, d (12.1)  | 62.7, CH2 | 5.22, d (13.2)  |
| 9a  | 119.8, C |                  | 120.2, C |                  | 108.9, C |                  |
| 9b  | 158.6, C |                  | 158.9, C |                  | 167.9, C |                  |
| 10  | 10.9, CH3 | 0.94, t (7.4)   | 115.3, CH2 | 5.39, d (9.1)   | 20.1, CH3 | 1.98, s         |
| 11  | 71.7, C  |                  | 71.8, C  |                  | 72.6, C  |                  |
| 12  | 24.3, CH3 | 1.14, s         | 24.2, CH3 | 1.17, s         | 24.1, CH3 | 1.20, s         |
| 13  | 26.7, CH3 | 1.36, s         | 26.6, CH3 | 1.36, s         | 26.2, CH3 | 1.36, s         |

Figure 2. Key 2D NMR correlations for 1–7.

Table 1. {1H} (500 MHz) and {13C} (125 MHz) NMR data of compounds 1–3 in CDCl3 (δ in ppm).

The absolute configuration of the C-6 was determined by the modified Mosher’s method [21]. The ΔδSR values between 1a and 1b (S- and R-MTPA esters of 1 on 6-OH) were negative for H-4/H-5 and positive for H2-7/H3-10, which indicated the 6R configuration (Figure 3). Then the other stand-alone stereogenic center C-2 of 1 was deduced and confirmed by comparison of its experimental and theoretical electronic circular dichroism (ECD) spectra, the latter calculated at the b3lyp/6-311+G (d,p) level (Figure 4). On the basis of the matching of the experimental and computed ECD spectra, the absolute configuration of 1 was defined as 2R, 6R (Figure 1).
The molecular formula of asperfuranoid B (2) was established as C_{15}H_{20}O_{4} on the basis of the ion peak at m/z 263.12854 [M − H]− in the HRESIMS spectrum, accounting for six degrees of unsaturation. The \(^1\)H NMR data (Table 1) of 2 were similar to those of compound 1, except for the presence of a terminal olefinic methylene [\(\delta_H 5.39\, (d, J = 9.1\, \text{Hz})\), and 5.27 (d, J = 10.5 Hz)] and an olefinic proton resonating at \(\delta_H 6.13\) (H-7), and the absence of two aliphatic signals at \(\delta_H 1.87\) and 1.78 (H-2,7), and \(\delta_H 0.94\) (H-3,10), suggesting a double bond at \(\Delta^7\). This deduction was further corroborated by the cross-peaks of H-2,10/H-7/H-6 in the COSY spectrum and the HMBC correlations (Figure 2) from H-6 to C-7 (\(\delta_C 139.3\)) and C-10 (\(\delta_C 115.3\)), and from H-7 to C-5a (\(\delta_C 141.0\)). In addition, the CD curve and specific rotation of 2 was similar to the CD spectrum and specific rotation of 1 in MeOH (Figure 4), respectively. Therefore, the absolute configuration of compound 2 was also determined as 2R, 6R.

Asperfuranoid C (3) has a molecular formula of C_{15}H_{16}O_{4}, as determined by the HRESIMS and NMR data, requiring eight degrees of unsaturation. The 2D NMR data (Figure 2) provided the structure of 3 to be partially related to 1, possessing a benzofuran ring with the substitution of hydroxyisopropyl at C-2. These functionalities accounted for six sites of unsaturation. The remaining NMR resonances (Table 1) were attributed to two olefinic carbons (\(\delta_C 101.7\) and 160.7) for a double bond, a ketone group (\(\delta_C 199.9\)), and a methyl group (\(\delta_C 20.1\)), providing two additional sites of unsaturation. The HMBC correlations from the hydroxymethyl protons (H-2,9) at \(\delta_H 5.28\) and 5.22 to C-5a (\(\delta_C 142.6\)), C-7 (\(\delta_C 160.7\)), C-9a (\(\delta_C 108.9\)), and C-9b (\(\delta_C 167.9\)), from the olefinic proton H-6 (\(\delta_H 5.69\)) to C-5 (\(\delta_C 117.6\)), C-5a, and C-9a, and from the methyl protons at \(\delta_H 1.98\) (s, H3-10) to C-6 (\(\delta_C 101.7\)), and C-7 (Figure 2) allowed the establishment of a 7-methyl-9H-pyran ring between C-5a and C-9a, accounting for the remaining one site of unsaturation. Additionally, the HMBC correlations from the H-2 and H-4 to C-3 (\(\delta_C 199.9\)) (Figure 2) confirmed the presence of the ketone group, which was located at C-3a. Thus, the planar structure of 3 was confirmed and its absolute configuration was designated as 2S by experimental and theoretical ECD data (Figure 4).

