Anti-inflammatory, Antiplatelet Aggregation, and Antiangiogenesis Polyketides from *Epicoccum sorghinum*: Toward an Understanding of Its Biological Activities and Potential Applications

Chi-Ying Li,††† Ching-Chia Chang,††† Yi-Hong Tsai, Mohamed El-Shazly, Chin-Chung Wu, Shih-Wei Wang, Tsong-Long Hwang, Chien-Kei Wei, Judit Hohmann, Zih-Jie Yang, Yuan-Bin Cheng, Yang-Chang Wu, and Fang-Rong Chang*†

ABSTRACT: The ethyl acetate extract of an endophyte *Epicoccum sorghinum* exhibited anti-inflammatory activity at a concentration of <10 μg/mL. By bioassay-guided fractionation, one new compound, named epicorepoxydon A (1), and one unusual bioactive compound, 6-(hydroxymethyl)benzene-1,2,4-triol (6), together with six known compounds, were isolated from *E. sorghinum*. The structures of all isolates were established by spectroscopic analyses. The relative configuration of 1 was deduced by the NOESY spectrum and its absolute configuration was determined by X-ray single-crystal analysis. The biological activities of all isolates were evaluated using four types of bioassays including cytotoxicity, anti-inflammatory, antiplatelet aggregation, and antiangiogenesis activities. Compounds 4 and 6 showed potent anti-inflammatory activity, compound 2 possessed potent antiplatelet aggregation and antiangiogenesis activities, and compound 6 demonstrated antiangiogenesis activity. This fungal species can cause a human hemorrhagic disorder known as onyalai. In this study, we identified the active components with antiplatelet aggregation and antiangiogenesis activities, which may be related to the hemorrhagic disorder caused by this fungus. Moreover, we proposed a biosynthetic pathway of the isolated polyketide secondary metabolites and investigated their structure–activity relationship (SAR). Our results suggested that *E. sorghinum* is a potent source of biologically active compounds that can be developed as antiplatelet aggregation and anti-inflammatory agents.

INTRODUCTION

Natural products from terrestrial plants, microbial organisms, and marine organisms played a key role in drug discovery and development throughout the last two centuries. Many current therapeutic drugs have their origins from natural products or their derivatives. Among the major sources of biologically active compounds are fungi. They produce diverse groups of bioactive secondary metabolites, which have been utilized in food, agriculture, or pharmaceutical industries such as cyclosporine, lovastatin, and penicillin. Endophytic fungi, which reside in inter- and/or intracellular parts of plants, are known to enhance host growth and generate numerous prominent bioactive secondary metabolites. These fungi may improve plants’ ability to stand for various types of living stresses and increase their resistance to insects and pests. Therefore, the chemical and biological exploration of endophytic fungi continued to be helpful for the discovery of bioactive natural products. Continuing our effort in discovering the chemical diversity and biological activities of natural compounds, the fungal strain *Epicoccum sorghinum*, isolated from the stem of *Arundo donax* Linn, was investigated due to the potent anti-inflammatory activity of its ethyl acetate (EtOAc) crude extract at a concentration of <10 μg/mL. Few phytochemical investigations have focused on this plant and its endophytic fungi. Indole derivatives were isolated from *A. donax*, which was collected from the Dr. Cecilia Koo Botanic Conservation Center (KBCC) after seasonal pruning. *E. sorghinum* is considered as a major component of the sorghum grain-mold disease complex and potent producer of tenuazonic acid (TeA). This mycotoxin is a tetramereric acid derivative and has the potential to inhibit protein biosynthesis. Sorghum is regarded as the fifth most important cereal crop around the world. Fungal contamination is deemed as one of the major problems associated with cereal crop production because some...
fungal species such as *Epicoccum* species can generate mycotoxins, which are harmful to humans and animals.\(^{13,15}\) Certain species of *Epicoccum* cause a human hemorrhagic disorder known as onyalai.\(^{13}\) *Epicoccum* species can contaminate various foods and beverages and cause leaf spot disease in different types of plants.\(^{13,15}\)

Onyalai is a hemorrhagic disorder that is characterized by the presence of blood blisters in the mouth and a form of thrombocytopenic purpura.\(^{13,16}\) This disease results from eating sorghum grains contaminated with *E. sorghinum*. However, no direct correlation was identified between onyalai and compounds isolated from *E. sorghinum*. Explaining the pathology of onyalai disease needs rigorous, comprehensive, and even long-term investigations. Advanced evidence needs to be explored in the future.

