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Research Article

Pestalotiopamide E and pestalotiopin B from an endophytic fungus *Aureobasidium pullulans* isolated from *Aloe vera* leaves

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ABSTRACT

A detailed chemical investigation of the mycelial extract of the endophytic fungus *Aureobasidium pullulans* isolated from leaves of the Moroccan *Aloe vera* yielded one new amide pestalotiopamide E (**1**) and its corresponding new acid pestalotiopin B (**2**) together with two indole metabolites (**3** and **4**), isoochracinic acid (**5**) and two hydronaphthalene derivatives (**6** and **7**). All isolated compounds were tested for their antiproliferative activity against mouse lymphoma L5178Y cell line.

1. Introduction

"Endophytes" refers to all organisms inhabiting internal plant tissues at some time during their lives without causing apparent harm and/or overt symptoms to the host (Ghimire and Craven, 2013). Many studies confirmed that most plants in natural ecosystems are symbiotic with mycorrhizal fungi and/or fungal endophytes (Aly et al., 2010). Endophytes have been found in virtually every organ of every plant species examined, although their distribution between individual plants is heterogeneous (Bacon and White, 2000). These endophytes also have a profound effect on plants as it provides a range of growth, health and defence enhancements (Tetard-Jones and Edwards, 2016). Endophytic fungi are a major source of phytochemicals and other bioactive natural products (Machavariani and Terekhova, 2014), which have roles in a variety of cellular processes, such as transcription, development, and intercellular communication (Tudzynski, 2014). Furthermore, numerous studies reported the potential use of endophytic compounds in different fields as medicine, industry and agriculture (Strobel and Daisy, 2003). Many antibiotics, antifungal, antimicrobial, antimycotics, immunosuppressant and anticancer compounds have been isolated from different classes of endophytes (Mousa and Raizada, 2013).

Genus *Aureobasidium* comprises 27 taxa (species and varieties), with *Aureobasidium pullulans* being one of the most studied species. *Aureobasidium pullulans*, commonly known as black yeast due to their melanin production, is cosmopolitan yeast-like fungus (Gostinčar et al., 2014). *A. pullulans* is one of the most widely used microorganism for the production of different varieties of compounds exhibiting a wide range of medical, pharmaceutical and biotechnological applications. These compounds include different polysaccharides as pullulan (Cheng et al., 2011) and β-glucan (Aoki et al., 2015), acids as malic acid and poly-β-L-malic acid (PMA) (Leathers and Manitchotpisit, 2013), peptides as aureobasidin A (Tiberghien et al., 2000), lipids as exophilin A and heavy oils as liamocins (Manitchotpisit et al., 2014). *A. pullulans* also has a remarkably large range of extracellular enzymatic activities (Molnarova et al., 2014). Several of these are of biotechnological interest; these include amylase, cellulase, lipase, protease, xylanase, β-fructofuranosidase, maltosyltransferase, α-L-arabinofuranosidase, mannanase, and lactase (Liu et al., 2008).

Endophytic compounds showed a wide range of medicinal uses such as antitumour (Ding et al., 2010), antifungal (Awazu et al., 1995), antiallergy (Sato et al., 2012), antibacterial and immunomodulatory effects (Bischoff et al., 2015). In addition to their therapeutic applications, they displayed significant pharmaceutical and biotechnological industrial importance (Ding et al., 2011).

In our ongoing research on endophytic fungi from different sources, we have investigated a mycelial extract of an endophytic fungus *Aureobasidium pullulans* derived from the Moroccan medicinal plant *Aloe versa*. Results revealed a new amide (**1**)

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and its corresponding acid (**2**); two indole metabolites namely, indole-3-carbaldehyde (**3**) (Abdel-Lateff et al., 2014) and indole-3-carboxylic acid (**4**) (Wang et al., 2016); isoochracinic acid (**5**) (Kameda and Namiki, 1974) and two hydroxylated isosclerone derivatives (**6** and **7**) (Andolfi et al., 2000; Borgschulte et al., 1991; Cimmino et al., 2011).

2. Results and discussion

Compound (**1**) was isolated as yellow oil and its UV spectrum revealed one absorption maximum (λ_{max}) at 221 nm. LRESIMS of **1** exhibited a pseudomolecular ion peaks at *m/z* 257.9 $[M+H]^+$ and 256.0 $[M-H]^-$ suggesting its molecular weight to be 257 g/mol and indicating the possible existence of an odd number of nitrogen atoms (Fig. 1).

