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Antiprotozoan sesterterpenes and triterpenes isolated from two Ghanaian mushrooms

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ABSTRACT

Bioassay-guided compound isolation led to the discovery of two new scalarane sesterterpenes (**1** and **2**) and two new triterpenes (**3** and **4**) from two mushroom species, *Pleurotus ostreatus* (edible) and *Scleroderma areolatum*, collected from Ghana. Their structures, including absolute stereochemistry, were established by spectroscopic methods, particularly (+)-ESI-TOF mass spectrometry and 1D and 2D NMR. The four compounds exhibited IC_{50} values of 1.65–7.63 μM against *Plasmodium falciparum* 3D7 and 5.04–13.65 μM against *Trypanosoma cruzi* Tulahuen C4 parasites and were also non-cytotoxic against HepG2 tumoral human liver cells. This is the first report describing the isolation of sesterterpenes belonging to the scalarane structural class from a terrestrial source.

1. Introduction

Malaria and kinetoplastid diseases remain global health issues that affect close to half of the world's population. The protozoan parasites implicated in these diseases are responsible for > 200 million reported cases per year causing over 700, 000 deaths of poor people living in endemic countries particularly in Africa, Asia and Latin America [1–3]. Most of the current drugs against these debilitating diseases are out of date, whilst others have problems including resistance, toxicity and logistics with respect to their cumbersome administration [3–9]. There is an urgent need for global collaborations in research efforts to discover the next-generation of drugs in the fight towards reducing the burden due to these diseases and their possible future eradication. In African, Asian and South American countries where the effects of these tropical diseases are mostly felt, it is estimated that 80% of the population use traditional medicines (plants, mushrooms etc.) for their primary health care [10,11]. Herein lies a unique opportunity of already existing relevant observational clinical data, that can form the basis of collaborative focused screening programs in more logistically endowed parts of the world for the discovery of the active ingredients contained in such traditional medicinal plants [12]. Mushrooms are generally good sources of bioactive metabolites with antimicrobial, antioxidant, antitumoral, nematocidal, and antiprotozoal applications [13–18]. *Ganoderma* is an example of a mushroom genus which has had an extensive application in Chinese herbal medicine [19,20]. From *Ganoderma lucidum*, seven antiplasmodial lanostanes (three of them new) were isolated, with IC_{50s} values from 6 to over 20 μM [19,20]. Antiprotozoal activities of crude methanol and chloroform extracts of *Phellinus linteus* and *Lentinus edodes* (Shiitake) have also been reported [13,21]. *Pleurotus ostreatus* (Jacq. ex. Fr.) *P. Kumm.* and *Scleroderma areolatum* Ehrenb, are two mushrooms commonly found in the wild in Ghana. The low fat, high dietary fiber, protein, vitamin and mineral-content edible *P. ostreatus* is commonly cultivated by farmers in Ghana [22,23]. Other edible cultivated species of the *Pleurotus* genus (family Pleurotaceae) include *P. pulmonarius*, *P. cornucopiae*, *P. sajor-caju*, *P. eryngii* and *P. ostreatus* [24]. The inedible *S. areolatum* (commonly called "earth balls") of the family Sclerodermataceae has a worldwide distribution [25,26]. Although there are reports on the antimicrobial, antioxidant, anti-inflammatory and anti-cancer properties of species of *Pleurotus* and *Scleroderma*, work regarding their antiprotozoal potencies is generally lacking [23,27–29]. Within the frame of a collaborative effort between our institutions, four new antiprotozoal bioactive

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metabolites were isolated from *P. ostreatus* and *S. areolatum* mushroom species collected from Ghana.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured using a Jasco P-2000 polarimeter. UV spectra were obtained with an Agilent 1100 DAD. IR spectra were recorded on a JASCO FT/IR-4100 spectrometer equipped with a PIKE MIRacle single reflection ATR accessory. NMR spectra were recorded in CD_3OD on a Bruker Avance III spectrometer (500 and 125 MHz for ^1H and ^{13}C NMR, respectively) equipped with a 1.7 mm TCI MicroCryoProbe. Chemical shifts were reported in ppm using the signals of the residual solvent as internal reference (δ_H 3.31 and δ_C 49.15 for CD_3OD). Semipreparative and preparative HPLC separations were performed on Gilson HPLC systems with UV detection at 210 and 280 nm. (+)-ESI-TOF mass spectra were acquired using a Bruker maXis QTOF mass spectrometer.