Several studies on *Epicoccum* species demonstrated a diversity of chemical components and a broad spectrum of biological activities.\(^{17−19}\) However, few studies focused on the secondary metabolites and their biological activities of *E. sorghinum*.\(^{20,21}\) Therefore, we performed an extensive chemical and biological investigation on this fungus and identified eight secondary metabolites, including one new compound, epicorepoxydon A (1), one unusual bioactive compound, 6-(hydroxymethyl)benzene-1,2,4-triol (6), one known ethyl phenyl-skeleton derivative, and five known benzyl-skeleton derivatives (Figure 1). The structure of 6 was illustrated in the PubChem database and no reference was reported.

Herein, we report the structural elucidation of a new compound (1) and its absolute configuration, which was deduced by X-ray single crystal analysis. We evaluated the cytotoxicity, anti-inflammatory, antiplatelet aggregation, and antiangiogenesis activities of all isolates to identify the secondary metabolites responsible for onyalai and all possible potential applications of this fungus. We also proposed a biosynthetic pathway of the isolated polyketide secondary metabolites and the structure–activity relationship (SAR) of the isolates.

## RESULTS AND DISCUSSION

Compound 1 was obtained as brown acicular crystals. The molecular formula of C\(_{15}\)H\(_{16}\)O\(_{7}\) was suggested for 1 based on a deprotonated molecular ion at \(m/z\) 307.0822 [M – H]\(^−\) in the negative mode HR-ESI-MS and \(^{13}\)C NMR data indicating eight degrees of unsaturation. The IR spectrum absorption bands at 3448 and 1635 cm\(^−1\) implied hydroxyl and carbonyl functionalities, respectively. The \(^{1}H\) NMR spectrum (Table 1) showed the presence of one methyl group at \(\delta_{H} 2.56\) (s), one methylene at \(\delta_{H} 4.27\) (s), three oxygenated methines at \(\delta_{H} 4.50\) (dd, \(J = 5.7, 2.4\) Hz), \(\delta_{H} 4.80\) (d, \(J = 5.7\) Hz), and \(\delta_{H} 5.80\) (d, \(J = 2.4\) Hz), as well as four aromatic or olefinic methines at \(\delta_{H} 6.75\) (m), \(\delta_{H} 6.78\) (m, 2H), and \(\delta_{H} 7.26\) (t, \(J = 7.6\) Hz). Fifteen signals were observed in the \(^{13}\)C NMR (Table 1) and DEPT spectra of 1. The structure of 6 was illustrated in the PubChem database and no reference was reported.

### Table 1. \(^{1}H\) and \(^{13}\)C NMR Data of 1 in CD\(_{3}\)OD\(^a\)

| no | \(\delta_{H}\) (mult, \(J\) in Hz) | \(\delta_{C}\), type |
|----|-------------------------------|----------------|
| 1  | 193.3, C                       |                |
| 2  | 138.1, C                       |                |
| 3  | 6.78, m                        | 145.5, CH      |
| 4  | 4.80, d (5.7)                  | 69.3, CH       |
| 5  | 4.50, dd (5.7, 2.4)            | 75.6, CH       |
| 6  | 5.80, d (2.4)                  | 78.8, CH       |
| 7  | 4.27, s                        | 593, CH\(_{4}\)|
| 1’ | 116.2, C                       |                |
| 2’ | 161.1, C                       |                |
| 3’ | 6.75, m                        | 115.7, CH      |
| 4’ | 7.26, t (7.6)                  | 134.4, CH      |
| 5’ | 6.78, m                        | 123.5, CH      |
| 6’ | 142.1, C                       |                |
| 7’ | 2.56, s                        | 22.8, CH\(_{4}\)|
| 8’ | 170.2, C                       |                |

\(^{1}H\) and \(^{13}\)C NMR data (\(\delta\)) were measured at 400 and 100 MHz, respectively; chemical shifts are in ppm; \(J\) values in Hz are in parentheses.