![Figure 3. \(\Delta \delta = \delta_S - \delta_R\) values for (S)- and (R)-MTPA esters of 1.](image)

![Figure 4. Experimental electronic circular dichroism (ECD) spectra of 1–3 in MeOH and the calculated ECD spectra of 1 and 3 at the B3LYP/6-311 + G (d, p) level.](image)
Compounds 4 and 5 were obtained as pale yellow powders with the molecular formula of C_{16}H_{18}O_{4} based on HRESIMS data. Analysis of the NMR data (Table 2) led to the identification of 4 and 5 as penicisochroman A, a previously reported and structurally characterized compound isolated from *Penicillium* sp. [13]. The near zero optical rotation, congruent with the crystal data (centrosymmetric space group P1) which have been reported from our research [15], indicated a racemic mixture. The chiral HPLC separation of (+)-penicisochroman A was further performed on an Acchrom S-Chiral A column to yield a pair of enantiomers, (−)-penicisochroman A (4) and (+)-penicisochroman A (5), with the opposite Cotton effects and the opposite optical rotations. To determine the absolute configurations of (−)-4 and (+)-5, the ECD spectra of (−)-4 and (+)-5 were measured in MeOH and compared with the calculated ECD spectra of the enatiomers (Figure 5). Thus, the absolute configurations of (−)-4 and (+)-5 were determined as 7R and 7S (Figure 1), respectively.

**Table 2.** ¹H (500 MHz) and ¹³C (125 MHz) NMR data of compounds 4 and 5 in CDCl₃ (δ in ppm).

| No. | 4          | 5          |
|-----|------------|------------|
|     | δ_C, Type  | δ_H, mult (J/Hz) | δ_C, Type  | δ_H, mult (J/Hz) |
| 2   | 145.5, C   |              | 145.5, C   |              |
| 3   | 183.6, C   |              | 183.6, C   |              |
| 3a  | 121.3, C   |              | 121.3, C   |              |
| 4   | 122.1, CH  | 7.53, d (7.9)| 122.1, CH  | 7.53, d (7.9) |
| 5   | 123.2, CH  | 6.84, d (7.9)| 123.2, CH  | 6.94, d (7.9) |
| 5a  | 140.9, C   |              | 140.9, C   |              |
| 6   | 39.5, CH₂  | 3.00, d (16.2)| 39.4, CH₂  | 2.92, d (16.5) |
| 7   | 97.6, C    |              | 97.6, C    |              |
| 9   | 57.9, CH₂  | 4.93, d (15.6)| 57.9, CH₂  | 4.72, d (15.6) |
| 9a  | 118.4, C   |              | 118.4, C   |              |
| 9b  | 160.5, C   |              | 160.5, C   |              |
| 10  | 23.1, CH₃  | 1.53, s      | 23.1, CH₃  | 1.53, s      |
| 11  | 131.8, C   |              | 131.8, C   |              |
| 12  | 17.6, CH₃  | 2.36, s      | 17.6, CH₃  | 2.36, s      |
| 13  | 20.3, CH₃  | 2.09, s      | 20.3, CH₃  | 2.09, s      |
| 7-OCH₃| 49.2, CH₃ | 3.34, s  | 49.2, CH₃  | 3.34, s |

