Fitoterapia 130 (2018) 190–197

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Polyketide derivatives from the sponge associated fungus *Aspergillus europaeus* with antioxidant and NO inhibitory activities

Xiaowen Du[a], Dong Liu[a], Jian Huang[a], Chanjuan Zhang[a], Peter Proksch[b], Wenhan Lin[a,*]

[a] State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing, 100191, PR China
[b] Institute für Pharmazeutische Biologie und Biotechnologie, Heinrich-Heine- Universität Düsseldorf, 40225 Düsseldorf, Germany

ARTICLE INFO

Keywords:
Aspergillus europaeus
Polyketides
Structure elucidation
Radical scavenging activity
Inhibition of NO production
Inhibition of NF-κB activation

ABSTRACT

DPPH assay of the in-house marine-derived fungi uncovered that the EtOAc extract of the cultured fungus *Aspergillus europaeus* WZXY-SX-4-1, which was isolated from the marine sponge *Xestospongia testudinaria*, possesses radical scavenging activity. Chromatographic separation of the bioactive extract resulted in the isolation of 20 polyketide derivatives, including six new compounds namely eurobenzophenones A-C (**1–3**), euroxanthones A-B (**4–5**), and (+)1-*O*-demethylvariecolorquinones A (**6**). The structures of new compounds were determined on the basis of the analyses of spectroscopic data, including the Snatzke method for the configurational assignment. Benzophenones **3**, **9** and **10** exhibited potent radical scavenging activity against DPPH. All polyketides were evaluated for the inhibitory effects toward the LPS induced nitric oxide (NO) production in mouse microglia BV2 cells and the NF-κB activation in human colon carcinoma cell line SW480. Compound **9** with the significant DPPH radical scavenging activity is corresponded to the potent inhibition against NF-κB in SW480 cells induced by LPS. Compounds **2**, **4**, **16–18** exerted remarked down-regulation of NF-κB in LPS-induced SW480 cells with weak inhibitory effects against NO production and the DPPH radical scavenging activity.

1. Introduction

Xanthone-related derivatives represent a large class of natural polyphenolic compounds, commonly occurring in plants and fungi, and exhibiting a broad spectrum of bioactivities [1–4]. The structure variation is highly owing to functionalization with diverse substituents at various positions. Basically, natural xanthone-based analogues are classified into simple xanthones, xanthone glycosides, prenylated xanthones, xanthonolignoids, bisxanthones, and miscellaneous xanthones. Initial interest in xanthone-related derivatives stemmed from their antimicrobial activities [5], while this aspect has expanded over the years to include antiproliferative properties as well as antidepressant, antiviral, antitubercular, cardiotonic, diuretic, choleretic, and cytotoxic activities. The pharmaceutical activities of xanthone derivatives are related to the structural patterns. Seven-ring polycyclic xanthone MDN-0185 exerted potent antiplasmodial activity against *Plasmodium falciparum* parasites [6]. Prenylated xanthones such as mangostanin and owaxanthones are strongly inhibitory effects toward sensitive and methicillin-resistant strains of *Staphylococcus aureus* [7]. Xanthone dimers including phomoxanthones A and B exhibited significant in vitro antimalarial and antitubercular activities [8]. Sterigmatocystin and its analogues are the environmental toxins, but they are also the inhibitors of the growth of transplanted leukemias P-388 and L1210 in mice [9]. Marine-derived fungi are considered as the potential source to produce the polyketide metabolites, of which chlorination, heterocyclic substitution, and dimerization are commonly occurred in some of the marine derived xanthone-related compounds [10–12]. In the course of search for antioxidant compounds from marine-derived fungi, a DPPH assay of the in-house marine fungal strains was performed, finding the EtOAc extract of the marine sponge (*Xestospongia testudinaria*) associated fungus *Aspergillus europaeus* WZXY-SX-4-1 to have radical scavenging activity. The OSMAC method [13] was used to optimize the culture mediums, while the EtOAc extract from the fungus cultured in salty rice showed stronger radical scavenging activity in the DPPH assay (Fig. 1). Thus, the fermentation of the fungus in solid phase on large scale was performed. Subsequently, the EtOAc extract of the fungus was chromatographed using HPLC separation to obtain 20 compounds, including six new compounds (Fig. 2).

* Corresponding author.
E-mail address: whlin@bjmu.edu.cn (W. Lin).

https://doi.org/10.1016/j.fitote.2018.08.030
Received 23 July 2018; Received in revised form 30 August 2018; Accepted 31 August 2018
Available online 05 September 2018
0367-326X/