Analyzing its \(^{1}H\) and \(^{13}\)C NMR data, this compound showed high similarity to 2.\(^{20}\) Thus, the structure of 1 was suggested as one methylene at \(\delta_{H} 4.27\) (s), three oxygenated methines at \(\delta_{H} 4.50\) (dd, \(J = 5.7, 2.4\) Hz), \(\delta_{H} 4.80\) (d, \(J = 5.7\) Hz), and \(\delta_{H} 5.80\) (d, \(J = 2.4\) Hz), as well as four aromatic or olefinic methines at \(\delta_{H} 6.75\) (m), \(\delta_{H} 6.78\) (m, 2H), and \(\delta_{H} 7.26\) (t, \(J = 7.6\) Hz). Fifteen signals were observed in the \(^{13}\)C NMR (Table 1) and DEPT spectra of 1. These signals resulted from one methyl (\(\delta_{C} 22.8\)), one methylene (\(\delta_{C} 59.3\)), three oxygenated methines (\(\delta_{C} 69.3, 75.6, and 78.8\)), four olefinic methines (\(\delta_{C} 115.7, 123.5, 134.4, and 145.5\)), four quaternary carbons (\(\delta_{C} 116.2, 138.1, 142.1, and 161.1\)), and two carbonyl carbons (\(\delta_{C} 170.2 and 193.3\)).
one of the stereoisomers of the polyketide secondary metabolite 2.

The COSY correlations of H-3’ (δ_H 6.75)/H-4’ (δ_H 7.26)/H-5’ (δ_H 6.78) and the HMBC correlations of CH_3-7’ (δ_C 2.56) with C-1’ (δ_C 116.2), C-2’ (δ_C 161.1), C-5’ (δ_C 123.5), and C-6’ (δ_C 142.1) as well as H-4’ (δ_H 7.26) with C-2’ (δ_C 161.1) and C-6’ (δ_C 142.1) suggested hydroxy and methyl groups attached to a benzene ring moiety. This partial structure of 1 was deduced and showed similarity to 7 with an ester carbonyl moiety. The HMBC correlation of H-6 (δ_H 5.80) with C-8’ (δ_C 170.2) suggested the connection of the ester carbonyl unit. Other parts of the COSY correlations, H-4 (δ_H 4.80)/H-5 (δ_H 4.50)/H-6 (δ_H 5.80), and HMBC correlations, H-5 (δ_H 5.80) with C-1 (δ_C 193.3) as well as H-7 (δ_H 4.27) with C-1 (δ_C 193.3), C-2 (δ_C 138.1), and C-3 (δ_C 145.5), established the linkage of a carbonyl functionality (Figure 2).

The relative configuration of 1 was deduced by the analysis of NOESY correlations and comparison with the published shifts with the published compounds.20 The NOESY correlations of H-4/H-5/H-6 suggested that these protons showed the same orientation (Figure 3).

Compared with 2, the missing correlation between H-5 and H-6 suggested that these two protons were pointed at different orientations. The proposed stereochemistry of 1 was further confirmed by X-ray single crystallographic analysis (Figure 4), and the name of the new compound, epicorepoxydon A, was given. Crystallographic data for 1 have been deposited at the Cambridge Crystallographic Data Centre with the deposition number CCDC 1993524.

The structure of compound 6 was only displayed on the PubChem database with no reference nor spectroscopic data. The ^1H and ^13C NMR data of 6 are summarized in Table S1. Interestingly, a series of unusual simple phenolic/polyphenolic benzyl/benzoic compounds with no common para-hydroxyl substitution, such as compounds 3–7, were identified from this species for the first time (Table S4).

According to previous reports in the literature, E. sorghinum is regarded as the major component of the sorghum grain-mold disease complex and a producer of tenuazonic acid (TeA) that inhibits protein biosynthesis in sorghum.13–15 In the past, many researchers considered Alternaria sp. as the major producer of TeA causing food contamination. But in recent years, some researchers found that E. sorghinum (formerly identified as Phoma sorghium) produces TeA even more than Alternaria sp.13,23 These findings encouraged us to investigate if TeA was available in our E. sorghinum extract. After examining the extract by HPLC, TeA was observed under the standard separation condition (Figure S1). However, TeA was not separated because it was present in minute quantity. Such observation suggested that the isolated endophytic strain E. sorghinum did not produce large amounts of TeA under liquid PDB media cultivation, and the type of media significantly affected the metabolic profile of the fungal extract.