**Figure 5.** Experimental and calculated ECD spectra of 4 and 5.

Asperpanoid A (6) had a molecular formula of C₁₀H₁₄O₃, as determined by the HRESIMS and NMR data, requiring four degrees of unsaturation. The ¹H NMR data (Table 3) and HSQC spectrum (Supplementary material, Figure S49) provided the resonances for a methyl group [δ_H 0.91 (3H, t, J = 7.3 Hz)], two methylene signals [δ_H 2.50 (2H, m) and 1.51 (2H, m)], a hydroxymethyl group [δ_H 4.83 (2H, s)], and a 1,2,3,4-tetrasubstituted benzene [δ_H 6.66 and 6.51 (each 1H, d, J = 8.1 Hz)]. Analyses of the 1D and 2D NMR data (Figure 2) established the gross structure of 6 to be similar as a known
phenylpropanoid analogue, 2-(hydroxymethyl)-3-propylphenol (8) [16]. The signals at δH 6.66 (d, J = 8.1 Hz) and 6.51 (d, J = 8.1 Hz) indicated the presence of a 1,2,3,4-tetrasubstituted benzene system in 6, instead of the trisubstituted benzene in 8. The 13C NMR spectrum (Table 3) revealed that a deshielded aromatic nonprotonated carbon (δC 144.1, C-1) in 6 replaced an aromatic methine carbon (δC 114.4, C-1) in 8. These observations coupled with the MS data suggested that the position of the additional hydroxy group was located at C-1. The COSY correlation between H-2 and H-3, and the HMBC correlations from H-3 to C-1 and C-5 confirmed the above deductions. Thus, compound 6 was the 1-hydroxylated analogue of 8 (Figure 1).

Table 3. NMR spectroscopic data for compounds 6 and 7 (δ in ppm).

| No | δC, Type | δ_H, mult (J/Hz) | δC, Type | δ_H, mult (J/Hz) |
|----|----------|------------------|----------|------------------|
| 1  | 144.1, C | 111.0, CH        | 6.93, d  | 8.3              |
| 2  | 114.7, CH| 6.66, d (8.1)    | 129.7, CH| 7.25, t (7.9)    |
| 3  | 121.1, CH| 6.51, d (8.1)    | 122.8, CH| 6.74, d (7.6)    |
| 4  | 132.6, C | 139.0, C         |          |                  |
| 5  | 125.2, C | 127.7, CH        |          |                  |
| 6  | 145.3, C | 159.6, C         |          |                  |
| 7  | 35.2, CH₂| 2.50, m          | 130.2, CH| 6.79, d (11.5)   |
| 8  | 25.6, CH₂| 1.51, m          | 133.4, CH| 5.94, dt (6.7, 11.5) |
| 9  | 14.2, CH₃| 0.91, t (7.3)    | 59.5, CH₂| 4.10, dd (1.3, 6.8)|
| 10 | 59.2, CH₂| 4.83, s          | 56.6, CH₂| 4.64, s          |
| 6-OCH₃ | 56.1, CH₃ | 3.85, s        |          |                  |

Asperpanoid B (7) was assigned the molecular formula C_{11}H_{12}O₃ on the basis of HRESIMS data. The ¹H and ¹³C NMR data (Table 3) of 7 matched well with those for the known compound peniciphenol (9) and indicated the same structural features present in 9 except for the presence of methoxy group at C-6, which is consistent with the difference in the molecular formula [13]. Accordingly, the signal for the additional methoxy group at δH 3.85 and δC 56.1 were observed in the NMR spectra of 7. These observations coupled with the MS data indicate that the hydroxyl group at C-6 in 9 was replaced by a methoxy group in 7. The location of the methoxy group at C-6 was further confirmed by the HMBC correlation from the methoxy proton to C-6. The similar NOESY coupled patterns (Figure 2) and coupling constant between H-7 and H-8 (J₇,₈ = 11.5 Hz) to 9 assigned the same Z-Δ7-double bond in 7. Therefore, compound 7 was the methoxy derivative of 9 (Figure 1).

2.2. Biological Activity

The isolated compounds 1–14 were evaluated for their inhibitory activity against α-glucosidase in vitro, and acarbose was selected as the positive control (IC₅₀ = 453.3 µM) (Table 4). Compounds exhibiting inhibitory activity against α-glucosidase with values greater than 40% at 100 µM were further tested and IC₅₀ values calculated. Compound 6 was more potent than acarbose, with an IC₅₀ value of 12.4 µM. Additionally, the other compounds revealed weak or no inhibitory effects at a concentration of 100 µM.

