Secondary Metabolites with α-Glucosidase Inhibitory Activity from the Mangrove Fungus *Mycosphaerella* sp. SYSU-DZG01

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Abstract: Four new metabolites, asperchalasine I (1), dibefurin B (2) and two epicoccine derivatives (3 and 4), together with seven known compounds (5–11) were isolated from a mangrove fungus *Mycosphaerella* sp. SYSU-DZG01. The structures of compounds 1–4 were established from extensive spectroscopic data and HRESIMS analysis. The absolute configuration of 1 was deduced by comparison of ECD data with that of a known structure. The stereostructures of 2–4 were further confirmed by single-crystal X-ray diffraction. Compounds 1, 8 and 9 exhibited significant α-glucosidase inhibitory activity with IC₅₀ values of 17.1, 26.7 and 15.7 µM, respectively. Compounds 1, 4, 6 and 8 showed antioxidant activity by scavenging DPPH with EC₅₀ values ranging from 16.3 to 85.8 µM.

Keywords: secondary metabolites; *Mycosphaerella* sp.; asperchalasine; α-glucosidase

1. Introduction

Diabetes mellitus (DM), a chronic metabolic disorder disease, is caused by the lack of insulin secretion (type I diabetes mellitus) or insufficient insulin sensitivity (type II diabetes mellitus) [1,2], and the typical characteristic of the latter is post-prandial hyperglycemia. α-Glucosidase is a kind of membrane-bounded enzyme which is mainly found in intestinal epithelium cells and leads to the increase of blood glucose levels by hydrolyzing the glycosidic bonds of a polysaccharide [3–5]. As a result of that, α-Glucosidase inhibitors (AGIs), such as acarbose, miglitol and voglibose have become a widespread medical treatment in type II diabetes mellitus according to their glycemic control ability [6,7]. Nevertheless, existing AGIs often cause many side effects including abdominal pain, flatulence, diarrhea and other gastrointestinal disorders [8,9]. Hence, many natural medicine chemists were attracted to develop α-glucosidase inhibitors with lower toxicity and side effects for potential use. Some new α-glucosidase inhibitors have been researched like flavipesolides A–C [10], asperteretal E [11] and so on [12,13], and the discovery of better α-glucosidase inhibitors is still an urgent need.

Marine fungi are proved to be rich sources of structurally unique and bioactive secondary metabolites. *Mycosphaerella* sp., which contributes the largest genus of Ascomycota, is a common plant pathogen widely distributed in terrestrial plant and marine environment [14–16]. As part of our ongoing investigation on new secondary metabolites from marine fungi in the South...
2. Results

2.1. Structure Elucidation

Asperchalasine I (1), the molecular formula, C_{33}H_{41}O_{7}N, was determined on the basis of the HRESIMS ion at m/z 562.2798 ([M – H]^{-} calcd. for C_{33}H_{40}O_{7}N: 562.2799). As shown in Table 1, the 1H NMR data indicated characteristic signals of two double-bond protons (δH 5.93 and 5.36), nine methine protons (δH 5.52, 5.06, 4.46, 3.76, 3.21, 3.05, 2.88, 2.54 and 1.62), four methylene protons (δH 2.09, 1.90, δH 2.01, 1.58, δH 1.53 and δH 1.21) and six methyl groups (δH 2.05, 1.78, 1.26, 1.17, 0.96 and 0.94). Subsequently, the 13C NMR data showed the presence of 33 carbon signals, according to the DEPT and HSQC data, which were identified as three carbonyls (δC 211.6, 203.6 and 176.3), an aromatic ring (δC 141.3, 133.7, 132.6, 132.5, 123.7 and 111.9), two trisubstituted double bonds (δC 141.8, 137.4, 125.8 and
125.1), nine methines, four methylenes, six methyls and a quaternary carbon (δC 67.3). The 1H-1H COSY (Figure 2) correlations of H_3-23/H_2-22/H_2-10/H_3-4/H_5/H_2-11, H-7/H-8/H-13, H-21/H_2-16/H_2-17 and H-19/H-20, together with the HMBC correlations from H-4 to C-1, C-9 and C-21, from H_3-12 to C-5, C-6 and C-7, from H-13 to C-25, from H_3-25 to C-15, from H_2-17 and H-19 to C-18, and from H-20 to C-21, suggested the presence of an cytochalasan moiety. Meanwhile, the epicoccine moiety was inferred by HMBC correlations from H-1' to C-3', from H-8' to C-2' and from H_3-9' to C-5', C-6' and C-7'. The key linking relation of the cytochalasan moiety and epicoccine moiety through C-19/C-8' and C-20/C-1' C-C bonds was suggested from the 1H-1H COSY cross-peak of H-19 (δH 3.70, δC 61.0), suggesting the replacements of methine at C-17 and a methoxy group at C-4' in asperchalasine B with a methylene and hydroxy group in 1, respectively.