The structural similarities of the isolated compounds encouraged us to propose a plausible biosynthetic pathway of the isolated polyketide secondary metabolites based on the previously isolated analogs with similar partial structures (Figure S5).22,24 One acetyl-CoA and three malonyl-CoA combine to form a polyketide precursor that undergoes dehydration to afford 7. Compound 7 undergoes decarboxylation followed by hydroxylation to produce 3 and 4. Compound 4 undergoes dehydrogenation and hydroxylation to yield isoeoxydon.24 Isoeoxydon undergoes hydrolysis to form (−)−gabosine C and (−)−gabosine E, respectively. (−)-Gabosine C reacts with 7 to obtain 1. On the other hand, (−)−gabosine E reacts with 7 to furnish 2. Theoretically, the hydrolysis that occurred on the epoxide at the C-5 and C-6 of isoeoxydon would form four stereoisomers. The rule of acid-catalyzed hydroxylation of epoxides should result in a trans dihydroxy rather than a cis arrangement. However, biosynthesis has frequently shown that arrangements of chemical functions may not be transmitted acceding to chemical priority. Therefore, rare cis products may be available in the biosynthetic pathway.

Fungi generated many secondary metabolites that demonstrated a plethora of biological activities.1,25 The isolated compounds were subjected to a panel of biological assays. The crude extract was subjected to anti-inflammatory assay and by utilizing bioassay-guided fractionation, two potential candidates were purified and identified. Compound 6 exhibited promising inhibitory activity on both superoxide anion generation (IC$_{50}$ 0.25 ± 0.02 μM) and elastase release (IC$_{50}$ 1.60 ± 0.05 μM). Compound 4 also showed an anti-inflammatory effect by inhibiting superoxide anion generation with an IC$_{50}$ value of 4.54 ± 0.52 μM. The presence of 4 and 6 in large quantities in the extract suggested that these
components are responsible for the anti-inflammatory activity of the extract. Certain secondary metabolites from endophytic fungi such as Epicoccum nigrum exhibited anti-inflammatory and inhibited the platelet-activating factor (PAF)-induced release of β-glucuronidase from rat polymorphonuclear leukocytes in vitro. Bisdethiobis-(methylthio)-gliotoxin from Penicillium terlikowskii was found to inhibit the platelet-activating factor (PAF)-induced rabbit platelet aggregation with IC_{50} 8.4 μM. Fungal toxins such as trichothecenes were found to induce hematological disorders including thrombopenia, neutropenia, and aplastic anemia in animals and humans. E. sorghinum causes a human and animal hematological disease, which is known as onyalai, after eating sorghum grains contaminated with the fungus. Onyalai is characterized by the presence of blood blisters in the mouth and a form of thrombocytopenic purpura. Platelets are the smallest blood cells that play an indispensable role in maintaining hemostasis. Dysfunction in the platelet activation process is manifested in hemorrhagic and thrombotic related diseases. Collagen is a part of the primary hemostatic agonists, whereas thrombin, ADP, and TXA2 are secondary stimulants. Angiogenesis plays an important role in physiological conditions such as bone remodeling, embryonic development, reproduction, and tissue repair. The process of angiogenesis involves endothelial cell proliferation, migration, and tube formation to form new blood vessels. Until now, no one has investigated the relation between onyalai and the natural components isolated from E. sorghinum.
isolates, 2 displayed potent activity against two platelet aggregation factors, collagen and U46619. The IC$_{50}$ values of 2 were 168.74 and 181.85 μM, respectively, while aspirin was the positive control. It also possessed significant antiangiogenic activity with an IC$_{50}$ value of 11.0 ± 0.50 μM (Figure 6). Although 1 is the C-6 position epimer of 2, it was inactive in cytotoxicity, anti-inflammatory, antiplatelet aggregation, and antiangiogenesis assays. Compound 6 exhibited potent antiangiogenic activity with an IC$_{50}$ 65.0 ± 5.50 μM. The cytotoxicity of all isolates was not significant against the tested cancer cells of A549, Hep-G2, and MDA-MB-231.