Table 4. Inhibitory effects of compounds 1–14 against α-glucosidase.

| Compounds ⁴ | % Inhibition (100 µM) | IC₅₀ (µM) |
|-------------|-----------------------|-----------|
| 6           | 98                    | 12.4 ± 1.0|
| Acarbose ⁵  | 19                    | 453.3 ± 1.0|

⁴ Compounds 1–5 and 7–14 showed weak or no activity (IC₅₀ > 100 µM); ⁵ acarbose was tested as positive control.
All isolated compounds (1−14) were also tested for their antibacterial effects against four pathogenic bacteria (S. aureus, E. coli, B. subtilis, and P. aeruginosa) (Table 5). Compounds 8 and 11 showed activities against S. aureus, E. coli, and B. subtilis, with MIC values in the range of 4.15 to 12.5 µg/mL, while the other compounds exhibited weak or no antibacterial activities (MIC values > 100 µg/mL). None of the compounds were active against P. aeruginosa (MIC values > 100 µg/mL).

Table 5. Antibacterial activities of compounds 1−14.

| Compounds  | MIC (µg/mL) | S. aureus | E. coli | B. subtilis | P. aeruginosa |
|------------|-------------|-----------|---------|-------------|---------------|
| 8          | 4.15 ± 1.12 | 8.3 ± 1.0 | 8.3 ± 1.1 | >100        |
| 11         | 12.5 ± 1.1  | 12.5 ± 1.2| 12.5 ± 1.0| >100        |
| ciprofloxacin | 1.25 ± 1.10| 1.25 ± 1.12| 2.5 ± 1.1 | 2.5 ± 1.2   |

* Compounds 1−7, 9, 10, and 12−14 showed no activity (MIC > 100 µg/mL); * ciprofloxacin was tested as positive control.

3. Materials and Methods

3.1. General Experimental Procedures

The melting points were recorded on a SGW X-4B micro melting point apparatus (Shanghai Precision Scientific Instrument Co., Ltd, Shanghai, China) and were uncorrected. Optical rotations were determined using an MCP 300 polarimeter (Anton Paar, Graz, Austria) at 25 °C. UV data were recorded on a TU-1900 spectrophotometer (Persee, Beijing, China) in MeOH solution. ECD spectra were recorded using a Chirascan CD spectrometer (Applied Photophysics, London, UK). IR spectra were recorded on a Nicolet Nexus 670 spectrophotometer (Thermo Nicolet Corporation, Madison, WI, USA) in KBr discs. All NMR experiments were measured with Bruker Avance 500 spectrometers (500 and 125 MHz) (Bruker BioSpin, Switzerland), and the residual solvent peaks of CDCl$_3$ (δC 77.1/δH 7.26), acetone-d$_6$ (δC 29.8 and 206.3/δH 2.05), or methanol-d$_4$ (δC 49.0/δH 3.31) were used as references. HRESIMS data were acquired on a Thermo Fisher LTQ Orbitrap Elite high-resolution mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Column chromatography (CC) was carried by silica gel (200–300 mesh, Qingdao Marine Chemical Factory) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Stockholm, Sweden). Semipreparative HPLC was performed on a Primaide HPLC system (Hitachi Instrument Dalian Co., Ltd, Dalian, China) with an Ultimate XB-C18 column (10 × 250 mm, 10 µm).

3.2. Fungal Material

The fungus Aspergillus sp. ZJ-68 (Supplementary material, Figure S67) was isolated from fresh leaves of the mangrove plant Kandelia candel, which were collected in July 2016 from the Zhanjiang Mangrove Nature Reserve in Guangdong Province, China. The fungal strain was identified according to a molecular biology protocol by rDNA amplification and sequencing of the internal transcribed spacer (ITS) region [22]. A BLAST search result showed that it was most similar (99%) to the sequence of Aspergillus sp. (compared to JF312217.1). The sequence data have been submitted to GenBank with accession number MK629267. The isolate was stored on PDA medium (potato 200 g, dextrose 20 g, sea salt 3 g, and agar 15 g in 1.0 L of H$_2$O, pH 7.4–7.8) slants at 4 °C.

3.3. Fermentation

The fungus Aspergillus sp. ZJ-68 was cultured on PDA agar plate at 28 °C for 7 days. The fungal colony was further inoculated into the PDB medium (potato 200 g, dextrose 10 g, and sea salt 3 g in 1.0 L of H$_2$O, pH 7.4–7.8) at 28 °C for 3 days on a rotating shaker (140 rpm). Then, a large-scale fermentation of the strain was performed. The fungal seed broth (10 mL) was added to one hundred flasks (1000 mL), each containing 300 mL of liquid medium that was composed of potato 200 g, dextrose...
20 g, and sea salt 30 g in 1.0 L of H2O, pH 7.4–7.8. These flasks were incubated at 28 °C for 30 days under static conditions.

