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Cytotoxic secondary metabolites from the endophytic fungus *Aspergillus versicolor* KU258497

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ABSTRACT

Two new isocoumarin dimers (**1** and **2**) and one new dihydroquinolone derivative (**3**) were isolated from *Aspergillus versicolor*, an endophyte derived from leaves of the Egyptian water hyacinth *Eichhornia crassipes* (Pontederiaceae), together with ten other known metabolites. Chemical structures of the isolated metabolites were determined based on HRESIMS, extensive 1D and 2D NMR spectroscopy. The relative and absolute configurations of the new natural products were established by ROESY and electronic circular dichroism (ECD) spectroscopy, respectively. The axial chirality of the isocoumarin 7,7′-homodimers (**1** and **2**) was deduced by TDDFT-ECD calculations. All isolated compounds were assessed for their antimicrobial, antitubercular and cytotoxic activities. Several tested compounds revealed significant cytotoxic activity against mouse lymphoma L5178Y cell line with IC_{50} values ranging from < 0.36 to 16.3 μM.

1. Introduction

Endophytic fungi are a renewable source for many new biologically active secondary metabolites and these metabolites have now skyrocketed (Aly et al., 2011; Ebrahim et al., 2016). *Aspergillus versicolor* is a wide-spread species of the genus *Aspergillus* previously isolated from plants, soil and marine habitat or even from air dust (Fomicheva et al., 2006). *A. versicolor* like other species belonging to this genus can withstand a wide temperature range (4–40 °C), pH and/or salinity. However, it usually reveals optimum growth at a temperature between 22 and 26 °C (Pasanen et al., 1997, Fomicheva et al., 2006, Piontek et al., 2016). It can also survive in very dry habitats due to its moderate xerophillic feature (Pasanen et al., 1997).

Aspergillus is a genus comprising about 250 species of highly aerobic fungi growing in almost all oxygen-rich environments (Geiser et al., 2007) with many of them known as oligotrophic species capable of growing in key nutrients-depleted environments (Lee et al., 2013). Moreover, many species have been successfully cultivated over a wide range of temperatures (10–50 °C), pH (2–11) and salinity (0–34%) (Meyer et al., 2011).

A. versicolor is known to produce a vast array of structurally diverse bioactive secondary metabolites such as sesquiterpenoid nitrobenzoyl esters (Belofsky et al., 1998), xanthones (sterigmatocystin), anthraquinones (averantin, methyl-averantin and nidurufin) (Lee et al., 2010), lipopeptides (fellutamides) (Lee et al., 2011), and isocoumarins (versicoumarin A) (Ye et al., 2014) that exhibited potent antiproliferative activities against several human tumor cell lines. Other metabolites exhibited antibacterial and/or antifungal activities including anthraquinones (averantin and nidurufin) against Gram-positive bacteria (Lee et al., 2010), diketopiperazine (DKP) alkaloids (brevianamide S) against Bacille Calmette-Guérin (BCG) bacteria with a promising mechanism of action towards tuberculosis (Song et al., 2012), oxepine-containing DKP alkaloids (versicoloids A and B) with a strong antifungal activities against phytopathogenic fungus *Colletotrichum acutatum* that is even higher than that of cycloheximide (positive control) (Wang et al., 2016), and aspergillomarasmine A (AMA) against metallo-β-lactamases specially NDM-1and VIM-2, which are responsible for carbapenem antibiotic resistance (King et al., 2014). Versicoumarin A showed potent anti-tobacco mosaic virus (anti-TMV) activity (Ye et al., 2014), while the aromatic polyketide aspergillin A displayed strong antioxidant

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potential which was higher than that of the positive control butylated hydroxytoluene (BHT) (Lee et al., 2013).

As a part of our ongoing research aiming at discovering new bioactive fungal metabolites from endophytes derived from the aquatic plant *Eichhornia crassipes* (Ebrahim et al., 2016), the fungus *A. versicolor* was isolated from the fresh healthy leaves of this plant collected in Egypt, and fermented on solid rice medium. In this study, we report the structure elucidation of two new isocoumarin dimers (**1** and **2**) and one new dihydroquinolone derivative (**3**) together with ten known compounds (see Supporting information, Fig. S1) which were identified as diorcinol (Takenaka et al., 2003), 4-carboxydiorcinal (Wang et al., 2012; Liu et al., 2017) and its hydroxy derivative (gerfelin) (Zenitani et al., 2003), several dihydroquinolone derivatives namely, aflaquinolones A, B and D (Neff et al., 2012), aniduquinolone A (An et al., 2013a) and aniquinazoline D (An et al., 2013b) in addition to the polyketide toxic metabolites sterigmatocystin (Cai et al., 2011) and alternariol (Aly et al., 2008). All isolated compounds were subjected to antimicrobial, antitubercular, and cytotoxicity (MTT) assays against mouse lymphoma L5178Y cell line and the screening results are presented.