■ CONCLUSIONS

The chemical and biological investigation of the endophytic fungal strain E. sorghinum was carried out resulting in the isolation of one new and seven known compounds; the biosynthetic pathway of epicorepoxidon A (1) was proposed. The biological activities of 2, 4, and 6 in this study were reported for the first time. Interestingly, the different stereochemistry at the C-6 position between 1 and 2 resulted in a significant difference in their biological activities. Our findings suggested the first insights into the antiplatelet aggregation and antiangiogenesis activities of E. sorghinum components.

■ MATERIALS AND METHODS

Isolation of Compounds. In the current study, the culture broth of E. sorghinum was cultivated using 120 Erlenmeyer flasks (500 mL); each flask contained 300 mL of potato dextrose broth (PDB) media. The flasks were incubated for 7 days using a rotatory shaker (150 rpm and 25 °C). After incubation, 36 L of the whole culture broth was filtered to separate the filtrate from the mycelia. The filtrate was extracted with ethyl acetate (EtOAc) and concentrated under reduced pressure to obtain the EtOAc extract (9.3 g). The EtOAc extract was subjected to a series of column chromatography procedures to yield the new compound, epicorepoxidon A (1), along with seven known compounds including 4,5-dihydroxy-6-(6'-methylsalicyl oxy)-2-hydroxymethyl-2-cyclohexen-1-one (2), 1,2,4-triol (3), 4,5-dihydroxybenzyl alcohol (4), 3-hydroxybenzyl alcohol (5), 6-(hydroxymethyl)benzene-1,2,4-triol (6), 2-hydroxy-6-methyl benzoic acid (7), and hydroxytyrosol (8). All isolates were deduced by analyzing and comparing their spectroscopic data with the literature values.

Fungal Material. The fungus E. sorghinum was isolated from the leaves of A. donax collected from the Dr. Cecilia Koo Botanic Conservation Center (KBCC), Pingtung, Taiwan, which deposits over 30,000 living plants. The leaves of A. donax were washed and air-dried. To clean the surface, the dried leaves were immersed in 0.01% Tween 20(aq), dd-H$_2$O, and 0.01% bleach(aq) for 1 min. The leaves were treated with 75% ethanol then the central parts (5 × 5 mm$^2$) of the leaves were sliced by sterilized scissors and seeded on the potato dextrose agar. The fungal strains were maintained in potato dextrose agar media at 25 °C. After duplicated purification, the mycelia of the pure strain were deposited in 2 mL tubes containing 1.5 mL of potato dextrose broth media as well as 0.2 mL of sterilized glycerol and kept at ~80 °C. The fungal strain was identified by Chi-Ying Li and Ching-Chia Chang. A voucher specimen (code number: K060107S-B) was deposited at the Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.

Species Identification. The fungal sample was preserved in phosphate-buffered saline (PBS) at ambient temperature. The DNA extraction was accomplished by utilizing Azyprep Multisource Genomic DNA Miniprep Kit (Azyprep, #02815KC1) according to the manufacturing company’s instructions. A pair of primers of the internal transcribed spacer, ITS 4 and ITS 5 (ITS 4: 5′-TCCCTCGCTTATTGATTATGC3′/ITS 5: 5′GGAGATTAAGTGGTAAAGG-3′), was selected for amplifying the 18S rRNA. Polymerase chain reaction (PCR) amplifications were carried out by FlexCycler (Analytik, Jena, Germany) under the following conditions: 95 °C (5 min), 30 cycles of 95 °C (30 s), 55 °C (30 s), and 72 °C (40 s), with the last extension at 72 °C (7 min). The amplified PCR products were further delivered to the Mission Biotech Co., Ltd. (Taipei, Taiwan) for sequencing services and blasted with the National Center for Biotechnology Information (NCBI) database. The blasting results displayed that the sample shared 99.5% sequence identity with E. sorghinum (GenBank accession number: KX611667.1).