3.4. Extraction and Isolation

The whole fermentation broth (30 L) was filtered by cheesecloth to separate the mycelia from the filtrate. The mycelia were extracted three times by CH2Cl2, while the filtrate was extracted three times by the equivalent volume of EtOAc. The CH2Cl2 and EtOAc solutions were concentrated under reduced pressure to give an organic extract. This extract was fractionated by silica gel (200–300 mesh) column chromatography using a petroleum ether (PE, 60–90 °C) and EtOAc gradient system (from 1:0 to 0:1) to give 10 fractions (F1 to F10). Fraction F2 was applied to Sephadex LH-20 eluting with CH2Cl2–MeOH (1:1, v/v) and purified by semipreparative reversed-phase (RP) HPLC column (80% MeOH/H2O) to afford the mixture containing 4 and 5 (10.1 mg, tR: 20.6 min), 12 (31.4 mg, tR: 18.0 min), and 13 (23.4 mg, tR: 19.1 min). The chiral resolutions of 4 (3.6 mg) and 5 (4.1 mg) were performed on an Acchrom S-Chiral A column (10 × 250 mm, 5 μm) using hexanes–isopropyl alcohol (90:10, v/v) as the eluent (Supplementary material, Table S66). Fraction F3 was separated into two subfractions (F3a and F3b) by CC on silica gel eluting with a step gradient of PE/EtOAc (80:20 to 70:30, v/v). Compounds 3 (3.4 mg, tR: 20.1 min), 7 (6.8 mg, tR: 19.6 min), and 10 (8.9 mg, tR: 18.0 min) were obtained from the F3a which was subjected to RP-HPLC column (70% MeOH–H2O). F3b was submitted to silica gel CC (CH2Cl2–MeOH, 2:100, v/v) to yield 9 (4.5 mg), 11 (9.8 mg), and 14 (7.4 mg). Fraction F4 was fractionated by CC on silica gel into two subfractions (F4a and F4b) eluting with gradient CH2Cl2 and MeOH (2:100 to 3:100, v/v). Compounds 1 (10.8 mg, tR: 19.7 min) and 2 (5.3 mg, tR: 20.8 min) were purified by RP-HPLC column (70% MeOH–H2O) from the F4b. F4a was submitted to silica gel CC eluting with CH2Cl2–MeOH (3:100, v/v) and further purified by Sephadex LH-20 eluting with CH2Cl2–MeOH (1:1, v/v) to yield compounds 6 (6.8 mg) and 8 (5.9 mg).

Asperfuranoid A (1): Pale yellow oil; [α]D25 23.8 (c 0.03, MeOH); UV (MeOH) λmax (log ε) 205 (3.68), 290 (1.02) nm; ECD (0.15 mM, MeOH) λmax (Δε) 206 (−6.59), 234 (−2.11), 260 (−0.54), and 288 (+0.49) nm; IR (KBr) νmax 3340, 2956, 2867, 1752, 1592, 1446, 1253, 991, 786 cm−1; 1H and 13C NMR data, Table 1; HRESIMS m/z 289.14070 [M + Na]+ (calcd for C15H22NaO4, 289.14103).

Asperfuranoid B (2): Colorless oil; [α]D25 21.7 (c 0.02, MeOH); UV (MeOH) λmax (log ε) 206 (3.53), 291(1.05) nm; ECD (0.05 mM, MeOH) λmax (Δε) 202 (+1.83), 213 (−3.87), 237 (−1.63), 265 (−0.73), and 288 (+0.51) nm; IR (KBr) νmax 3340, 2956, 2867, 1752, 1592, 1446, 1253, 991 cm−1; 1H and 13C NMR data, Table 1; HRESIMS m/z 263.12854 [M − H]+ (calcd for C15H19O4, 263.12888).

