Diversified Polyketides With Anti-inflammatory Activities From Mangrove Endophytic Fungus Daldinia eschscholtzii KBJYZ-1

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In total, five new polyketide derivatives: eschscholin B (2), dalditone A and B (3 and 4), (1R, 4R)-5-methoxy-1,2,3,4-tetrahydronaphthalene-1,4-dio (5), and daldilene A (6), together with 10 known as analogs (1, 7–15) were isolated from the mangrove endophytic fungus Daldinia eschscholtzii KBJYZ-1. Their structures and absolute configurations were established by extensive analysis of NMR and HRESIMS spectra data combined with ECD calculations and the reported literature. Compounds 2 and 6 showed significant cell-based anti-inflammatory activities with IC₅₀ values of 19.3 and 12.9 µM, respectively. In addition, western blot results suggested that compound 2 effectively inhibits the expression of iNOS and COX-2 in LPS-induced RAW264.7 cells. Further molecular biology work revealed the potential mechanism of 2 exerts anti-inflammatory function by inactivating the MAPK and NF-κB signaling pathways.

Keywords: mangrove endophytic fungus, Daldinia eschscholtzii, anti-inflammatory activity, NF-κB, MAPK

INTRODUCTION

Mangrove endophytic fungi have proven to be a promising source of novel chemical backbones and bioactive metabolites owing to extreme environments (tidal flooding, high salinity, anaerobic soil, and high temperature) of mangroves (Chen S. et al., 2022; Chen Y. et al., 2022). Daldinia eschscholtzii is an endophytic fungus isolated commonly from mangrove plants (Yang et al., 2017). The diverse bioactivity metabolites, including tetralones (Liao et al., 2019a), lactones (Kongyen et al., 2015), naphthoquinones (Wutthiwong et al., 2021), chromones (Barnes et al., 2016), and polyphenols (Zhang et al., 2016), have attracted much attention. For instance, naphthoquinones 5-hydroxy-2-methoxy-6,7-dimethyl-1,4-naphthoquinone form D. eschscholtzii HJ004 showed antibacterial activity (Liao et al., 2019b), and chromones 5-hydroxy-8-methoxy-2-methyl-4H-chromen-4-one from D. eschscholtzii GsE13 showed phytotoxicity (Flores-Reséndiz et al., 2021).