Table 1. 1H (500 MHz) and 13C (125 MHz) NMR data for 1 in CDCl₃.

| Position | 1H δ (in Hz) | 13C δ (in Hz) |
|----------|--------------|---------------|
| 1        | 176.3, C     | 176.3, C      |
| 2        | 51.9, CH     | 51.9, CH      |
| 3        | 3.21, m      | 3.21, m       |
| 4        | 3.05, dd (3.9, 5.0) | 3.05, dd (3.9, 5.0) |
| 5        | 2.54, m      | 2.54, m       |
| 6        | 141.8, C     | 141.8, C      |
| 7        | 5.36, s      | 5.36, s       |
| 8        | 2.88, d (11.1)| 2.88, d (11.1) |
| 9        | 43.3, CH     | 43.3, CH      |
| 10       | 67.3, C      | 67.3, C       |
| 11       | 48.5, CH₂    | 48.5, CH₂     |
| 12       | 13.6, CH₃    | 13.6, CH₃     |
| 13       | 5.93, d (11.0)| 5.93, d (11.0) |
| 14       | 125.8, CH    | 125.8, CH     |
| 15       | 40.7, CH₂    | 40.7, CH₂     |
| 16       | 190, d (4.3) | 190, d (4.3)  |
| 17       | 22.5, CH₂    | 22.5, CH₂     |
| 18       | 81.3, CH     | 81.3, CH      |
| 19       | 11.9, CH     | 11.9, CH      |
| 20       | 35.1, CH₂    | 35.1, CH₂     |
| 21       | 37.3, CH     | 37.3, CH      |
| 22       | 203.6, C     | 203.6, C      |
| 23       | 253, CH      | 253, CH       |
| 24       | 23.6, CH₃    | 23.6, CH₃     |
| 25       | 121.6, C     | 121.6, C      |
| 1'       | 5.06, s      | 5.06, s       |
| 2'       | 123.7, C     | 123.7, C      |
| 3'       | 132.5, C     | 132.5, C      |
| 4'       | 133.7, C     | 133.7, C      |
| 5'       | 141.3, C     | 141.3, C      |
| 6'       | 111.9, C     | 111.9, C      |
| 7'       | 132.6, C     | 132.6, C      |
| 8'       | 81.3, CH     | 81.3, CH      |
| 9'       | 11.9, CH₃    | 11.9, CH₃     |
The electron circular dichroism (ECD) spectrum (Figure S8) showed two positive Cotton effects (CE) at 228 nm and 306 nm, which were also consistent with those of asperchalasine B. Therefore, the absolute configuration of 1 was suggested to be 3S, 4R, 5S, 8S, 9S, 19S, 20S, 1’S, 8’R.

Dibefurin B (2), a colorless monocrystal, was assigned a molecular formula of C_{18}H_{20}O_{6} by the HRESIMS ion at m/z 331.1187 ([M – H]^− calcld. for C_{18}H_{19}O_{6}: 331.1187). For the monomer, the $^1$H, $^13$C NMR (Table 2) and HSQC data of 2 revealed diagnostic signals for nine carbons, including two carbyls (δ_{C} 200.6 and 192.6), two disubstituted olefin carbons (δ_{C} 157.6 and 131.4), an oxygen-bearing carbon [δ_{H} 6.67 (OH-6), δ_{C} 91.7 (C-6)], three methyls (δ_{C} 19.1, 12.2 and 12.1) and a quaternary carbon (δ_{C} 60.4). The HMBC correlations from H_{3}-7 to C-1, C-2, C-3 and C-6’, from H_{3}-8 to C-2, C-3 and C-4, from H_{3}-9 to C-3, C-4 and C-5, revealed that Me-7, Me-8 and Me-9 were connected at C-2, C-3 and C-4, respectively. Similarly, the hydroxyl was attached to C-6, as evidenced by the HMBC correlations from OH-6 to C-1, C-2’, C-5 and C-6. In view of the HRESIMS data and X-ray (Figure 4), it could confirm that compound 2 was a symmetric dimer and the absolute configuration of 2 was assigned as 2S, 6R, 2′R, 6′S.