Asperfuranoid C (3): Pale yellow oil; [α]D25 23.8 (c 0.5 MeOH); UV (MeOH) λmax (log ε) 215 (3.31), 280 (2.01), 352 (1.02) nm; ECD (0.14 mM, MeOH) λmax (Δε) 216 (−21.01), 227 (+3.01), 313 (+2.02), and 343 (−0.96) nm; IR (KBr) νmax 3423, 2927, 1710, 1604, 1440, 1376, 1274, 1170, 1072 cm−1; 1H and 13C NMR data, Table 1; HRESIMS m/z 259.09756 [M − H]+ (calcd for C15H15O3, 259.09758).

(−)-Penicisochroman A (4): Pale yellow powder; mp 171–172 °C; [α]D25 220° −111 (c 0.5 MeOH); UV (MeOH) λmax (log ε) 220 (3.23), 286 (2.98), 351 (1.02) nm; ECD (0.11 mM, MeOH) λmax (Δε) 231 (+2.34), 282 (+1.71), and 348 (−0.35) nm; IR (KBr) νmax 3417, 2921, 2850, 1712, 1602, 1437, 1375, 1265, 1093 cm−1; 1H and 13C NMR data, Table 2; HRESIMS m/z 275.12767 [M + H]+ (calcd for C16H19O4, 275.12779).

(+)-Penicisochroman A (5): Pale yellow powder; mp 170–171 °C; [α]D25 220° +105 (c 0.5 MeOH); UV (MeOH) λmax (log ε) 220 (3.23), 286 (2.98), 351 (1.02) nm; ECD (0.11 mM, MeOH) λmax (Δε) 233 (−2.62), 291 (−1.70), and 343 (+0.16) nm; IR (KBr) νmax 3403, 2927, 2856, 1712, 1610, 1436, 1376, 1268, 1085 cm−1; 1H and 13C NMR data, Table 2; HRESIMS m/z 275.12770 [M + H]+ (calcd for C16H19O4, 275.12779).

Asperpanoid A (6): Colorless oil; UV (MeOH) λmax (log ε) 224 (2.36), 281 (2.01) nm; IR (KBr) νmax 3340, 2956, 2925, 2867, 1585, 1461, 1253, 987, 786 cm−1; 1H and 13C NMR data, Table 3; HRESIMS m/z 181.08690 [M − H]+ (calcd for C10H13O3, 181.08702).
Acarbose was used as the positive control and all assays were performed in three replicates. Asperpanoid B (R.C. performed the experiments for the isolation, structure elucidation, inhibitory activity)

Author Contributions:

ff

The absolute configurations of 1 were unambiguously established by a combination of Mosher’s method, and experimental and calculated ECD data. Compounds 4 and 5 were a pair of enantiomers and their absolute configurations were established as 7R and 7S for the first time. This study further expanded the structural diversity of naturally occurring benzofuranoid and phenylpropanoid derivatives. In the bioactivity assays, compound 6 exhibited potent inhibitory activity against α-glucosidase with an IC₅₀ value of 12.4 μM, and compounds 8 and 11 showed significant activities against S. aureus, E. coli, and B. subtilis with MIC values in the range of 4.15 to 12.5 μg/mL. Compounds 6, 8, and 11 may be considered as potential new drug leads.

3.5. Inhibitory Activity Against α-Glucosidase

α-Glucosidase inhibitory activity was assessed in 96-well plates using 0.01 M KH₂PO₄–K₂HPO₄ (pH 7.0) buffer solution, and the enzyme solutions were prepared to give 2.0 units/mL in buffers. The assay was conducted in the 200 µL reaction system which contains 168 µL of buffers, 10 µL of diluted enzyme solution, and 2 µL of dimethyl sulfoxide (DMSO) or sample (dissolved in DMSO). The detailed methodology for biological testing has already been described in a previous report [12]. Acarbose was used as the positive control and all assays were performed in three replicates.

3.6. Antibacterial Assay

Two Gram-positive bacteria B. subtilis (ATCC 6633) and S. aureus (ATCC 25923), and two Gram-negative bacteria E. coli (ATCC 25922) and P. aeruginosa (ATCC 27853) were used. The antibacterial assay and the determination of the MIC were assessed according to continuous dilution method in 96-well plates which has been described in our published paper [23]. The tested substances were dissolved in DMSO and ciprofloxacin was the positive control.