It is well known that excessive inflammation could lead to tissue damage, loss of function, and many more related diseases, such as arthritis, systemic lupus erythematosus, ulcerative colitis, and cancer (Zhang Y. et al., 2021). The most often used therapeutic medicines, such as non-steroidal anti-inflammatory drugs (NSAIDs), have been shown to significantly reduce prostaglandin production by reducing the activity of cyclooxygenase (COX) enzymes (Bindu et al., 2020). Whereas,
various side effects might be caused by NSAIDs, including gastrointestinal mucosal injury, liver, and kidney toxicity (Wang et al., 2020). As a result, the discovery of new anti-inflammatory medications has become an unavoidable trend. The metabolites from mangrove endophytic fungus were the key sources of the anti-inflammatory lead compounds due to their novel structure, low toxicity, and significant inhibitory effect (Chen et al., 2021). As part of our continuing investigation into searching for novel anti-inflammatory natural compounds derived from mangrove endophytic fungi, a fungus *D. eschscholtzii* KBJYZ-1, which was isolated from *Plutea indica* Less., aroused our interest because the ethyl acetate extract of the fungal culture displayed excellent anti-inflammatory activity. As a result, five new compounds (2–6) and ten known compounds (1, 7–15) were isolated (Figure 1). The anti-inflammatory activity of all isolated compounds was evaluated by the lipopolysaccharides (LPSs) induced NO production in RAW264.7 macrophages. Moreover, a silica gel column was used for chromatography (CC) was used with petroleum ether/ethyl acetate gradient elution from 0:10 to 2:8, to obtain initial ten fractions (Fr.1–Fr.10) were obtained. Fr.2 (200.6 mg) was separated by Sephadex LH-20 to obtain 8 (3.2 mg). The subfraction Fr.2.1 (60.3 mg) was further subject to silica gel CC (CH₂Cl₂/PE v/v, 2:1) to obtain 1 (2.6 mg). The subfraction Fr.2.1.4 and Fr.2.3 were pooled and purified by Sephadex LH-20 CC (CH₂Cl₂/MeOH v/v, 1:1) to get 5 (2.1 mg) and 6 (3.3 mg), respectively. In total, 7 (4.1 mg) and 9 (2.3 mg) were obtained from subfraction Fr.2.4 (40.9 mg) which was purified by silica gel CC (CH₂Cl₂). Fr.3.2 (100.3 mg) was separated by Sephadex LH-20 CC (CH₂Cl₂/MeOH v/v, 1:1) to get two subfraction Fr.3.1–3.2. Subfraction Fr.3.2 (80.3 mg) was further purified to obtain six subfractions (3.2.1–3.2.6). Fr.2.2 (2.2 mg) was obtained by purification Fr.3.2.6 (6.5 mg) using sephadex LH-20 CC (CH₂Cl₂/MeOH v/v, 1:1). Fr.4 (450.0 mg) was purified and fractionated into four subfractions (4.1–4.4) by Sephadex LH-20 CC (CH₂Cl₂/MeOH v/v, 1:1). Fr.4.1 (125.3 mg) was again fractionated using silica gel CC (CH₂Cl₂/MeOH v/v, 125:1~80:1), and subfractions Fr.4.1.2 (15.3 mg) and Fr.4.1.4(10.3 mg) were purified by Sephadex LH-20 CC (CH₂Cl₂/MeOH v/v, 1:1) to obtain 11 (1.8 mg) and 15 (1.3 mg), respectively. Fr.5 (200.3 mg) was purified by Sephadex LH-20 CC (CH₂Cl₂/MeOH v/v, 1:1) to yield 10 (5 mg) and 12 (4.3 mg), and other subfractions (5.1–5.8). Fr.5.3 (20.4 mg) was separated into four subfractions (Fr.5.3.1–Fr.5.3.4) using silica gel CC (CH₂Cl₂/MeOH v/v, 100:1, 95:1, 90:1, 80:1), and subfractions Fr.5.3.2 furnished 11 (3.5 mg). Subfractions Fr.5.3.4 purified by Sephadex LH-20 furnished 14. Fr.6 (585.0 mg) was purified by Sephadex LH-20 to furnish five fractions (6.1–6.5). Fraction of Fr.6.4 (221.0 mg) purified by silica gel CC resulted four fractions (6.4.1–6.4.4), purification of subfraction Fr.6.4.2 (15.3 mg) and Fr.6.4.3 (10.9 mg) by Sephadex LH-20 furnished 3 (4.2 mg) and 4 (3.5 mg), respectively.

**ESCHSCHOLIN B (2):** yellow oil; \( [\alpha] = -15.1 (c 0.26, MeOH); UV (MeOH) \( \lambda_{max} (\log \varepsilon) : 210 (1.68) \text{ nm} \); IR (KBr) \( \nu_{max} : 2,936, 2,845, 2,355, 1,702, 1,464, 1,378, 1,284, 1,053 \text{ cm}^{-1} \); 1H and 13C NMR (CDCl₃) data (Table 1); HRESIMS m/z 269.2470 [M + H]⁺ (calcd for C₁₂H₁₇O₃, 269.2468).

**Dalditone A (3):** yellow solid; \( [\alpha] = +0.01 (c 0.12, MeOH); UV (MeOH) \( \lambda_{max} (\log \varepsilon) : 266 (1.77), 205 (1.89) \text{ nm} \); IR (KBr) \( \nu_{max} : 3,381, 2,928, 2,395, 1,760, 1,650, 1,463, 1,064 \text{ cm}^{-1} \); 1H NMR (MeOH-d₄) data (Table 1); 13C NMR (MeOH-d₄) data (Table 2); HRESIMS m/z 243.0627 [M + Na]⁺ (calcd for C₁₂H₁₀O₄Na, 243.0620).
Dalditone B (4): yellow solid; $\left[\alpha\right] = +10.5\, (c\, \text{0.33}, \text{MeOH});$
UV (MeOH) $\lambda_{\text{max}} (\log \varepsilon): 260\, (1.23), 224\, (1.84), 201\, (1.71)$ nm; IR (KBr) $\nu_{\text{max}}: 3,288, 2,928, 2,867, 2,200, 1,671, 1,556,$ $1,460, 1,299, 1,133, 1,039$ cm$^{-1}; ^1H$ and $^{13}C$ NMR (MeOH-$d_4$) data (Table 2); HRESIMS $m/z 219.0649\, [M - H]^-$ (calcd for $C_{12}H_{11}O_4, 219.0643$).