2.2. Sample collection and extract preparation

Fruiting bodies of *Pleurotus ostreatus* (Jacq. ex. Fr.) *P. Kumm.* were collected from wood trees, decaying woods, soil and leaf litters from farms and forests in Ayeduase (latitude 6°40′33 N, longitude 1°33′36 W, altitude 252 m) and Jachie Pramso (latitude 6°36′N, longitude 1°44′W, altitude 266 m) in the Ashanti Region of Ghana. Those of *Scleroderma areolatum* were collected from Abura Kwaman (latitude 5°05′N to 5°25 N, longitude 1°5′W to 1°20′W, altitude 75 m) in the Central Region of Ghana. The two mushroom species were authenticated by a mycologist in the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana and deposited in the Herbarium of the Department of Pharmacognosy, KNUST, Kumasi, Ghana with the voucher specimens KNUST/HMI/2015/S0026 and KNUST/HMI/2015/S0010 for *P. ostreatus* and *S. areolatum*, respectively. The crude extracts from the two mushrooms were prepared by oven-drying each sample at 30 °C to constant dry weight after which they were grounded to powder. About 200 g of the powder was added to 1 L of 70% methanol/water and allowed to stand at room temperature (RT) for 3 days with frequent agitation. This was then filtered through a Whatman No. 10 filter paper and the filtrate evaporated at 40 °C, lyophilized and stored. The yield for the methanol extracts were 14.50% w/w and 19.47% w/w for *P. ostreatus* and *S. areolatum*, respectively.

2.3. Bioassay-guided isolation

Ten miligrams per milliliter (10 mg/mL) solutions of dried *P. ostreatus* and *S. areolatum* extracts were prepared in 100% DMSO, 20% DMSO, 100% methanol and 70% methanol. The crude samples were then filtered and sonicated for 30 min. Two hundred microliters volumes of the samples were transferred into 96-well AB-gene plates and eight-point ½ serial dilutions performed for each. The prepared plates were used as stocks, aliquots of which were tested in the two antiprotozoal assays according to previously described procedures [30,31] and both extracts were found to be active against *T. cruzi* and *P. falciparum* parasites. For *the P. falciparum* assay, the stocks with DMSO were replaced with stocks prepared in milliQ water. DMSO at 100% was the most suitable solvent for initial dissolution of the extracts. For bioassay-guided isolation, 300 µL of a freshly prepared 20 mg/mL DMSO stock solution of the *P. ostreatus* extract was filtered (0.34 µm membrane) and fractionated by semi-preparative High Performance Liquid Chromatography (HPLC) with a linear gradient of 5–100% acetonitrile/H_2O on an agilent zorbax RX-C8 (9.4 × 250 mm, 5 µm) column at 3.6 mL/min flow rate with UV detection at 210 and 280 nm and 80 fractions were collected every 30 s. From the 80 fractions generated, two fractions **F53** and **F55** displayed activity against both *T. cruzi* and *P. falciparum* parasites. Aliquots of the above-mentioned active fractions were analysed by LC-(+)-ESI-TOF mass spectrometry and Proton Nuclear Magnetic Resonance Spectroscopy (^1H NMR). The active components in the fractions **F55** and **F53** were designated compounds **1** (rt 26.3 min) and **2** (rt 25.4 min) respectively. For scale-up purification of the two compounds for biological tests, about 2.5 g of the *P. ostreatus* dried crude extracts were each dissolved in 15 mL methanol and sonicated for 30 min. An aliquot of the crude (3 µL) was analysed by LCMS to ascertain that the peaks/mass profiles of the compounds of interest were present. The sample was then centrifuged at low speed and the resulting supernatant passed through a 0.34 µm filter. The filtrate was dried and re-weighed to obtain 545.8 mg. The dried extracts was then re-dissolved in 1.5 mL methanol, centrifuged, filtered, and then injected and fractionated by preparative HPLC on an agilent Zorbax SB-C8 (21.2 × 250 mm, 7 µm) with a linear gradient of 5–100% acetonitrile/H_2O at 20 mL/min flow rate with the same UV detection stated above. Compounds **1** and **2** were identified by LCMS. Further purification of these peaks by the semi-preparative HPLC with same conditions as described above yielded 3.4 and 2.8 mg of compounds **1** and **2** respectively.