Figure 2. The key 2D NMR correlations of 1–4.

Figure 3. Molecular model of 1 (1’β, 8’β-oxygen bridge).
that compound 2 was a symmetric dimer and the absolute configuration of 2 was assigned as 2S, 6R, 2′R, 6′S.

Table 2. 1H (400 MHz) and 13C (100 MHz) NMR data for 2 in DMSO-d6.

| Position | δH, mult (J in Hz) | δC, Type |
|----------|---------------------|-----------|
| 1 (1′)   | 200.6, C            |           |
| 2 (2′)   | 60.4, C             |           |
| 3 (3′)   | 157.6, C            |           |
| 4 (4′)   | 131.4, C            |           |
| 5 (5′)   | 192.6, C            |           |
| 6 (6′)   | 91.7, C             |           |
| 7 (7′)   | 1.21, s             | 12.1, CH3 |
| 8 (8′)   | 2.02, s             | 19.1, CH3 |
| 9 (9′)   | 1.73, s             | 12.2, CH3 |

Figure 4. Single-crystal X-ray structures of 2–4.

Compound 3 was purified as a colorless crystal whose molecular formula was deduced as C_{10}H_{12}O_{3} based on HRESIMS data (179.0716 [M – H]−, calcd. 179.0714). Analysis of the 1H NMR spectrum of 3 (Table 3) displayed three aromatic proton resonances (δH 7.13, 6.82, and 6.69), an oxygenated methylene (δH a 5.12, δH b 5.02), two oxygenated methines (δC 5.06 and 3.97), and a methyl (δH 1.20). The 13C spectrum revealed 10 signals, indicating an aromatic ring, a methylene, two methines and a methyl. In the 1H-1H COSY spectrum, the ortho-trisubstitution on the aromatic ring was confirmed by the cross-peaks of H-4/H-5/H-6. Moreover, the 1H-1H COSY spectrum showed correlations from H-10 to H-9 and H-11, and the chemical shift of C-10 (δC 70.6) showed the hydroxyl was attached to C-10. Subsequently, the HMBC correlations between H-6 and C-1/C-2 determined the linkage of 1-OH to C-1, and the correlations between H-7 and C-1/C-2/C-3, H-9 and C-2/C-3/C-10 established the presence of a phthalan ring. The same relative configuration of C-9 and C-10 was clearly deduced under the guidance of single-crystal X-ray (Figure 4). Hence, the absolute configuration of 3 was determined as 9R, 10R.

Compound 4 was deduced to have a molecular formula of C_{10}H_{10}O_{6} from its HRESIMS spectrum with a deprotonated molecular ion at m/z 225.0407. The 1H NMR (Table 3) in MeOH-d4 showed three singlets at δH 9.71, 3.90, and 2.08, according to the 13C NMR and HSQC data, which were attributed to an aldehyde group (δC 194.7), a methoxy group (δC 52.9) and a methyl (δC 12.4), respectively. In addition, resonances of a carbonyl and an aromatic ring were observed in the 13C NMR data. In the HMBC spectrum, the correlations of H-9 to C-2, C-3 and C-4 supported the connection of Me-9 to C-3, the correlations of H-7 to C-1 and C-6 indicated the linkage of aldehyde group and C-1. Meanwhile, the carbonyl (δC 169.9, C-8) had the HMBC correlation from H-10 (δH 3.90), further indicated the presence of methyl ester. With the assistance of single-crystal X-ray (Figure 4), the structure of compound 4 was clearly confirmed.
Table 3. $^1$H and $^{13}$C NMR data for 3 and 4 in MeOH-$d_4$.