(1R, 4R)-5-methoxy-1,2,3,4-tetrahydronaphthalene-1,4-dio (5): colorless solid; $\left[\alpha\right] = +23.2\, (c\, 0.60, \text{MeOH});$ UV (MeOH) $\lambda_{\text{max}} (\log \varepsilon): 254\, (1.80), 210\, (1.44)$ nm; IR (KBr) $\nu_{\text{max}}: 3,389, 3,004, 2,945, 1,728, 1,580, 1,463, 1,269, 1,018, 993, 754$ cm$^{-1}; ^1H$ and $^{13}C$ NMR (CDCl$_3$) data (Table 3); HRESIMS $m/z 194.0498\, [M - H]^-$ (calcd for $C_{11}H_{13}O_3, 194.0490$).

Daldilene A (6): yellow solid; UV (MeOH) $\lambda_{\text{max}} (\log \varepsilon): 258\, (1.5), 206\, (2.1)$ nm; IR (KBr) $\nu_{\text{max}}: 2,917, 2,949, 1,765, 1,560, 1,460, 1,299, 1,039$ cm$^{-1}; ^1H$ and $^{13}C$ NMR (CDCl$_3$) data (Table 3); HRESIMS $m/z 214.0649\, [M - H]^-$ (calcd for $C_{12}H_{11}O_4, 214.0643$).

![FIGURE 1](https://example.com/figure1.png)

**TABLE 1** $^1H$ and $^{13}C$ NMR data of 2 in CDCl$_3$.

| No. | $\delta_C$ | $\delta_H$ [mult, $J$ (Hz)] | No. | $\delta_C$ | $\delta_H$ [mult, $J$ (Hz)] |
|-----|------------|-----------------------------|-----|------------|-----------------------------|
| 1   | 69.3, CH$_2$ | 3.43, s                      | 10  | 27.2, CH$_2$ | -                           |
| 2   | 49.0, C      | -                           | 11  | 29.4, C     | -                           |
| 3   | 216.9, C     | -                           | 12  | 39.6, CH    | 1.29, m                     |
| 4   | 37.3, CH$_2$ | 2.38, (1.7)                 | 13  | 71.1, CH$_2$ | 3.55, td (6.3, 10.7)        |
| 5   | 23.5, CH$_2$ | 1.43, dd (7.0, 14.0)        | 14  | 14.2, CH$_2$ | 0.75, d (6.8)               |
| 6   | 29.8, CH$_2$ | 1.30, m                     | 15  | 20.0, CH$_2$ | 1.0, d (6.3)                |
| 7   | 29.4, CH$_2$ | 1.15, overlap               | 16  | 21.5, CH$_2$ | 1.02, s                     |
| 8   | 29.1, CH$_2$ | 1.15, overlap               | 17  | 21.5, CH$_2$ | 1.02, s                     |
| 9   | 32.4, CH$_2$ | 1.15, overlap               | 18  | -           | -                           |

**TABLE 2** $^1H$ and $^{13}C$ NMR data of 3 and 4 in MeOH-$d_4$.

| No. | $\delta_C$ | $\delta_H$ [mult, $J$ in Hz] | No. | $\delta_C$ | $\delta_H$ [mult, $J$ in Hz] |
|-----|------------|-----------------------------|-----|------------|-----------------------------|
| 1   | 163.2, C   | -                           | 10  | 161.7, C   | -                           |
| 2   | 116.3, CH  | 6.88, d (8.6)               | 11  | 114.7, CH  | 6.85, d (8.6)               |
| 3   | 132.7, CH  | 7.85, dd (2.1, 8.6)         | 12  | 130.7, CH  | 7.79, dd (2.2, 8.6)         |
| 4   | 123.7, C   | -                           | 13  | 123.4, C   | -                           |
| 5   | 136.3, CH  | 7.98, d (2.1)               | 14  | 134.9, CH  | 7.92, d (2.2)               |
| 6   | 111.3, C   | -                           | 15  | 110.8, C   | -                           |
| 7   | 169.6, C   | -                           | 16  | 168.1, C   | -                           |
| 8   | 80.0, C    | -                           | 17  | 76.3, C    | -                           |
| 9   | 97.4, C    | -                           | 18  | 96.3, C    | -                           |
| 10  | 69.8, C    | 3.56, dd (6.5, 10.5)        | 19  | 29.8, CH   | 2.85, dd (6.7, 13.5)        |
| 11  | 71.1, CH$_2$ | 3.61, s                   | 20  | 65.8, CH$_2$ | 3.65, dd (6.5, 10.5)        |
| 11a | -          | -                           | 21  | 3.56, dd (6.5, 10.5) | -               |
| 12  | 26.2, CH$_3$ | 1.53, s                   | 22  | 16.2, CH$_3$ | 1.27, d (1.9)               |
Western Blot

Briefly, RAW264.7 cells (1×10⁶ cells/well) were inoculated into the 6-well plates and incubated with 2 ml DMEM at 37°C. The spent cell culture medium was discarded when the cell fusion reached about 70–80%. Then, cells were stimulated with compounds (25, 12.5, and 6.25 µM), and incubated for 24 h. Western blot was carried out and the assay was done as described previously (Niu et al., 2021). Blots were visualized using enhanced chemiluminescence (ECL) detection kits and analyzed using Image J software.

