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Monochlorinated calocerins A-D and 9-oxostrobilurin derivatives from the basidiomycete *Favolaschia calocera*

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ABSTRACT

Eight previously undescribed compounds were isolated and characterised from the supernatant and mycelium of a culture of the basidiomycete *Favolaschia calocera* originating from Kakamega equatorial rainforest in Kenya. These were: 9- oxostrobilurins A, G, K and I and the four monochlorinated calocerins A, B, C and D. The calocerins extend our knowledge of halogenated compounds obtained from natural sources. Four further known compounds were also identified: strobilurin G, favolon, pterulinic acid and 2,3 -dihydro-1-benzoxepin derivative. The four oxostrobilurins exhibited prominent antifungal and cytotoxic activities while the four calocerins only showed cytotoxic activity.

1. Introduction

Fungi are one of the most diverse groups of organisms with tropical regions considered to harbour most of their species (Hawksworth, 2001). The Basidiomycota in particular are known to be rich in unique bioactive metabolites but many of their species have not been studied extensively (Stadler and Hoffmeister, 2015). Important medicinal mushrooms like *Agaricus subrufescens, Ganoderma sichuanense, Grifola frondosa, Phellinus linteus*, and *Hericium erinaceus* have been reported to possess a variety of therapeutic treatments (De Silva et al., 2013; Richter et al., 2015; Thongbai et al., 2015). In recent years, exploitation of mushrooms in quest for novel metabolites with therapeutic properties has been on the rise, resulting in isolation of several bioactive compounds. These compounds include cyathins, striatins, sarcodonins, scabronines, chlorinated orcinols, erinacines, corallocins, laschiatrion and pleuromutilins with different biological activities such as antimicrobial, anti-inflammatory, antiproliferative, osteoclast-forming suppressing properties, nerve growth factor activating activities, or agonistic effects toward the kappa-opioid receptor (De Silva et al., 2013; Mudalungu et al., 2016; Stadler and Hoffmeister, 2015; Richter et al., 2016; Wittstein et al., 2016). While most of the aforementioned compounds were found from basidiomycetes of Asian, American and European origin the mycobiota of Africa remains largely untapped with regard to studies on their bioactive metabolites. In this paper we describe the isolation and identification of several novel molecules from a strain that was recently obtained and characterized in the course of a survey of Kenyan basidiomycetes that had shown pronounced biological activities in a preliminary screening for novel antifungal compounds.

2. Results and discussion

Favolaschia calocera R. Heim (Mycenaceae) was identified to species level by sequencing parts of the rDNA (5.8S gene region, including the internal transcribed spacers (ITS1 and ITS2) and part of the large subunit LSU) as described in the Experimental. A BLAST search in GenBank confirmed the identity of the fungal culture as *Favolaschia calocera,* since the most homologous sequences were derived from this species.

This fungus was investigated for active secondary metabolites as its crude extract exhibited strong antifungal activity against *Candida tenuis* and *Mucor plumbeus* during antimicrobial screening.

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Chemical profiling of the supernatant and mycelium extracts led to the isolation of eight previously undescribed compounds: 9-oxostrobilurin A (**1**), 9-oxostrobilurin G (**2**), 9-oxostrobilurin K (**3**), 9-oxostrobilurin I (**4**), calocerin A (**5**), calocerin B (**6**), calocerin C (**7**) and calocerin D (**8**). Four known compounds: strobilurin G, favolon, pterulinic acid and 2,3 −dihydro-1-benzoxepin were also isolated (Anke et al., 1995; Engler et al., 1997; Fredenhagen et al., 1990; Kornsakulkarn et al., 2010).

From the supernatant extract, compound **1** (Fig. 1) was isolated as yellow oil. Its molecular formula was determined to be $C_{16}H_{18}O_4$ from the HRMS. A doublet for methyl group protons (δ 1.30), two methoxy groups singlets (δ 3.73 and 3.85) and aromatic protons signals were observed in the ^{1}H NMR (Table 1). H-15(δ 3.85) showed correlation to C-12 (δ 160. 2) while H-16 (δ 3.73) was correlating to C-13 (δ 167.9) in the HMBC spectra. Further, HMBC correlations observed between H-12 to C-13/C-11/C-15/C-10 suggested the presence of a β-methoxyacrylate moiety in the molecule. The methyl protons (H-14) occurring at δ 1.30 showed HMBC correlation to C-9/C-10/C-11 as well as coupling constant J = 6.87 Hz which was equivalent to that of the C-10 proton implying that C-14 and C-10 were connected to each other. Furthermore, H-8 (δ 6.78) correlation to C-6 (δ 134.9)/C-9 (δ 198.9) and H-7 (δ 7.78) correlations to C-1/5 (δ 128.2)/C-8 (δ123.8)/C-9 (δ 198.9) in the HMBC established the connection of the side chain to the aromatic ring. Further, COSY correlation was observed between H-7 and H-8. The olefinic bond between C-7 and C-8 was assigned as *trans* based on its coupling constant (16.02 Hz). Compound **1** had been reported before as a semisynthetic derivative (Engler et al., 1999), but we report it for the first time as a natural product.