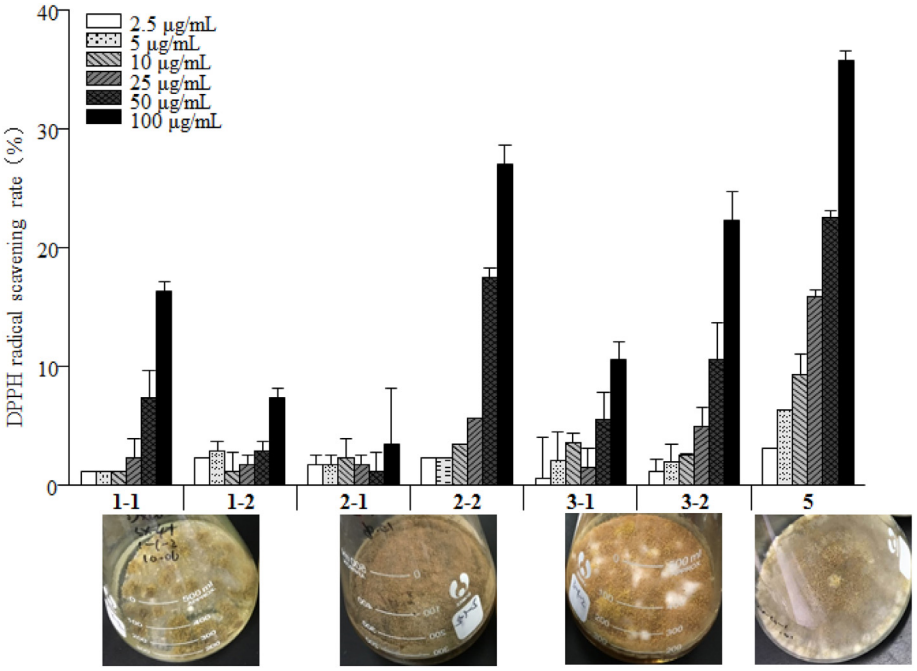

Fig. 1. DPPH radical scavenging activities for the EtOAc extracts of *Aspergillus europaeus* cultured in different mediums.
1–1: liquid germ cultured by PE medium; **1–2**: mycelium cultured by PE medium; **2–1**: liquid germ cultured by MnPY medium; **2–2**: mycelium cultured by MnPY medium; **3–1**: liquid germ cultured by ISP2 medium; **3–2**: mycelium cultured by ISP2 medium; **5**: rice cultured fungus.

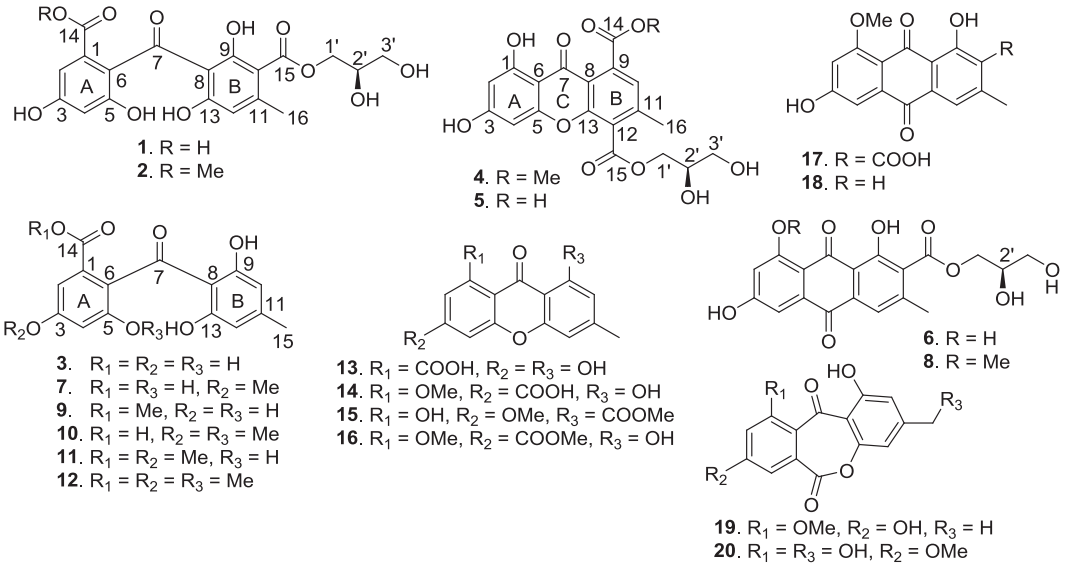

Fig. 2. Structures of the isolated compounds.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a Rudolph IV Autopol automatic polarimeter. IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrometer. ECD spectra were obtained on a Jasco *J*-810 spectropolarimeter. ^{1}H and ^{13}C NMR as well as 2D NMR spectra were recorded on a Bruker Avance III 400 NMR spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively). Chemical shifts were expressed in δ (ppm) and were referenced to the solvent peaks at δ_H 2.50 and δ_C 39.5 for DMSO-*d*6, and coupling constants are in *Hz*. The temperature for NMR measurement is 298 K, the coupling constant for HMBC experiments is optimized as 8 Hz, and the mixing time used for ROESY experiments is 2 s. HRESIMS spectra were measured on a Thermo Scientific LTQ Orbitrap XL spectrometer. Silica gel (160–200 and 200–300 mesh) used for column chromatography was purchased from Qingdao Marine Chemistry Co., Ltd., Sephadex LH-20 (18–110 μm, Pharmacia) and ODS (50 μm, YMC) were involved in the column chromatography. TLC analysis was carried out on precoated silica gel plates (0.20–0.25 mm, GF_{254}, Qingdao Marine Chemistry Co. Ltd.). Semi-preparative HPLC was performed on semi-preparative column (YMC-pack ODS-A C_{18}, 5 μm, 10 × 250 mm) with Alltech 426-HPLC pump and Alltech UVis-200 detector. DAD-HPLC was performed on Waters e2695 separations module with Waters 2998 photodiode array detector and Waters Symmetry C_{18} column (5 μm 4.6 × 250 mm). $Mo_2(OAc)_4$ (molybdenum reagent) for Snatzke method was purchased from Alfa Aesar Co. Ltd. Fetal bovine serum (FBS) was purchased from Gibco Co. Ltd. Dulbecco's modified Eagle's medium (DMEM) and phosphate buffer saline (PBS) was purchased from M and C Gene Tech Co. Ltd. Nitric oxide (NO) concentration was detected by a Thermofisher Multiskan FC microplate reader using Applygen E1030 NO assay kit. Bioluminescence intensity was detected by a Berthold LB960 microporous plate chemo-luminescence detector using Beyotime