4. Conclusions

In summary, three new benzofuranoids, asperfuranoids A–C (1–3), and two new phenylpropanoid derivatives (6 and 7) were isolated from the mangrove endopytic fungus Aspergillus sp. ZJ-68. The absolute configurations of 1–5 were unambiguously established by a combination of Mosher’s method, and experimental and calculated ECD data. Compounds 4 and 5 were a pair of enantiomers and their absolute configurations were established as 7R and 7S for the first time. This study further expanded the structural diversity of naturally occurring benzofuranoid and phenylpropanoid derivatives. In the bioactivity assays, compound 6 exhibited potent inhibitory activity against α-glucosidase with an IC₅₀ value of 12.4 μM, and compounds 8 and 11 showed significant activities against S. aureus, E. coli, and B. subtilis with MIC values in the range of 4.15 to 12.5 μg/mL. Compounds 6, 8, and 11 may be considered as potential new drug leads.

Supplementary Materials: The following are available online at http://www.mdpi.com/1660-3397/17/8/478/s1. HREIMS, NMR, IR, UV of the new compounds 1–3, 6, and 7; ECD calculation details (Methods and Results) for compounds 1 and 3–5; preparation of MTPA esters of 1 by the modified Mosher’s method.

Author Contributions: R.C. performed the experiments for the isolation, structure elucidation, inhibitory activity against α-glucosidase, and antibacterial evaluation, and prepared the manuscript; H.J. and Z.Z. performed the 1D and 2D NMR experiments; C.L. supervised the research and contributed to part of the structure determination; Z.S. supervised the research work and revised the manuscript.

Funding: We thank the Guangdong Special Fund for Marine Economic Development (GDME-2018C004), Guangdong MEPF Fund (GDOE-2019A21), the National Natural Science Foundation of China (2187713, 21472251), the Key Project of Natural Science Foundation of Guangdong Province (2016A040403091), the Special Promotion Program for Guangdong Provincial Ocean and Fishery Technology (A201701C06) for their generous support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wu, J.; Xiao, Q.; Xu, J.; Li, M.Y.; Pan, J.Y.; Yang, M.H. Natural products from true mangrove flora: Source, chemistry and bioactivities. Nat. Prod. Rep. 2008, 25, 955–981. [CrossRef] [PubMed]

2. Thatoi, H.; Behera, B.C.; Mishra, R.R.; Dutta, S.K. Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: A review. Ann. Microbiol. 2013, 63, 1–19. [CrossRef]

3. Kusari, S.; Spiteller, M. Are we ready for industrial production of bioactive plant secondary metabolites utilizing endophytes. Nat. Prod. Rep. 2011, 28, 1203–1207. [CrossRef] [PubMed]
4. Aly, A.H.; Debbab, A.; Proksch, P. Fungal endophytes: unique plant inhabitants with great promises. Appl. Microbiol. Biotechnol. 2011, 90, 1829–1845. [CrossRef] [PubMed]

5. Ebrahim, W.; El-Neketi, M.; Lewald, L.I.; Orfali, R.S.; Lin, W.; Rehberg, N.; Kalscheuer, R.; Daletos, G.; Proksch, P. Metabolites from the fungal endophyte Aspergillus australasicanus in axenic culture and in fungal–bacterial mixed cultures. J. Nat. Prod. 2016, 79, 914–922. [CrossRef] [PubMed]

6. Frisvad, J.C.; Larsen, T.O. Extrolites of Aspergillus fumigatus and other pathogenic species in Aspergillus section Fumigati. Front. Microbiol. 2016, 6, 1485. [CrossRef] [PubMed]

7. Lubertozzi, D.; Keasling, J.D. Developing Aspergillus as a host for heterologous expression. Biotechnol. Adv. 2009, 27, 53–75. [CrossRef] [PubMed]

8. Novak, N.; Gerdin, S.; Berovic, M. Increased lovastatin formation by Aspergillus terreus using repeated fed-batch process. Biotechnol. Lett. 1997, 19, 947–948. [CrossRef]

9. Chen, S.; Chen, D.; Cai, R.; Cui, H.; Long, Y.; Lu, Y.; Li, C.; She, Z. Cytotoxic and antibacterial preussomerins from the mangrove endophytic fungus Lasiodiplodia theobromae ZJ-HQ1. J. Nat. Prod. 2016, 79, 2397–2402. [CrossRef]