Initial bioassay-guided purification of the *S. areolatum* extract performed as described above resulted in two active HPLC fractions **F48** and **F53** designated compounds **3** (rt 25.5 min) and **4** (rt 28.6 min) respectively. For scale-up purifications 2.2 g of the crude was initially dissolved 15 mL methanol, sonicated, centrifuged, filtered and dried as previously described to yield 347.7 mg extract. This was dissolved in 1.5 mL methanol and used to perform HPLC purifications as previously described, finally resulting in 1.8 and 2.8 mg of compounds **3** and **4** respectively.

2.3.1. Compound *1*

White, amorphous solid; $[\alpha]_D^{25}$ + 31.0 (c 0.08, MeOH); IR (ATR) ν_{max} 3389, 2926, 2850, 1736, 1670, 1451, 1388, 1240, 1076, 1029 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; (+)-ESI-TOF MS *m/z* 445.2949 $[M + H]^+$ (calcd. for $C_{27}H_{41}O_5$, 445.2949) and 906.6104 $[2\,M + NH_4]^+$ (calcd. for $C_{54}H_{84}NO_{10}$, 906.6090).

2.3.2. Compound *2*

White, amorphous solid; $[\alpha]_D^{25}$ + 93.3 (c 0.08, MeOH); IR (ATR) ν_{max} 3172, 2937, 2868, 1731, 1669, 1450, 1391, 1238, 1146, 1024 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; (+)-ESI-TOF MS *m/z* 548.3219 $[M + NH_4]^+$ (calcd. for $C_{30}H_{46}NO_8$, 548.3218); 1078.6119 $[2\,M + NH_4]^+$ (calcd. for $C_{60}H_{88}NO_{16}$, 1078.6099).

2.3.3. Compound *3*

White, amorphous solid; $[\alpha]_D^{25}$ + 118.1 (c 0.06, MeOH); IR (ATR) ν_{max} 3408, 2934, 1721, 1661, 1598, 1448, 1372, 1295, 1253, 1207, 1175, 1132, 1049, 1024, 987 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; (+)-ESI-TOF MS *m/z* 539.3005 $[M + H]^+$ (calcd. for $C_{32}H_{43}O_7$, 539.3003); 479.2790 $[M\text{-}CH_3COOH + H]^+$ (calcd. for $C_{30}H_{39}O_5$, 479.2792); 461.2680 $[M\text{-}CH_3COOH\text{-}H_2O + H]^+$ (calcd. for $C_{30}H_{37}O_4$, 461.2686).

2.3.4. Compound *4*

White, amorphous solid; $[\alpha]_D^{25}$ + 35.2 (c 0.06, MeOH); IR (ATR) ν_{max} 3508, 2945, 2879, 2835, 1734, 1715, 1653, 1455, 1374, 1239, 1154, 1097, 1025, 970, 940 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; (+)-ESI-TOF MS m/z 590.4054 $[M + NH_4]^+$ (calcd. for $C_{34}H_{56}NO_7$, 590.4051); 513.3563 $[M\text{-}CH_3COOH + H]^+$ (calcd. for $C_{32}H_{49}O_5$, 513.3575); 453.3353 $[M\text{-}2CH_3COOH + H]^+$ (calcd. for $C_{30}H_{45}O_3$, 453.3363).