| Position | $^1$H, mult (J in Hz) | $^1$C, Type | $^1$H, mult (J in Hz) | $^1$C, Type |
|----------|-----------------------|-------------|-----------------------|-------------|
| 1        | 152.8, C              |             | 112.2, C              |             |
| 2        | 127.3, C              |             | 117.4, C              |             |
| 3        | 142.1, C              |             | 129.9, C              |             |
| 4        | 6.82, d (7.4)         | 114.3, CH   | 152.5, C              |             |
| 5        | 7.13, t (7.7)         | 129.9, CH   | 134.1, C              |             |
| 6        | 6.69, d (7.9)         | 115.0, CH   | 151.2, C              |             |
| 7        | 5.12, dd (2.8, 11.8)  | 72.4, CH$_2$| 9.71, s               | 194.7, CH   |
|          | 5.02, d (11.9)        |             |                      |             |
| 8        |                      |             | 169.9, C              |             |
| 9        | 5.06, d (3.3)         | 89.4, CH    | 2.08, s               | 12.4, CH$_3$|
| 10       | 3.97, qd (3.9, 6.4)   | 70.6, CH    | 3.90, s               | 52.9, CH$_3$|
| 11       | 1.20, d (6.4)         | 18.6, CH$_3$|                      |             |

$^a$ $^1$H and $^{13}$C NMR recorded at 500 MHz and 125 MHz; $^b$ $^1$H and $^{13}$C NMR recorded at 400 MHz and 100 MHz.

2.2. Biological Evaluation

Compounds 1–11 were tested for their inhibitory effects against α-glucosidase, and antioxidant activity. As seen in Table 4, the results indicated that compounds 1, 8 and 9 showed significant inhibitory effects against α-glucosidase with IC$_{50}$ values of 17.1, 26.7 and 15.7 µM, respectively, which were better than the positive controls acarbose (610.2 µM) and 1-deoxynojirimycin (71.5 µM). Beyond that, all of the compounds were tested for their antioxidant activity based on DPPH· (2, 2-diphenyl-1-picrylhydrazyl radical) scavenging. The results showed the antioxidant activity of 8 was 89% at the concentration of 100 µM and compound 8 possessed more potent capacity than positive control ascorbic acid in scavenging DPPH· with an EC$_{50}$ value of 16.3 µM. Compounds 1, 4 and 6 also exhibited weak DPPH-scavenging activity with respective EC$_{50}$ values of 77.8, 85.8 and 59.1 µM.

Table 4. The α-glucosidase inhibitory and antioxidant activities of compounds 1–11.

| Compounds       | α-Glucosidase Inhibitory | Antioxidant |
|-----------------|--------------------------|-------------|
|                 | IC$_{50}$ (µM) | % Inhibition (100 µM) | EC$_{50}$ (µM) |
| 1               | 17.1          | 56            | 77.8          |
| 2               | >50           | <50           | -             |
| 3               | >50           | <50           | -             |
| 4               | >50           | 57            | 85.8          |
| 5               | >50           | <50           | -             |
| 6               | >50           | 65            | 59.1          |
| 7               | >50           | <50           | -             |
| 8               | 26.7          | 89            | 16.3          |
| 9               | 15.7          | <50           | -             |
| 10              | -             | -             | -             |
| 11              | >50           | <50           | -             |
| Acarbose        | 610.2         | -             | -             |
| 1-Deoxynojirimycin| 71.5         | -             | -             |
| Ascorbic acid   | 92            | 22.4          | -             |

- means no test; $^a$ positive control.

3. Experimental Section

3.1. General Experimental Procedures

UV data were measured on a UV-Vis-NIR spectrophotometer (Perkin Elmer, Waltham, UK). IR spectrum data were recorded using a Bruker Vector spectrophotometer 22. Melting points...
were tested on a Fisher-Johns hot-stage apparatus which were uncorrected. Optical rotations were recorded using an MCP300 (Anton Paar, Shanghai, China). HRESIMS data were conducted on an Ion Mobility-Q-TOF High-Resolution LC-MS (Synapt G2-Si, Waters). The ECD experiment data were measured with J-810 spectropolarimeter (JASCO, Tokyo, Japan). The NMR spectra were recorded on Bruker Avance spectrometer (Bruker, Beijing, China) (Compounds 1 and 3: 500 MHz for $^1$H and 125 MHz for $^{13}$C, respectively; compounds 2 and 4: 400 MHz for $^1$H and 100 MHz for $^{13}$C). Column chromatography (CC) was carried out on silica gel (200–300 mesh, Marine Chemical Factory, Qingdao, China) and sephadex LH-20 (Amersham Pharmacia, Piscataway, NJ, USA).