Fermentation, Extraction, and Isolation. The whole fermented broth (36 L) was filtered through filter paper to separate the supernatant from the mycelia. The filtrate was extracted by ethyl acetate (EtOAc) and concentrated by a rotary evaporator to obtain the EtOAc crude extract (9.3 g). This EtOAc crude extract (9.3 g) was subjected to Sephadex LH-20 column chromatography eluted via MeOH to yield five fractions (Fr. 1–Fr. 5). Fraction 3 (3020.3 mg) was separated using silica gel column chromatography and stepwise eluted by CH$_2$Cl$_2$/MeOH (29:1 to 9:1) to afford five subfractions (Fr. 3-1–Fr. 3-5). Fraction 3-2 (613.8 mg) was subjected to silica gel column and stepwise eluted with CH$_2$Cl$_2$/MeOH (49:1 to 9:1) to give eight subfractions (Fr. 3-2-1–Fr. 3-2-8). Fraction 3-2-1 (459.5 mg) was further isolated by silica gel open column and eluted stepwise with CH$_2$Cl$_2$/MeOH (17:1 to 9:1) to get 3 (386.8 mg). Fraction 3-2-2 (17.3 mg) was further purified by reversed-phase (RP) HPLC (Luna 5 μm Phenyl-Hexyl, 250 × 10 mm, Phenomenex, flow rate = 2.0 mL/min, UV detector) eluted with 45% MeOH$_{(aq)}$ to afford 5 (10.8 mg). Fraction 3-3 (1178.6 mg) was separated using silica gel open column chromatography with a gradient of CH$_2$Cl$_2$/MeOH (33:1 to 9:1) to furnish seven subfractions (Fr. 3-3-1–Fr. 3-3-7). Fraction 3-3-3 (448.2 mg) was chromatographed on a silica gel open column eluted stepwise with CH$_2$Cl$_2$/MeOH (33:1 to 9:1) to obtain five subfractions (Fr. 3-3-3-1–Fr. 3-3-3-5). Fraction 3-3-3-1 (67.1 mg) was further isolated by RP-HPLC (Luna 5 μm Phenyl-Hexyl, 250 × 10 mm, Phenomenex, flow rate = 2.0 mL/min, UV detector) using 30% MeOH$_{(aq)}$ as the eluent to yield 1 (7.6 mg) and 8 (2.1 mg). Fraction 3-3-3-2 (297.4 mg) was fractionated by silica gel column chromatography and eluted stepwise with CH$_2$Cl$_2$/MeOH (24:1 to 9:1) to afford eight subfractions (Fr. 3-3-3-2-1–Fr. 3-3-3-2-8). Fraction 3-3-3-2-1 (149.4 mg) was further separated by RP-HPLC (Luna 5 μm Phenyl-Hexyl, 250 × 10 mm, Phenomenex, flow rate = 2.0 mL/min, UV detector) and eluted with 37% MeOH$_{(aq)}$ to obtain 2 (70.5 mg) and 6 (45.3 mg). Fraction 3-3-4 (263.2 mg) was subjected to silica gel column chromatography with a gradient elution of CH$_2$Cl$_2$/MeOH (29:1 to 9:1) to furnish eight subfractions (Fr. 3-3-4-1–Fr. 3-3-4-7). Fraction 3-3-4-3 (18.3 mg) was further purified by RP-HPLC (Luna 5 μm Phenyl-Hexyl, 250 × 10 mm, Phenomenex, flow rate = 2.0 mL/min, UV detector) eluted
with 30% MeOH to afford 4 (6.2 mg). Fraction 3-5 (500.3 mg) was separated using silica gel column chromatography eluted stepwise with CH2Cl2/MeOH (21:1 to 9:1) to give 7 (346.1 mg).

Epicerebroxylon A (1): Brown acicular crystals; [α]D25 −25 (c 0.05, MeOH); UV (MeOH) λmax (log ε) 211 (3.79), 241 (3.42), 308 (2.91) nm; IR (neat) νmax 3448, 1635 cm−1; 1H NMR (CD3OD, 400 MHz) and 13C NMR (CD3OD, 100 MHz) data shown in Table 1; HR-ESI-MS m/z 307.0822 [M − H]− (calcd for C15H16O7, 307.0823).