Statistical Analysis

All the experiments were repeated at least three times and statistical analyses were evaluated using the GraphPad Prism 7 program. The data were expressed as a means ± SD. p < 0.05 indicates statistical significance. A one-way ANOVA analysis was used to determine statistical significance.

RESULTS AND DISCUSSION

Structure Elucidation

Compound 1 was identified as eschscholin A (Liu et al., 2017) by comparing the 1H and 13C NMR (Supplementary Table S1). Here, the absolute configuration of 12S was first determined by ECD calculation (Figure 2).

Compound 2, a yellow oil, had a molecular formula of C27H32O2. As established by high-resolution electrospray ionization mass spectrometry (HRESIMS), it showed two degrees of unsaturation. The 1H NMR spectrum (Table 1), provided signals for four methylenes at δH 0.75 (d, J = 6.8 Hz, H3-14), 0.7 (d, J = 6.8 Hz, H3-15), 1.02 (s, H3-16), and 1.02 (s, H3-17); one oxygenated methine at δH 3.43 (s, H2-1), 2.38 (t, J = 7.3 Hz, H2-15); an oxygenated methine group at δH 3.55 (td, J = 6.3 Hz, 10.3 Hz, H-13); 13C NMR (Table 1) and HSQC spectra data of 2 exhibited 17 carbon signals, including four methylenes, ten methylenes, two methines, and one carbonyl carbon. Moreover, the spin system of H3-4/H3-5/H3-6/H3-7/H3-8/H3-9/H3-10/H3-11/H3-12/H3-13/H3-14/H3-15/H3-16 from COSY data (Figure 3), together with the HMBC correlations (Figure 3) from H3-16 to C-2 and C-1, from H3-17 to C-2 and C-3, from H3-4 to C-3, established the preliminary structure. Finally, except for a carbonyl group, the remaining indices of hydrogen deficiency were determined as 14-membered macrocycle. Comparing the NMR data indicated the structure of 2 was a resemblance to eschscholin A (Liu et al., 2017). Thus, the structure of 2 was established as presented in Figure 1. The relative configuration of 2 was confirmed by the NOESY correlation of H-13/H3-14, together with the large coupling constant JH-12H-13 = 10.7 Hz (Figure 4). Furthermore, the absolute configuration was confirmed by the ECD calculation. The identical experimental and calculated ECD curves (Figure 2) assigned the 12S, 13R configuration of 2.

Compound 3 was obtained as a yellow solid. The molecular formula was determined as C12H10O4, based on the HRESIMS data. The 1H NMR spectrum (Table 2) provided signals for one methyl δH 1.53 (s, H3-12), one oxygenated methylene δH 3.61 (s, H2-11), three methines δH 6.88 (d, J = 8.6 Hz, H-2), 7.83 (d, J = 8.6 Hz, H-3), 7.88 H 8.0 Hz, H-4), three methylene δH 1.85 (d, J = 7.2 Hz, H-5), and one methylene δH 2.15 (d, J = 6.1 Hz, H-6).

**TABLE 3** 1H and 13C NMR data of 5 and 6 in CDCl3.

| No. | 5 δC [ppm] | δH [mult, J (Hz)] | 6 δC [ppm] | δH [mult, J (Hz)] |
|-----|-----|-----|-----|-----|
| 1 | 63.2 | 5.6 (4.7) | 1 | 24.5 | 3.5 (4.7) |
| 2a | 25.7 | 1.8 (4.7) | 2 | 37.7 | 3.05 (4.7) |
| 2b | 2.2 | 3.10 | 198.3 |
| 3a | 27.7 | 1.8 (4.7) | 4 | 126.4 | 8.95 (8.3) |
| 3b | 2.1 | 5.8 | 126.5 | 7.79 (7.6) |
| 4 | 67.7 | 4.7 (4.7) | 6 | 128.7 | 8.38 (7.4) |
| 4a | 139.9 | C | 3a, 9a | 129.2, C |
| 5 | 120.9 | 7.07 (7.8) | 6a, 6b | 128.7, C |
| 6 | 129.0 | 7.29 (8.0) | 12a, 12b | 131.2, C |
| 7 | 109.7 | 6.85 (8.2) | 3b, 9b | 129.0, C |
| 8 | 159.5 | C |
| 8a | 126.7, C |
| 9 | 55.5 | CH3 | 3.89, s |