3.2. Fungal Materials

The fungus used in this research was isolated from the fruit of the marine mangrove plant *Bruguiera* collected in 2014 in Hainan Dongzhai Harbor Mangrove Reserve by using the standard protocol. The strain was identified as *Mycosphaerella* sp. (compared to no. KX067865.1) upon the analysis of ITS sequence data of the rDNA gene. The ITS sequence data obtained from the fungal strain has been submitted to GenBank with accession no. MN194208. A voucher strain was deposited in our laboratory.

3.3. Fermentation, Extraction and Isolation

The fungus *Mycosphaerella* sp. SYSU-DZG01 was grown on solid cultured medium in 100 × 1000 mL Erlenmeyer flasks at room temperature for 30 days under static conditions, each containing 80 g rice and 120 mL 0.3% saline. After incubation, the former was extracted with methanol twice and concentrated to yield 10.9 g of crude extract under reduced pressure. The crude extract was subjected to LC-HRESIMS analysis (Figure S29). Then, the residue was eluted by using gradient elution with petroleum ether/EtOAc from 9:1 to 0:10 (v/v) on silica gel CC to get ten fractions (Fr.1–Fr.10). Fr.2 (630 mg) was further eluted by silica gel CC using CH$_2$Cl$_2$/MeOH (40:1) to obtain Fr.2.1–Fr.2.3. Fr.2.3 (301 mg) was purified by Sephadex LH-20 CC and eluted with MeOH to obtain compound 1 (3.5 mg), 6 (11.1 mg) and 10 (3.6 mg). Fr.4 (217 mg) was applied to silica gel CC using CH$_2$Cl$_2$/MeOH (20:1) to obtain Fr.4.1–Fr.4.7. Fr.4.1 (8.1 mg) was further purified by Sephadex LH-20 CC using MeOH to obtain compound 1 (2.3 mg), 3 (2.2 mg), 8 (20.8 mg) and 9 (2.7 mg). Fr.5 (817 mg) was eluted (by CH$_2$Cl$_2$/MeOH, 25:3) to obtain Fr.5.1–Fr.5.5. Fr.5.1 (13.3 mg), Fr.5.2 (27.7 mg) and Fr.5.4 (10.9 mg) was purified by Sephadex LH-20 CC using CH$_2$Cl$_2$/MeOH (1:1) to yield compound 4 (4.3 mg), 5 (2.0 mg), 7 (3.7 mg) and 11 (2.7 mg).

Asperchalasine I (1): White powder; [α]$_D^{25}$ = +61.4 (c 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 206 (4.53) nm; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3369, 1747, 1664, 1645, 1238, 1037; HRESIMS m/z 562.2798 [M − H]$^-$ (calcd. for C$_{33}$H$_{40}$O$_7$N: 562.2799); $^1$H and $^{13}$C NMR data: see Table 1.

Dibefurin B(2): Colorless crystal; m.p. 67.8–69.2 °C; [α]$_D^{25}$ = +0.3 (c 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 237 (3.98) nm; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3448, 1747, 1664, 1645, 1238, 1037; HRESIMS m/z 331.1187 [M − H]$^-$ (calcd. for C$_{18}$H$_{19}$O$_6$: 331.1187); $^1$H and $^{13}$C NMR data: see Table 2.

Compound 3: Colorless crystal; m.p. 89.8–91.9 °C; [α]$_D^{25}$ = −37.1 (c 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 204 (4.39), 269 (3.24) nm; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3261, 2887, 1601, 1471, 1297, 767, 706; HRESIMS m/z 179.0716 [M − H]$^-$ (calcd. for C$_{10}$H$_{12}$O$_3$: 179.0714); $^1$H and $^{13}$C NMR data: see Table 2.