**TeA Examination.** The TeA crude extract and TeA standard were monitored by RP-HPLC on a Cosmosil reversed-phase column (C-18, 250 × 4.6 mm2, 5 μm, 1.0 mL/min, Nacalai Tesque, Kyoto, Japan) with acetonitrile and water (0.1% H3PO4) as the mobile phase (0–5 min: 20:80, 5–20 min: from 20:80 to 0:100, 20–30 min: 0:100).

**Anti-inflammatory Activity Assay.** The method for anti-inflammatory activity assay was similar to the method previously described. In brief, human neutrophils were collected from healthy volunteers through venipuncture and separated by Ficoll centrifugation. Dextran was employed for sedimentation. After resuspension in calcium (Ca2+)−free HBSS buffer at pH 7.4, the isolated neutrophils were incubated at 4 °C before use.

**Measurement of Superoxide Generation.** The measurement of superoxide generation has been previously described. In brief, neutrophils (6 × 106 cell/mL) were balanced in ferricytochrome c (0.5 mg/mL) and Ca2+ (1 mM) at 37 °C for 5 min and then incubated with 0.1% DMSO or the tested samples for another 5 min. Cells were activated by utilizing fMLP (0.1 μM) for 10 min and treated with cytochalin B (CB, 1 μg/mL) for 3 min. The spectrophotometer (U-3010; Hitachi) was employed for continuous detection of the changes in absorbance at 550 nm.

**Measurement of Elastase Release.** The measurement of elastase release has been previously described. In brief, neutrophils (6 × 106 cell/mL) were balanced in MeO-Suc-Ala-Ala-Pro-Val-p-nitroanilide (100 μM) and Ca2+ (1 mM) at 37 °C for 5 min and then incubated with 0.1% DMSO or the tested samples for another 5 min. Cells were activated by utilizing fMLP (0.1 μM) for 10 min and treated with CB (0.5 μg/mL) for 3 min. The spectrophotometer (U-3010; Hitachi) was employed for continuous detection of the changes in absorbance at 550 nm.

**Antiangiogenesis Activity Assay.** Isolation and Cultivation of Human EPCs. Peripheral blood (80 mL) was collected from healthy volunteers with informed consent before collection. The peripheral blood mononuclear cells (PBMCs) were fractionated from other blood components by centrifugation on Ficoll-Paque Plus (Amersham Biosciences, Uppsala, Sweden) based on the manufacturer’s instructions. Utilizing CD34 MicroBead kit and MACS Cell Separation System (Miltenyi Biotec, Bergisch Gladbach, Germany), the CD34-positive progenitor cells were obtained from the separated PBMCs. The isolation and maintenance of CD34-positive EPCs were carried out as previously described.

**Tube Formation Assay.** Matrigel (BD Biosciences, Bedford, MA) was utilized to facilitate the differentiation of EPCs into a capillary tube-like structure. For polymerization, Matrigel was loaded into 96-well plates and maintained at 37 °C for 30 min. After gel formation, EPCs (1.5 × 104 cells) were seeded per well on the layer of polymerized Matrigel in MV2 medium (containing 2% FBS) with the presence of tested compounds and incubated at 37 °C for 24 h. The methods were performed as previously described.

**Cytotoxicity Assay.** EPCs were incubated using 96-well plates in a density of 5 × 103 cells in each well. Cells were primed with MV2 medium (containing 2% FBS) in the indicated concentration of the tested compounds for 24 h. The percentage of LDH release was measured by the ratio of LDH activity in the medium to LDH activity in the cell lysate.

**Antiplatelet Activity Assay.** Preparation of Washed Human Platelets. The platelet suspension was prepared on the basis of the previously described procedures. Briefly, human blood anticoagulated with acid citrate dextrose was collected from healthy donors, who had not taken any medicines during the previous 2 weeks. Platelets were suspended in Tyrode’s solution (2 mM Ca2+, 11.1 mM glucose, and 3.5 mg/mL bovine serum albumin) at a concentration of 3 × 108 platelets/mL.