1,650, 1,193, 1,068 cm⁻¹; 1H and 13C NMR (CDCl3) data (Table 3); HRESIMS m/z 287.1054 [M + H]+ (calc for C20H15O2, 287.1051).

ECD Calculations

The ECD calculations were performed according to the method described previously (Chen et al., 2015). The conformers of 1, 2, 4, and 5 were optimized using DFT calculations at B3LYP/6-31g (d) level in MeOH. Then, ECD calculations were conducted using time-dependent density functional theory (TD-DFT) at B3LYP/DGDZVP, PBEPBE/6-311+G, B3LYP/6-31G, and B3LYP/6-311G levels, respectively.

Anti-inflammatory Assay

Cell Culture

RAW264.7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C with 5% CO2.

Cell Viability Assay

The cell viability was evaluated by the MTT assay as described previously (Niu et al., 2021). Briefly, RAW264.7 cells (5×10⁴ cells/well) with logarithmic growth were inoculated in 96-well plates for 12 h at 37°C with 5% CO2. Cells were treated with different concentrations of L-NMMA or the test compounds (10, 20, 30, 40, and 50 µM) and LPS (1 µg/ml) for 24 h. Then, approximately 10 µl of MTT (0.5 mg/ml) was added to each well and incubated for 4 h at 37°C. After completion of the post-incubation, the absorbance was measured at 490 nm.

Measurement of NO Production

RAW264.7 cells were inoculated in 96-well plates and incubated for 14 h at 37°C. Period, different concentrations of L-NMMA or the test compound were added, and stimulated with LPS (1 µg/ml) for 24 h. The levels of NO were measured according to the instructions of the manufacturer. The absorbance was measured at 540 nm.
FIGURE 2 | Experimental and calculated ECD curves for 1 (A), 2 (B), 4 (C), 5 (D).

FIGURE 3 | Key HMBC and COSY correlations of 2-6.
$J = 2.1\ Hz, \ 8.6\ Hz, \ H-3),\ 7.98\ (d, J = 2.1\ Hz, \ H-5)$. The $^{13}$C NMR (Table 2) and HSQC spectra displayed 12 carbons, including one methyl, one methylene, six sp² carbons, two sp carbons, and one carboxyl carbon. The HMBC correlations (Figure 3) from H-5 to C-8, from H$_2$-11 to C-9, from H$_3$-12 to C-9, C-10, and C-11, together with the chemical shift at C-8 ($\delta_C\ 80.0$) and C-9 ($\delta_C\ 97.6$), supported that the alkynyl group is located at C-6. Furthermore, the weak HMBC correlation from H$_2$-11 to C-1 confirmed that C-11 and C-1 were connected by an oxygen atom. Compound 3 was determined to be the scalemic mixture as shown by the flat ECD spectra and tiny specific rotation value. The chiral-phase resolution under various circumstances was unsuccessful.

Compound 4, a yellow solid, its molecular formula determined to be C$_13$H$_12$O$_4$ by the HRESIMS, and indicated seven degrees of unsaturation. Comparing the NMR data (Table 2) disclosed a similar structure of 3 and 4, except for the absence of the hydroxy at C-10 in 4. The spin system of H-12/H-10/H-11 was observed from the COSY spectrum (Figure 3). The HMBC correlation (Figure 3) from H-10 to C-8 further confirmed the deduction. In addition, the HMBC correlation and HRESIMS supported that the ether bond between C-11 and C-1 was fractured. The 12S configuration was confirmed by the identical experimental and ECD calculation curves (Figure 2).