Compound 4: Colorless crystal; m.p. 95.9–97.8 °C; [α]$_D^{25}$ = +3.4 (c 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 241 (3.66), 305 (3.56) nm; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3375, 2962, 1711, 1641, 1261, 1150, 933; HRESIMS m/z 225.0407 [M − H]$^-$ (calcd. for C$_{10}$H$_{10}$O$_6$: 225.0407); $^1$H and $^{13}$C NMR data: see Table 2.
3.4. X-Ray Crystallographic Data

Colorless crystals of compounds 2–4 were obtained from MeOH-CH₂Cl₂ at room temperature by slow volatilization, and examined on an Agilent Xcalibur Nova single crystal diffractometer with Cu Kα radiation.

The crystallographic data for compound 2 has been deposited in the Cambridge Crystallographic Data Centre (CCDC number: 18022803)

Crystal data of 2: C₁₈H₂₆Cl₂O₆, Mr = 332.34, triclinic, a = 6.9740(4) Å, b = 7.9800(4) Å, c = 14.3659(6) Å, α = 101.148(4)°, β = 99.209(4)°, γ = 98.479(5)°, V = 761.05(7) Å³; space group P-1, Z = 2, Dc = 1.450 g/cm³, μ = 0.908 mm⁻¹ and F(000) = 352.0; Crystal dimensions: 0.40 × 0.30 × 0.02 mm³. Independent reflections: 4998 (Rint = 0.0269). The final R₁ was 0.0522, wR₂ = 0.1447 [l > 2σ (I)]. The goodness of fit on F² was 1.048.

The crystallographic data for compound 3 has been deposited in the Cambridge Crystallographic Data Centre (CCDC number: 1812103)

Crystal data of 3: C₁₀H₁₂O₃, Mr = 180.07, monoclinic, a = 4.7697(1) Å, b = 11.1895(3) Å, c = 9.1541(3) Å, α = 90°, β = 93.829(3)°, γ = 90°, V = 487.47(2) Å³; space group P2₁, flack 0.14(18), Z = 2, Dc = 1.350 g/cm³, μ = 0.872 mm⁻¹ and F(000) = 212.0; Crystal dimensions: 0.40 × 0.10 × 0.05 mm³. Independent reflections: 7438 (Rint = 0.0620). The final R₁ was 0.0445, wR₂ = 0.1258 [l > 2σ (I)]. The goodness of fit on F² was 1.046.

The crystallographic data for compound 4 has been deposited in the Cambridge Crystallographic Data Centre (CCDC number: 18120705)

Crystal data of 4: C₁₀H₁₄O₆, Mr = 226.04, orthorhombic, a = 15.9208(7) Å, b = 6.6849(3) Å, c = 18.5162(7) Å, α = 90°, β = 90°, γ = 90°, V = 1970.66(14) Å³; space group Pbcn, Z = 8, Dc = 1.525 g/cm³, μ = 1.108 mm⁻¹ and F(000) = 944.0; Crystal dimensions: 0.25 × 0.03 × 0.03 mm³. Independent reflections: 3833 (Rint = 0.0496). The final R₁ was 0.0485, wR₂ = 0.1328 [l > 2σ (I)]. The goodness of fit on F² was 1.050.

3.5. Biological Assays

3.5.1. Inhibitory Activity of α-Glucosidase

The α-glucosidase inhibitory activity was assayed according to the reported method [29]. The inhibitory activity of α-glucosidase was tested in the 96-well plated with 100 mm PBS (KH₂PO₄-K₂HPO₄, pH 7.0) buffer solution each. Compounds 1–11, acarbose and 1-deoxynojirimycin (positive control) were dissolved in DMSO, the substrate (p-nitrophenyl glucoside, 5 mM) were dissolved in PBS buffer solution and enzyme solutions (2.0 units/mL) were prepared. The assay was conducted in a 100 μL reaction system containing 20 μL enzyme stock solution, 69 μL PBS buffers and 1 μL of DMSO or testing materials. After 10 min incubation at 37 °C, 10 μL of the substrate was added and incubated for 20 min at 37 °C. The Absorbance which measured by a BIO-RAD (iMark) microplate reader at 405 nm was used to calculate the inhibitory activity according to the equation:

$$\eta(\%) = \frac{[B - S] / B}{B} \times 100\%$$  \hspace{1cm} (1)

η (%) is the percentage of inhibition; B is the assay medium with DMSO; S is the assay medium with compound. The results of IC₅₀ values were calculated by the nonlinear regression analysis. Acarbose and 1-deoxynojirimycin were used as positive controls.