The molecular formula of **1** was determined by HRESIMS to be $C_{12}H_{19}NO_5$ based on the prominent peak at *m/z* 258.1336 $[M+H]^+$ and at *m/z* 280.1154 $[M+Na]^+$ and indicated the presence of four degrees of unsaturation. ^{13}C NMR spectrum of **1** revealed the presence of twelve carbon resonances differentiated by DEPT experiment into four quaternary carbons including three carbonyl carbons at δ_c 177.0 (C-1"), 173.0 (C-1′) and 169.0 (C-1) together with one olefinic quaternary carbon at 151.3 (C-3) ppm. In addition, ^{13}C NMR and DEPT spectra also exhibited one olefinic tertiary carbon at δ_c 121.8 (C-2) ppm; five secondary carbons at δ_c 64.3 (C-5), 39.5 (C-4"), 33.3 (C-4), 32.4 (C-2") and 26.0 (C-3") ppm; and two primary methyl carbons at δ_c 25.5 (3-CH_3) and 20.9 (2′-CH_3) ppm. ^1H NMR spectrum of **1** revealed eight signals, five of them are involved in two spin systems as shown by ^1H-^1H COSY (Fig. 2), the first connects three signals at δ_H 3.21 (2H, d, 6.9), 2.32 (2H, t, 7.5) and 1.78 (2H, m) ppm ascribed to CH_2 = 4", CH_2 = 2" and CH_2 = 3", respectively. A second spin system was identified between two proton signals at δ_H 2.94 (2H, t, 6.8) and an oxymethylene moiety at δ_H 4.20 (2H, t, 6.8) ppm assigned for CH_2 = 4 and CH_2 = 5, respectively, in addition to a long range correlation between δ_H 5.76 (1H, s, H-2) and δ_H 1.89 (3H, d, 1.4, 3-CH_3) ppm. By comparison of the obtained spectral data of **1** with reports from the literature, a close resemblance of **1** to pestalotiopamide B was apparent (Xu et al., 2011). The major difference between both compounds was the chemical shift of C-4 in compound (**1**) at δ_C 33.3 ppm while in pestalotiopamide B it was δ_C 40.0 ppm. HMBC spectrum (Fig. 2) exhibited major key correlations which unambiguously determined the connections and the positions of carbonyl moieties within the structure. In addition, NOE spectrum (Fig. 2) displayed a correlation from H-2 and 3-CH_3 confirming that the double bond has a (*Z*) conformation and not (*E*) as in pestalotiopamide B. Based on the aforementioned data, compound (**1**) was unambiguously identified as (*Z*)-4-(5-acetoxy-3-methylpent-2-enamido) butanoic acid trivially named as pestalotiopamide E.

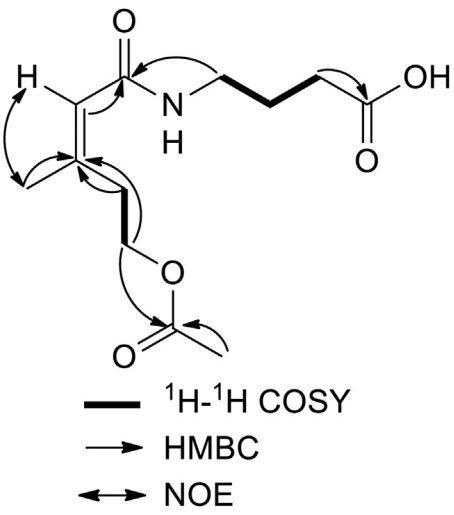

Fig. 2. Key ^1H-^1H COSY, HMBC and NOE correlations of compound (**1**).

The molecular formula of compound (**2**) was determined to be $C_8H_{12}O_4$ based on HRESIMS which exhibited a pseudomolecular ion peak at *m/z* 195.0621 $[M+Na]^+$ (calc. for $C_8H_{12}O_4Na$, *m/z* 195.0633). ^1H NMR and ^1H–^1H COSY spectra of **2** (Table 1) showed partial similarity to that of **1** except for the absence of three methylene groups assigned to CH_2 = 4", CH_2 = 2" and CH_2 = 3". ^{13}C NMR (Table 1) and HMBC spectra (see Supplementary materials) of **2** revealed similar key correlations to those exhibited by **1** which unambiguously indicated the positions of carbonyl groups at C-1 and C-1′. Overall the ^{13}C NMR data of **2** revealed close similarity with spectral data of pestalotiopin A (Xu et al., 2011), previously reported from the endophytic fungus *Pestalotiopsis* sp. except for the value of carbon resonance ascribed to C-4 which differed by 7 ppm compared to pestalotiopin. This difference, as in case of **1**, revealed the presence of a (*Z*) configured double bond unlike (*E*) conformation as in pestalotiopin A. Based on the obtained spectral data, compound (**2**) was identified as (*Z*)-5-acetoxy-3-methylpent-2-enoic acid which was given the trivial name pestalotiopin B.

All isolated compounds were subjected to cytotoxicity (MTT) assay against the mouse lymphoma (L5178Y) cell line but no apparent antiproliferative activity for any of the isolated compounds was detected.

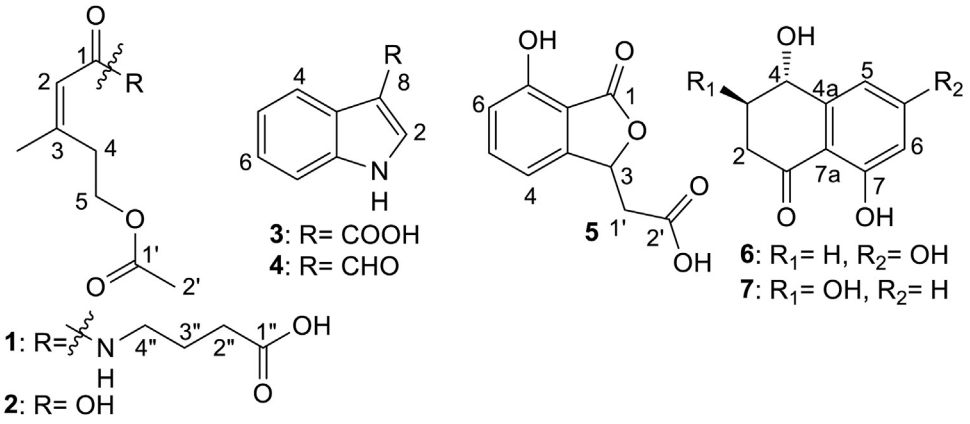

Fig. 1. Structures of compounds (**1**–**7**).