Compound 5, a colorless solid, had a molecular formula of C$_{11}$H$_{14}$O$_3$ by HRESIMS, and showed five degrees of unsaturation. $^1$H NMR (Table 3) showed three aromatic signal peaks at $\delta_H\ 7.07\ (d, J = 7.8\ Hz, \ H-5),\ 7.29\ (d, J = 8.0\ Hz, \ H-6),\ 6.85\ (d, 8.2\ Hz, \ H-7)$, two oxygenated methines signal peak $\delta_H\ 5.06\ (t, J = 5.1\ Hz, \ H-1),\ 4.79\ (m, H-4)$. Comparing the NMR data (Table 3) revealed that 5 and 12 (Talapatra et al., 1988) had a similar structure. Except in 5, where the carbonyl group at C-1 was converted to a hydroxy group. The above conclusion was verified by the H-1/H-2/H-3/H-4 correlation from the COSY spectrum (Figure 3), combine with the HMBC correlations (Figure 3) from H-1 to C-8 and C-8a. While, according to the HMBC correlation from H$_3$-9 to C-8, decided that methoxy was located in C-8. The absence of correlation of H-1 and H-4 in the NOESY spectrum showed the 1S, 4S configuration of 5. Thereafter, the identical test and calculated ECD curves (Figure 2) determined the absolute configuration of compound 5 as 1S, 4S.

Compound 6, a yellow solid, its molecular formula was identified as C$_{20}$H$_{14}$O$_2$, according to the HRESIMS. The $^1$H-NMR (Table 3) showed three aromatic protons at $\delta_H\ 8.95\ (d, J = 8.3\ Hz),\ 7.79\ (t, J = 7.6\ Hz),\ 8.38\ (d, J = 7.6\ Hz)$, two methylene peaks $\delta_H\ 3.54\ (t, J = 4.7\ Hz)$ and 3.05 (m). While the $^{13}$C NMR (Table 3) and HSQC spectra exhibited 20 carbons, including four methyls, six sp carbons, and the rest of the carbons, were quaternary carbon (including two carbonyls). Comparison of the NMR data (Table 3) of 6 and 7, showed a similar structure for 6 and 7, except for the absence of the hydroxy group at C-4 and C-9 in 6. The deduction was supported by the H-4/H-5/H-6 correlation from the COSY spectrum and the HMBC correlations (Figure 3) from H-4 to C-3 and C-3a. Thus, the structure of 6 was established.

![FIGURE 4 | NOESY correlation of 2.](image-url)
inhibiting the protein expression of iNOS, meanwhile inhibiting protein expression of COX-2 in LPS-induced RAW264.7 cells.

In macrophages, NF-κB and MAPK signaling pathways were the main signaling pathways controlling inflammatory responses. In NF-κB signaling, key signaling proteins, including
FIGURE 7 | Influences of compound 2 on the MAPK pathway detected by Western blotting. (A) The expression levels of p-JNK, p-ERK, p-P38, and GAPDH detected by the western blotting. (B) The proportion of p-JNK to GAPDH content. (C) The proportion of p-ERK to GAPDH content. (D) The proportion of p-P38 to GAPDH content. Data rendered are the mean ± SD, n = 3. In comparison to the control, ***P < 0.001. In comparison to the LPS, ###P < 0.001.

FIGURE 8 | Influences of compound 2 on p-P65, p-IκBα and GAPDH protein expression detected by the western blotting (A). The proportion of p-P65 to GAPDH content and p-IκBα to GAPDH content (B). Data rendered are the mean ± SD, n = 3. In comparison to the control, ***P < 0.001. In comparison to the LPS, #P < 0.05, ###P < 0.001.
IkBo and P65 phosphorylation forms, were chosen as markers of signaling activity; meanwhile, in MAPK signaling pathways, JNK, ERK, and P38 phosphorylation forms were chosen as indicators of signaling activation (Zhang H. et al., 2021). In Figure 7, LPS could significantly upregulate JNK, ERK, and P38 protein phosphorylation in RAW264.7 cells in comparison to the control group ($P < 0.001$). Compound 2 to varying degrees inhibited the expression of JNK, ERK, and P38 proteins phosphorylation in LPS stimulated RAW264.7 cells. In conclusion, the anti-inflammatory function of compound 2 might be connected to the suppressed MAPK signaling pathways in RAW264.7 cells. In Figure 8, LPS remarkably improves the phosphorylation of IkBo and P65 in RAW264.7 cells in comparison to the control group ($P < 0.001$). Compound 2 inhibited the expression of p-P65 and p-IkBo proteins in LPS-induced RAW264.7 cells. In conclusion, the anti-inflammatory effect of compound 2 may be connected to the suppressed NF-κB signaling pathways in RAW264.7 cells.