Compound **2** was isolated as yellow oil from both the supernatant and mycelium. Its molecular formula was established as $C_{26}H_{34}O_7$ from HRMS. The NMR data indicated the presence of a 1,2,4-trisubstituted benzene ring and the same 9-oxostrobilurin side chain fragment as in (**1**). Correlations between diastereotopic protons H-18 to C-3/C-20 were observed in the HMBC spectra. Further, COSY correlations between the same protons and H-19 (δ 3.53) were observed. H-19 showed HMBC correlation to C-20,/C-21/C-22/C-23. The two methyl singlets H-21 and H-22 correlated to C-20 in the HMBC spectrum. Thus, a partial structure of the molecule was established as an isoprene unit. Moreover, NOESY correlations between H-21(δ 1.26) to H_a-18 (δ 4.07) and between H-22 (δ 1.47), H_b-18 (δ 4.28) and H-19 (3.53) supported the presence of another isoprene unit fragment in the molecule. The NOE cross peak between H-19 and H-22 was observed, giving an indication of the stereochemistry at C-19. From the COSY correlation between H-23 and H-24 and HMBC correlations of the methyl protons H-26/H-27 to C-24/C-25 and the methylene proton H_b-23 to C-25, the structure of the second isoprene unit fragment was deduced. A HMBC correlation of H-23 to C-19 confirmed the linkage of this side chain to the rest of the molecule. Oxygen atoms were proposed to attach the isoprene units based on C-18 (δ 68.9), C-20 (δ 81.3) and C-23 (δ 67.3) chemical shifts. The NMR data of the 1,5-benzodioxepin part of the molecule were in agreement with those of relevant fragment of the known compounds strobilurin G and 9-methoxystrobilurin G. (Fredenhagen et al., 1990; Hellwig et al., 1999; Kornsakulkarn et al., 2010).

Compound **3** was isolated as yellow oil from both the supernatant and the mycelium of the culture. Its molecular formula was established as $C_{26}H_{34}O_7$ from HRMS. Subsequent examination of ^{1}H and ^{13}C data revealed close similarities with those of compound **2** with the main difference being on the 1,5-benzodioxepin side chain. All the 2D NMR correlations were similar to those of (**2**) accept for the side chain mention above. Compound **3** side chain had a quaternary carbon C-23 (δ 76.2), two chemically equivalent methyl group C-26 and 27 (δ 26.3) and vinyl carbons C-24 (δ143.4) and exo-methylene group C-25 (δ 114.5). The HMBC correlations of H-26/27 (δ 1.31) to C-23/C-24, H-24 (δ 5.9) to C-23/C-25/C-26/C-27 as well as those of H-25 (δ 5.16) to C-23/C- 24 established an isoprene side chain linked to the C-19 (δ 75.3) via an oxygen atom. This assignment was supported by the HMBC correlation of H-19 (δ 3.68) to C-23. The NMR data of (**3**) were similar to those of the already reported strobilurin K, the difference being the keto group

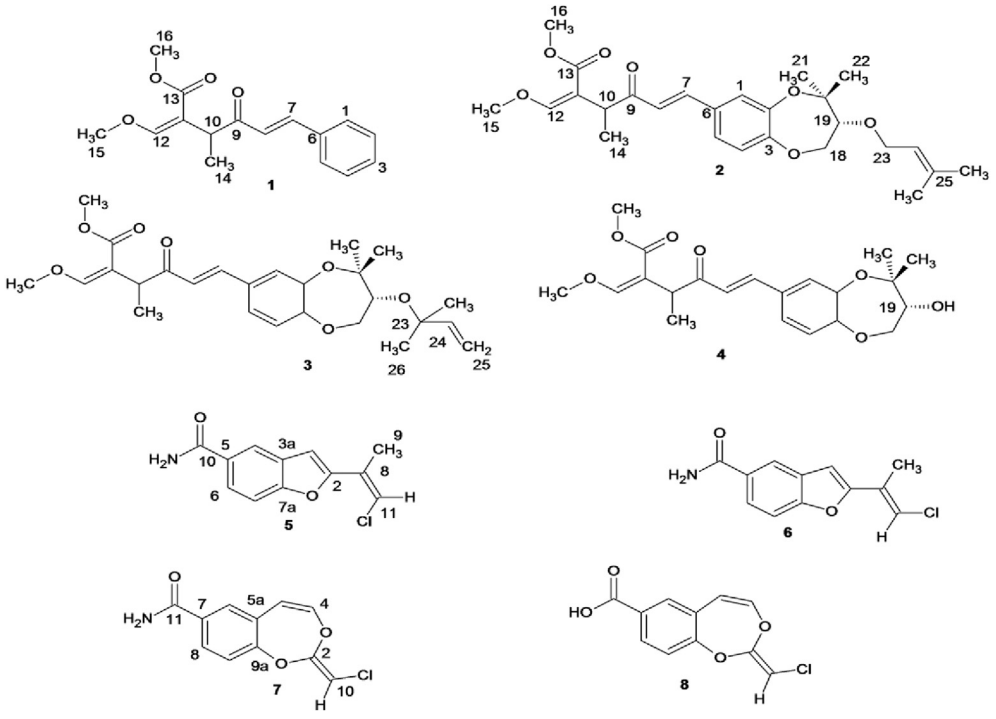

Fig. 1. Structures of compounds **1**-**8**.