**Measurement of Platelet Aggregation.** Before adding the platelet activators, the platelet suspension was incubated with dimethyl sulfoxide (DMSO) as a vehicle or with the tested samples at different concentrations at 37 °C for 5 min under stirring (80.5 × g). After adding the indicated concentration of platelet inducers (U46619 1 μM; collagen 5 μg/mL), the level of platelet aggregation was estimated as the maximal increase of light transmission within 5 min. The light-transmission aggregometer (Chrono-Log Co., Havertown, PA) was employed for measuring platelet aggregation.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c01000.

HR-ESI-MS, 1H, 13C, and DEPT and 2D NMR spectra of 1 as well as the 1H and 13C data of 6 (Table S1); results of HPLC examination of fungal crude extract and TeA standard; bioassay results of anti-inflammatory and antiangiogenesis activities (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**

Fang-Rong Chang — Graduate Institute of Natural Products, College of Pharmacy, Drug Development and Value Creation Research Center, and Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan; Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan; orcid.org/0000-0003-2549-4193; Phone: +886-7-312-1101; Email: aaronfrc@kmu.edu.tw

**Authors**

Chi-Ying Li — Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

Ching-Chia Chang — Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

Yi-Hong Tsai — Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

Mohamed El-Shazly — Department of Pharmacognosy, Faculty of Pharmacy, Ain-Shams University, Cairo 11566, Egypt; Department of Pharmaceutical Biology, Faculty of Pharmacy

11097 https://dx.doi.org/10.1021/acsomega.0c01000
ACS Omega 2020, 5, 11092–11099
and Biotechnology, German University in Cairo, Cairo 11835, Egypt; orcid.org/0000-0003-0050-8288

Chin-Chung Wu — Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

Shih-Wei Wang — Department of Medicine, Mackay Medical College, New Taipei City 252, Taiwan

Tsong-Long Hwang — Graduate Institute of Natural Products, College of Medicine, Chang Gung University, Taoyuan 333, Taiwan; Research Center for Chinese Herbal Medicine, Research Center for Food and Cosmetic Safety, and Graduate Institute of Health Industry Technology, College of Human Ecology, Chang Gung University of Science and Technology, Taoyuan 333, Taiwan; Department of Anesthesiology, Chang Gung Memorial Hospital, Taoyuan 333, Taiwan

Chien-Kei Wei — Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

Judit Hohmann — Department of Pharmacognosy, Interdisciplinary Excellence Center and Interdisciplinary Centre for Natural Products, University of Szeged, H-6720 Szeged, Hungary; orcid.org/0000-0002-887-6392

Zih-Jie Yang — Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

Yuan-Bin Cheng — Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan; orcid.org/0000-0001-6581-1320

Yang-Chang Wu — Graduate Institute of Integrated Medicine, China Medical University, Taichung 404, Taiwan; Chinese Medicine Research and Development Center, China Medical University Hospital, Taichung 404, Taiwan

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c01000

Author Contributions

C.-Y.L. and C.-C.C. contributed equally to this work.

Author Contributions

Antonella Carbone and Valentina Ricci contributed to the design of the study. C.-Y.L. and C.-C.C. contributed to the acquisition of the data. C.-Y.L. and C.-C.C. contributed to the interpretation of the results. C.-Y.L. and T.L. contributed to the drafting of the manuscript. C.-Y.L. and C.-C.C. contributed to the critical revision of the manuscript. Y.-H.T., C.-C.C., and T.L. contributed to the final approval of the manuscript.

Notes

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work was supported by grants from the Ministry of Science and Technology, Taiwan, awarded to F.-R.C. (Grant number: MOST 105-2628-B-037-001-MY3, MOST 106-2320-B-037-008-MY2, MOST 108-2320-B-037-022-MY3, 108-2811-B-037-511, and 109-2927-I-037-502). In addition, this research was funded by the Drug Development and Value Creation Research Center, Kaohsiung Medical University & Department of Medical Research, Kaohsiung Medical University Hospital, awarded to F.-R.C. (Grant number: KMU-TC108A03-11). We appreciate the Center for Research Resources and Development for providing 400 MHz NMR as well as ESI-MS instrumentation support at the Kaohsiung Medical University; HR-ESI-MS was supported by the National Sun Yat-sen University.

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