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Penialidins A–C with strong antibacterial activities from *Penicillium* sp., an endophytic fungus harboring leaves of *Garcinia nobilis*

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ABSTRACT

Three new polyketides named penialidins A–C (**1**–**3**), along with one known compound, citromycetin (**4**), were isolated from an endophytic fungus, *Penicillium* sp., harbored in the leaves of the Cameroonian medicinal plant *Garcinia nobilis*. Their structures were elucidated by means of spectroscopic and spectrometric methods (NMR and HRMS^n). The antibacterial efficacies of the new compounds (**1**–**3**) were tested against the clinically-important risk group 2 (RG2) bacterial strains of *Staphylococcus aureus* and *Escherichia coli*. The ecologically imposing strains of *E. coli* (RG1), *Bacillus subtilis* and *Acinetobacter* sp. BD4 were also included in the assay. Compound **3** exhibited pronounced activity against the clinically-relevant *S. aureus* as well as against *B. subtilis* comparable to that of the reference standard (streptomycin). Compound **2** was also highly-active against *S. aureus*. By comparing the structures of the three new compounds (**1**–**3**), it was revealed that altering the substitutions at C-10 and C-2 can significantly increase the antibacterial activity of **1**.

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1. Introduction

Exploring fungal biodiversity for isolation and characterization of bioactive molecules has been a topic of intense research in the recent years. Several success stories have been scripted by the discovery of valuable therapeutically-active compounds from relatively less investigated fungal microflora, which have shown great promise as biologically active lead compounds. For instance, the antifungal agents caspofungin, micafungin and anidulafungin [1] and anticancer drugs such as paclitaxel [2], camptothecin [3] and vincristine [4] have all been isolated from fungi inhabiting diverse ecological niches.

Endophytic fungi are microorganisms that colonize living, internal tissue of plants without causing any immediate, overt, negative effects or external symptoms [5]. They are recognized as potential source of bioactive secondary metabolites with a high level of structural diversity [6]. *Penicillium* species, known for their ability to produce a plethora of bioactive compounds are rich source of polyketides. Some important pharmaceutical agents from *Penicillium* sp., such as griseofulvin and mevastatin, are derived from the polyketide biosynthetic pathway [7].

In continuation of our search for bioactive compounds produced by endophytic fungi inhabiting Cameroonian medicinal plants, an endophytic fungal strain, *Penicillium* sp. CAMMC64, was isolated from the leaves of *Garcinia nobilis* (Clusiaceae) that is known to contain a wide variety of bioactive oxygenated and prenylated xanthones [8]. Herein we report the isolation, structural characterization and antibacterial activity of three new polyketides named penialidins A–C (**1**–**3**), along with the known citromycetin (**4**) [9] from *Penicillium* sp. CAMMC64.

2. Experimental

2.1. General procedure

The high resolution mass spectra were obtained with an LTQ-Orbitrap Spectrometer (Thermo Fisher, USA) equipped with a

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HESI-II source. The spectrometer was operated in positive mode (1 spectrum s^{-1}; mass range: 100–1500) with nominal mass resolving power of 60000 at m/z 400 with a scan rate of 1 Hz with automatic gain control to provide high-accuracy mass measurements within 2 ppm deviation using an internal standard; Bis(2-ethylhexyl)phthalate: $m/z = 391.28428$. The spectrometer was attached with an Agilent (Santa Clara, USA) 1200 HPLC system consisting of LC-pump, photodiode array (PDA) detector ($\lambda = 260$ nm), auto sampler (injection volume 5 μl) and column oven (30 °C). The following parameters were used for experiments: spray voltage 5 kV, capillary temperature 260 °C, tube lens 70 V. Nitrogen was used as sheath gas (50 arbitrary units) and auxiliary gas (5 arbitrary units). Helium served as the collision gas. The high resolution mass spectra (HRMS; Fig. 3 and Fig. S21) of compounds were performed on preparative HPLC using a Nucleodur C18 Gravity column (Macherey-Nagel, Düren, Germany; 50 × 2 mm, 1.8 μm particle size). Samples were analyzed by using a gradient program as follows: 95% A isocratic for 10.0 min, linear gradient to 100% B over 14 min, after 100% B isocratic for 4 min, the system returned to its initial condition (80% A) within 0.5 min, and was equilibrated for 4.5 min (flow rate 350 μl min^{-1}). Enantiomeric separation was achieved by means of a chiral column Lux 5u Cellulose-1 (Phenomenex, 150 × 4.60 mm) using a 60 min isocratic run with 30% H_2O (+0.1% HCOOH) (A)/70% MeOH (+0.1% HCOOH) (B) (flow rate 800 μl min^{-1}) (Fig. S24). The purification was carried out by preparative HPLC run for 20 min on a Gilson apparatus with UV detection at 220 nm using a Nucleodur C18 Isis column (Macherey-Nagel, Düren, Germany), 5 μm (250 × 16 mm) with a H_2O (A)/CH_3OH (B) gradient (flow rate 4 ml min^{-1}). The samples were separated by using a gradient program as follows: 60% A isocratic for 2 min, linear gradient to 100% B over 18 min, after 100% B isocratic for 5 min, the system returned to its initial condition (60% A) within 0.5 min, and was equilibrated for 4.5 min. The NMR spectra were recorded on a Bruker DRX-500 MHz spectrometer. Chemical shifts (δ) were quoted in parts per million (ppm) from internal standard tetramethylsilane and coupling constants (J) were in Hz. Silica gel [Merck, Kieselgel 60 (0.063–0.200 mm)] was used for column chromatography. Melting points were determined on a BÜCHI melting point b-545 apparatus. UV spectra were measured with the photodiode array detector. IR spectra were recorded with Nexus FT-IR spectrometer and optical rotation was recorded with a Krüss $P8_{000}$-T, $P8_{100}$-T, PS_{000}-T polarimeter.