CONCLUSION

In total, five new compounds, including eschscholin B (2), dallditone A-B (3-4), (1R, 4R)-5-methoxy-1,2,3,4-tetrahydroannaphthalene-1,4-dio (5), and dalldilene A (6), were isolated from mangrove endophytic fungus D. eschscholtzii. Their structures and absolute configurations were determined by spectroscopy data and ECD calculation. The absolute configuration of I was first determined by ECD calculation. Compounds 2 and 6 exhibited potent anti-inflammatory activities with IC$_{50}$ values of 19.3 and 12.9 µM, respectively. Compound 2 belongs to the family of macroyclic ether, which showed various biological activities. For example, euryalsolides B with immunosuppressive and adipogenesis inhibitory activities (Teng et al., 2021), 12S, 13S-epoxyobtusa-llene IV with cytotoxic activity (Gutiérrez-Cepeda et al., 2016), durumhemiketalolides A and C with anti-inflammatory activity (Cheng et al., 2009) have reported. In addition, further studies showed that compound 2 might play an anti-inflammatory role by inhibiting the activation of MAPK and NF-κB signaling pathways. This study will contribute to the chemical diversity of polyketide and the discovery of potential anti-inflammatory agents from extreme mangrove-derived fungi.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS

GW performed the experiments and wrote the article. ZY, SW, and YY participated in the experiments. YC and WK reviewed the article. WK designed and supervised the experiments. All authors have read and agreed to the published version of the article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.900227/full#supplementary-material

REFERENCES

Barnes, E. C., Jumpathong, J., Lumyong, S., Voigt, K., and Hertweck, C. (2016). Daldinion, an unprecedented binaphthyl derivative, and diverse polyketide congeners from a fungal orchid endophyte. Eur. J. Med. Chem. 47, 4551–4555. doi: 10.1002/chin.201631229

Bindu, S., Mazumder, S., and Bandypadhyay, U. (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: a current perspective. Biochem. Pharmacol. 180, 114147. doi: 10.1016/j.bcp.2020.114147

Chen, S., Cai, R., Liu, Z., Cui, H., She, Z. (2022). Secondary metabolites from mangrove-associated fungi: source, chemistry and bioactivities. Nat. Prod. Rep. 39, 560–595. doi: 10.1039/D1NP00041A

Chen, Y., Liu, Z. M., Huang, Y., Liu, L., He, J. G., Wang, L., et al. (2019). Ascomylactams A-C, cytotoxic 12- or 13-membered-ring macrocyclic alkaloids isolated from the mangrove endophytic fungus Didymella sp. CSYK-4, and structure revisions of phomapyrrolidones A and C. J. Nat. Prod. 82, 1752–1758. doi: 10.1021/acs.jnatprod.8b00918

Chen, Y., Liu, Z. M., Liu, H. J., Pan, Y. H., Li, J., Liu, C., and She, Z. G. (2018). Dichlorosoucoumarins with potential anti-inflammatory activity from the mangrove endophytic fungus Ascomycota sp. CSYK-4. Mar. Drugs 16, 54. doi: 10.3390/md16020054

Chen, Y., Wang, G. S., Yuan, Y. L., Zou, G., Yang, W. C., Tan, Q., et al. (2022). Metabolites with cytotoxic activities from the mangrove endophytic fungus Fusarium sp. 25T2. Front. Chem. 10, 842405. doi: 10.3389/fchem.2022.842405

Chen, Y., Zou, G., Yang, W. C., Zhao, Y. Y., Tan, Q., Chen, L., et al. (2021). Metabolites with anti-inflammatory activity from the mangrove endophytic fungus Diaporthe sp. QYM12. Mar. Drugs 19, 36. doi: 10.3390/md19020056

Cheng, S. Y., Wen, Z. H., Wang, S. K., Chiou, S. F., Hsu, C. H., Dai, C. F., et al. (2009). Unprecedented hemiketal embranolides with anti-inflammatory activity from the soft coral Lobophytum durum. J. Nat. Prod. 72, 152. doi: 10.1021/np080686k

Flores-Reséndiz, M., Lappe-Oliveras, P., and Macías-Rubalcava, M. L. (2021). Mitochondrial damage produced by phytotoxic chromene and chromane derivatives from endophytic fungus Dalldinia eschscholtzii strain GSE13. Appl. Microbiol. Biotechnol. 105, 4225–4239. doi: 10.1007/s00253-021-11318-7