3.5.2. Antioxidant Activity

The DPPH· scavenging was assayed according to the reported method [30]. The DPPH radical scavenging test was performed in 96-well microplates. Testing materials (compounds 1–11) were added to 150 μL (0.16 mmol/L) DPPH solution in MeOH at a range of 50 μL solutions of different
concentrations (2, 25, 50 and 100 μM). After 30 min, absorbance at 517 nm was measured and the percentage of activity was calculated. Ascorbic acid was used as a positive control.

4. Conclusions

In summary, four new metabolites, including one new asperchalasine I (1), dibefurin B (2), two epicoccine derivatives (3, 4) and seven known compounds were isolated from the fungus Mycosphaerella sp. SYSU-DZG01. The structures of 1–11 were established by spectroscopic data and the absolute configuration of compounds 1–3 was determined in this research. Compound 1 possesses a unique T-shaped skeleton. All of the compounds were tested for their biological activities. Compounds 1, 8 and 9 exhibited inhibitory effects against α-glucosidase with IC_{50} values of 17.1, 26.7 and 15.7 μM, respectively while compounds 1, 4, 6 and 8 showed antioxidant activity by scavenging DPPH· with EC_{50} values of 77.8, 85.8, 59.1 and 16.3 μM. These results suggested that the asperchalasine I may be a potential candidate for α-glucosidase inhibitor.

Supplementary Materials: The following are available online at http://www.mdpi.com/1660-3397/17/8/483/s1, Figure S1: 1H NMR spectrum of compound 1 (500 MHz, CDCl_{3}), Figure S2: 13C NMR spectrum of compound 1 (125 MHz, CDCl_{3}), Figure S3: DEPT 135, DEPT 90 and 13C NMR spectrum of compound 1 (125 MHz, CDCl_{3}), Figure S4: 1H-1H COSY spectrum of compound 1 (CDCl_{3}), Figure S5: HSQC spectrum of compound 1 (CDCl_{3}), Figure S6: HMBC spectrum of compound 1 (CDCl_{3}), Figure S7: NOESY spectrum of compound 1 (CDCl_{3}), Figure S8: HRESIMS spectrum of compound 1, Figure S9: Experiment ECD spectrum of I, Figure S10: 1H NMR spectrum of compound 2 (400 MHz, DMSO-d_{6}), Figure S11: 13C NMR spectrum of compound 2 (100 MHz, DMSO-d_{6}), Figure S12: 1H-1H COSY spectrum of compound 2 (DMSO-d_{6}), Figure S13: HSQC spectrum of compound 2 (DMSO-d_{6}), Figure S14: HMBC spectrum of compound 2 (DMSO-d_{6}), Figure S15: HRESIMS spectrum of compound 2, Figure S16: 1H NMR spectrum of compound 3 (500 MHz, MeOH-d_{4}), Figure S17: 13C NMR spectrum of compound 3 (125 MHz, MeOH-d_{4}), Figure S18: 1H-1H COSY spectrum of compound 3 (MeOH-d_{4}), Figure S19: HSQC spectrum of compound 3 (MeOH-d_{4}), Figure S20: HMBC spectrum of compound 3 (MeOH-d_{4}), Figure S21: NOESY spectrum of compound 3 (MeOH-d_{4}), Figure S22: HRESIMS spectrum of compound 3, Figure S23: 1H NMR spectrum of compound 4 (400 MHz, MeOH-d_{4}), Figure S24: 13C NMR spectrum of compound 4 (100 MHz, MeOH-d_{4}), Figure S25: 1H-1H COSY spectrum of compound 4 (MeOH-d_{4}), Figure S26: HSQC spectrum of compound 4 (MeOH-d_{4}), Figure S27: HMBC spectrum of compound 4 (MeOH-d_{4}), Figure S28: HRESIMS spectrum of compound 4. Figure S29: The LC-HRESIMS analysis profile of crude extract.

Author Contributions: P.Q. contributed to isolation, structure elucidation and wrote the paper; Z.L. contributed to the analysis of the NMR data and structure elucidation. Y.C. contributed to the analysis of the NMR data and biological tests. R.C. contributed to the spectral analysis. Z.S. and G.C. guided the whole experiment and revised the manuscript.

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