2.2. Fungal material

The fungus was isolated from the leaf of *G. nobilis* collected in Mount Etinde, Southwest region Cameroon. The plant material was identified by Mr. Victor Nana, botanist at the Cameroon National Herbarium (Yaoundé) and deposited under a voucher specimen (50779/HNC/Cam/Mt Zamangoué). Identification of the producing strain was carried out according to our previous description [10].

2.3. Fungal culture and extraction

The endophytic fungal strain CAMMC64 was cultivated on a shaker for 10 days at 25 °C in 500 ml × 10 Erlenmeyer flasks each containing 300 ml of sterile potato dextrose broth (PDB) medium. The culture was successively extracted with ethyl acetate to afford crude extract.

2.4. Fractionation and isolation

Screening of the crude extract of *Penicillium* sp. was performed by means of LC–HRMS. Subsequently the extract was chromatographed on a silica gel column. Successive elution

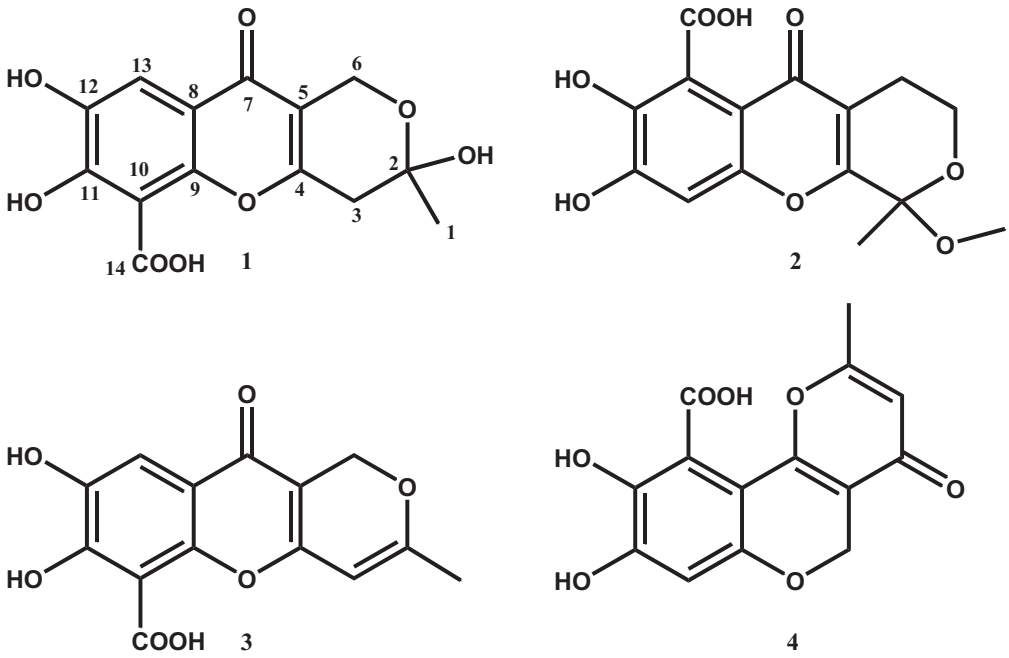

Fig. 1. Chemical structures of compounds **1**–**4** isolated from *Penicillium* sp. CAMMC64.

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