Gao, R. C., Shu, W. H., Shen, Y., Sun, Q. C., Jin, W. G., Li, D., et al. (2021). Peptide derivatives from endophytic fungus Didymella sp. QYM12. J. Nat. Prod. 84, 1752–1758. doi: 10.1021/acs.jnatprod.8b01080
Wang, Y., Zhou, Z. Y., Han, M. S., Zhai, J. X., Han, N., Liu, Z. H., et al. (2020). A new hydronaphthalene from the mangrove-derived *Daldinia eschscholtzii* HJ001. *Mar. Drugs* 17, 710. doi: 10.3390/md17120710

Liao, H. X., Shao, T. M., Mei, R. Q., Huang, G. L., Zhou, X. M., Zheng, C. J., et al. (2019a). Bioactive secondary metabolites from the culture of the mangrove-derived fungus *Daldinia eschscholtzii* HJ004. *Mar. Drugs* 17, 710. doi: 10.3390/md17120710

Liao, H. X., Zheng, C. J., Huang, G. L., Mei, R. Q., Nong, X. H., Shao, T. M., et al. (2019b). Bioactive polyketide derivatives from the mangrove-derived fungus *Daldinia eschscholtzii* HJ004. *J. Nat. Prod.* 82, 2211–2219. doi: 10.1021/acs.jnatprod.9b00241

Liu, H. X., Tan, H. B., Li, S. N., Chen, Y. C., Li, H. H., and Zhang, W. M. (2017). Two new metabolites from *Daldinia eschscholtzii*, an endophytic fungus derived from *Pogostemon cablin*. *J. Asian Nat. Prod. Res.* 19, 1–7. doi: 10.1080/10286020500246626

Liu, L. J., Li, W., Koike, K., Zhang, S. I., and Nikaido, T. (2004). New α-tetralonyl glucosides from the fruit of *Juglans madshurica*. *Chem. Pharm. Bull.* 52, 566–569. doi: 10.1248/cpb.52.566

Machida, K., Matsuoka, E., Kasahara, T., and Kikuchi, M. (2005). Studies on the constituents of *Juglans* species. I. Structural determination of (4S)- and (4R)-4-hydroxy-α-tetralone derivatives from the fruit of *Juglans mandshurica* MAXIM. var. siboldiana MAKINO. *Chem. Pharm. Bull.* 53, 934–937. doi: 10.1248/cpb.53.934

Niu, Y., Wang, B. G., Zhou, L., Ma, C. Y., Waterhouse, G. I., Liu, Z. H., et al. (2021). Nigella sativa: A dietary supplement as an immune-modulator on the basis of bioactive components. *Front. Nutr.* 8, 521. doi: 10.3389/fnut.2021.722813

Rao, C. R., and Venkateswarlu, V. (1956). Synthesis of 5-and 5,8-dimethoxy-2-methylchromones. *Recl. Trav. Chim. Pays-Bas* 75, 1321–1326. doi: 10.1021/acs.orglett.6b03435

Sun, Y. W., Liu, G. M., Huang, H., and Yu, P. Z. (2012). Chromone derivatives from *Halenia elliptica* and their anti-HBV activities. *Phytochemistry* 73, 1195–1201. doi: 10.1016/j.phyto.2011.09.015

Talapatra, S. K., Karmacharya, B., De, S. C., and Talapatra, B. (1988). (3R)-Regiolone, an α-tetralone from *Juglans regia*: structure, stereochemistry, and conformation. *Phytochemistry* 27, 3929–3932. doi: 10.1016/0031-9422(88)83047-4

Teng, L. L., Mu, R. F., Liu, Y. C., Xiao, C. J., Li, D. S., Guo, K., et al. (2021). Immunosuppressive and adipogenesis inhibitory sesterterpenoids with a macrocyclic ether system from *Euysolens gracilis*. *Org. Lett.* 23, 2223–2227. doi: 10.1021/acs.orglett.1c00369

Wang, Y., Zhou, Z. Y., Han, M. S., Zhai, J. X., Han, N., Liu, Z. H., et al. (2020). The anti-inflammatory components from the endogenous fraction of syringae folium (ESF) and its mechanism investigation based on network pharmacology. *Bioorg. Chem.* 99, 103764. doi: 10.1016/j.bioorg.2020.103764

Wutthiwong, N., Sithiphasilp, V., Pintatum, A., Suwannarach, N., Kumla, J., Lumyong, S., et al. (2021). A rare tricyclic polyketide having a chromone unit fused to a 3-lactone and its symmetrical biphenyl dimer, daldiniaeschone B, from an endophytic fungus *Daldinia eschscholtzii* SDBR-CMUNIKC745. *J. Fungi* 7, 358. doi: 10.3390/jof7050358

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