2. Results and discussion

Compound (**1**) was isolated as an off-white amorphous solid, $[\alpha]_D^{20}$ +4.0 (*c* 0.70, MeOH). HRESIMS of **1** revealed a pseudomolecular ion peak at *m/z* 457.0405 $[M+H]^+$ (calcd for 457.0402$C_{21}H_{13}O_{12}$) indicating the existence of 16° of unsaturation whereas its UV spectrum revealed absorption maxima at 202, 261 and 343 nm characteristic of isocoumarins (Larsen and Breinholt, 1999). The ^{13}C NMR spectrum of **1** (Table 1) exhibited 21 carbons divided into sixteen quaternary carbons including two α,β-unsaturated lactone/ester (isocoumarin) carbonyl ester (δ_C 166.5 and 166.9), an ester carbonyl (δ_C 161.9), a carboxylic carbonyl (δ_C 163.8), six oxygenated olefinic/aromatic carbons (δ_C 143.8, 145.7, 162.9, 163.0, 165.4 and 165.5), six non-oxygenated olefinic/aromatic carbons (δ_C 101.1, 101.2, 110.5, 111.1, 137.7 and 138.4); in addition to one methoxy group (δ_C 53.4) and four olefinic/aromatic tertiary carbons (δ_C 106.0, 106.4, 113.5 and 114.6). ^1H NMR data of **1** (Table 1, see Supporting information) displayed seven singlet proton resonances distinguished into two aromatic protons at δ_H 6.83 (s, H-5′) and 6.88 (s, H-5), two protons of α,β-unsaturated carbonyl groups at δ_H 7.61 (s, H-4′) and 7.73 (s, H-4), one methyl ester group at δ_H 3.89 (s, Me-10) together with two chelated hydroxyl groups at δ_H 11.00 (s, OH-8′) and 11.08 (s, OH-8) ppm. Based on ^1H and ^{13}C NMR data of **1** (Table 1) combined with the molecular formula information, compound **1** revealed a close structural similarity to a recently reported dimeric isocoumarin metabolite, bipenicilisorin (**1a**) (Chen et al., 2017) and its monomeric counterpart (Arunpanichlert et al., 2010). The main structural differences between **1** and **1a** were identified as the lack of methoxy groups at C-6 and C-6′ with the existence of only one methyl ester group in **1** rather than two groups in **1a** (Fig. 1).

These differences implied dissimilarity in the dimeric structure of **1** compared to bipenicilisorin (**1a**) with the former yielding separate proton signals for each aromatic proton within the structure. Being a dimeric derivative of penicilisorin was further confirmed by the disappearance of the proton resonances of H-7 and H-7′, the presence of quaternary carbon signals at δ_C 111.1 and 110.5 ascribed for C-7 and C-7′, respectively. Further confirmation of the depicted chemical structure and substituent distribution of **1** was provided via 2D NMR spectral analyses including ^1H-^1H COSY, HMQC and HMBC (Fig. 2) experiments. HMBC spectrum of **1** (Fig. 2) revealed key correlations between the methyl ester group at δ_H 3.89 (s, Me-10) and a carboxyl carbon at δ_C 161.9 (C-9) whose position was confirmed by key HMBC correlations with proton signal at δ_H 7.73 (s, H-4). Based on the aforementioned data, compound **1** was identified as the new 6,6′,9′-trinor-bipenicilisorin.

Compound **2**, isolated as a yellowish amorphous powder, $[\alpha]_D^{20}$ +4.0 (*c* 0.20, MeOH). HRESIMS revealed a pseudomolecular ion peak at *m/z* 471.0558 $[M+H]^+$ (calcd for 471.0558$C_{22}H_{15}O_{12}$). The UV (MeOH) spectrum of **2** revealed absorption maxima (λ_{max}) at 201, 262 and

Table 1
NMR data of compounds (**1** and **2**).

Position	**1**			**2**		
	δ_H[a] (multi, *J* value in Hz)	δ_H[b] (multi, *J* value in Hz)	δ_C, type[b,c]	δ_H[a] (multi, *J* value in Hz)	δ_H[b] (multi, *J* value in Hz)	δ_C, type[b,c]
1			166.5, C			166.5, C
2						
3			143.8, C			143.9, C
4	7.73 (1H, s)	7.53 (1H, s)	114.6, CH	7.72 (1H, s)	7.53 (1H, s)	114.6, CH
4a			137.7, C			137.8, C
5	6.88 (1H, s)	6.72 (1H, s)	106.4, CH	6.86 (1H, s)	6.72 (1H, s)	106.4, CH
6			165.5, C			165.5, C
7			111.1, C			111.0, C
8			163.0, C			163.0, C
8a			101.2, C			101.3, C
9			161.9, C			161.9, C
10	3.89 (3H, s)	3.95 (3H, s)	53.4, CH_3	3.89 (3H, s)	3.95 (3H, s)	53.4, CH_3
8-OH	11.08 (1H, br s)			11.02 (1H, br s)		
1′			166.9, C			166.5, C
2′						
3′			145.7, C			143.9, C
4′	7.61 (1H, s)	7.46 (1H, s)	113.5, CH	7.72 (1H, s)	7.53 (1H, s)	114.6, CH
4a′			138.4, C			137.8, C
5′	6.83 (1H, s)	6.69 (1H, s)	106.0, CH	6.86 (1H, s)	6.72 (1H, s)	106.4, CH
6′			165.4, C			165.5, C
7′			110.5, C			111.0, C
8′			162.9, C			163.0, C
8a′			101.1, C			101.3, C
9′			163.8, C			161.9, C
10′				3.89 (3H, s)	3.95 (3H, s)	53.4, CH_3
8′-OH	11.00 (1H, br s)			11.02 (1H, br s)		

[a] Measured in DMSO-d_6 at 600 MHz.
[b] Measured in methanol-d_4 (^1H at 600 MHz and ^{13}C at 150 MHz).
[c] Data were assigned and confirmed by HMBC and HMQC spectra.