10. Cui, H.; Liu, Y.; Nie, Y.; Liu, Z.; Chen, S.; Zhang, Z.; Lu, Y.; He, L.; Huang, X.; She, Z. Polycyketides from the mangrove-derived endophytic fungus Nectria sp. NN001 and their α-glucosidase inhibitory activity. Mar. Drugs 2016, 14, 86. [CrossRef] [PubMed]

11. Cui, H.; Lin, Y.; Luo, M.; Lu, Y.; Huang, X.; She, Z. Diaporisoindoles A–C: three isoprenylisoindole alkaloid derivatives from the mangrove endophytic fungus Diaiporthe sp. SYSU-HQ3. Org. Lett. 2017, 19, 5621–5624. [CrossRef] [PubMed]

12. Cai, R.; Wu, Y.; Chen, S.; Cui, H.; Liu, Z.; Li, C.; She, Z. Penicisoumarins A–J: isocoumarins from the endophytic fungus Penicillium sp. HNY29-2B. Phomopsis sp. 085242. Beilstein J. Org. Chem. 2016, 12, 1761–1767. [CrossRef]

13. Bunbamrung, N.; Intaraudom, C.; Boonyuen, N.; Rachtawee, P.; Laksanacharoen, P.; Pittayakhajonwut, P. α-Pyrone from the mangrove endophytic fungus Aspergillus pseudodeflectus. J. Nat. Prod. 2010, 73, 479–480. [CrossRef] [PubMed]

14. Kuramochi, K.; Tsubaki, K. Synthesis and structural characterization of natural benzofuranoids. J. Nat. Prod. 2015, 78, 1056–1066. [CrossRef]

15. Xiao, Z.; Chen, S.; Cai, R.; Lin, S.; Hong, K.; She, Z. New furoisocoumarins and isocoumarins from the mangrove endophytic fungus Aspergillus sp. 085242. Beilstein J. Org. Chem. 2016, 12, 2077–2085. [CrossRef] [PubMed]

16. Weber, D.; Gorzalczany, S.; Martino, V.; Acevedo, C.; Sterner, O.; Anke, T. Metabolites from endophytes of the medicinal plant Erythrina crista-galli. Z. Naturforsch. C 2005, 60, 467–477. [CrossRef]

17. Ahyun, M.; Noda, T.; Hase, Y.; Nishimura, Y.; Oda, K.; Ueda, Y.; Ichikawa, M.; Takahashi, S.; Terakawa, K.; Nakamura, T. α-Pyrones from the mangrove endophytic fungus Aspergillus fumigatus. Phytochem. Lett. 2014, 10, 13–18. [CrossRef]

18. Ayer, W.A.; Pena-Rodriguez, L.M. Metabolites produced by Alternaria brassicae, the black spot pathogen of canola. part 2, sesquiterpenoid metabolites. J. Nat. Prod. 1987, 50, 408–417. [CrossRef]

19. Ogawa, A.; Murakami, C.; Kamisuki, S.; Kuriyama, I.; Yoshida, H.; Sugawara, F.; Mizushima, Y. Pseudodeflectucin, a novel isocoumarin derivative from Aspergillus pseudodeflectus a parasite of the sea weed, Sargassum fusiform, as a selective human cancer cytotoxin. Bioorg. Med. Chem. Lett. 2004, 14, 3539–3543. [CrossRef]

20. Lu, Z.; Wang, Y.; Miao, C.; Liu, P.; Hong, K.; Zhu, W. Sesquiterpenoids and benzofuranoids from the marine-derived fungus Aspergillus ustus 094102. J. Nat. Prod. 2009, 72, 1761–1767. [CrossRef]

21. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. High-field FT NMR application of Mosher’s method. The absolute configurations of marine terpenoids. J. Am. Chem. Soc. 1991, 113, 4092–4096. [CrossRef]

22. Kjer, J.; Debbab, A.; Aly, A.H.; Proksch, P. Methods for isolation of marine-derived endophytic fungi and their bioactive secondary products. Nat. Protoc. 2010, 5, 479–490. [CrossRef] [PubMed]

23. Cai, R.; Chen, S.; Liu, Z.; Tan, C.; Huang, X.; She, Z. A new α-pyrene from the mangrove endophytic fungus Phomopsis sp. HNY29-2B. Nat. Prod. Res. 2017, 31, 124–130. [CrossRef]