SUPPLEMENTARY MATERIAL

Cytotoxic constituents of *Lasiosphaera fenzlii* on different cell lines and the synergistic effects with paclitaxel

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The fruit body of *Lasiosphaera fenzlii* was found to show cytotoxicity on cancer cells during a preliminary screening. Repeated column chromatography of the fungal methanol extract resulted in the isolation of six compounds identified as 5 α ,8 α -epidioxy-ergosta-6,22-dien-3 β -ol (**1**), 8 α -epidioxy-ergosta-6,9(11),22-trien-3 β -ol (**2**), 5 α -ergosta-7,22-dien-3 β -ol (**3**), 5 α -ergosta-7,22-dien-3-one (**4**), and ergosta-7,22-dien-3 β ,5 α ,6 β -triol (**5**), and 6-dihydroxy-2,3-dihydro-1H-isoindol-1-one (**6**). The two peroxide compounds, **1** and **2**, showed cytotoxic activity and compound **1** was selectively cytotoxic to cancer cells. Further more, compound **1** synergized the cytotoxicity of paclitaxel on Hela cells by increasing intracellular accumulation of paclitaxel in cancer cells but not in normal cells.

Keywords: *Lasiosphaera fenzlii*; constituents; ergosterol peroxide; cytotoxicity; paclitaxel; synergistic effect.

Experimental

Materials and equipments

The dry fruit body of *L. fenzlii* was purchased from Anguo drug market, Hebei province, China. The fungus was identified by the authors through carefully inspecting the fungal morphology and considering the information provided by the fungal supplier. Voucher specimen (number: NPFFL-1) was stored in the Lab of Natural Product & Functional Foods, College of Life Sciences, Inner Mongolia University. Column chromatography was performed on silica gel (100 to 200 mesh, Qingdao Marine Chemical Inc., China), RP-18 (38 to 63 μ m, Wako Pure Chemical Industries Ltd., Japan). NMR spectra were obtained from a Bruker Avance III 500 spectrometer. PTX, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich. Dimethyl sulfoxide (DMSO) was purchased from Amersco.

An agilent 1290 ultra high performance liquid chromatography with a 6430 triple quadrupole mass spectrometer (UHPLC-MS) [Agilent Technologies Singapore (International) Pte. Ltd., Singapore, Singapore] was used for qualitative and quantitative analysis.

Isolation and identification of compounds from *L. fenzlii*

L. fenzlii (2 Kg) were cut to small pieces and extracted with methanol under reflux for 3 times. The methanol extracts were combined and concentrated to get 38 g of a dark green mass. This was subjected to a silica gel column eluted with a solvent gradient of petroleum ether/ EtOAc from 100:0 to 0:100 and EtOAc/EtOH/H₂O from 8:2:1 to 5:2:1. Similar fractions were combined to get seven fractions. Fraction I (EtOAc/EtOH/H₂O 6:2:1 eluted part) was subjected to a C-18 column eluted with H₂O/MeOH to get fraction Ia from H₂O/MeOH 9:1 eluted part. Further purification of fraction Ia by silica gel column and preparative HPLC (PHPLC) to get compound **6** (3.6 mg). Fraction II (petroleum ether/ EtOAc 1:1 eluted) was subjected to C-18 column eluted with a solvent gradient of H₂O/MeOH to obtain fraction IIa, fraction IIb, and fraction IIc from 5:95, 1:9, and 0:100 eluted parts, respectively. Fraction IIa, IIb, IIc were further purified with a silica gel column eluted with petroleum ether/EtOAc. Compound **3** (75 mg), **4** (8 mg) and **5** (6.4 mg) were obtained from fraction IIa; Compound **5** (3.5 mg) was obtained from fraction IIb; Compound **1** (42 mg) was obtained from fraction IIc. (**Figure S1**)

Compound 1 White solid, ESIMS: m/z 451 [M+Na]⁺; ¹H NMR (500 MHz, CDCl₃): 0.81 (3H, d, J =6.0, H-26), 0.83 (3H, d, J =6.0, H-27), 0.82 (3H, s, H-18), 0.89 (3H, s, H-19), 0.91 (3H, d, J =6.5, H-28), 1.00 (3H, d, J =6.5, H-21), 3.97 (1H, m, H-3), 5.13 (1H, dd, J =8.5, 17.0), 5.22 (1H, dd, J =8.0, 17.0) (H-22, 23), 6.24 (1H, d, J =8.5, H-6), 6.51 (1H, d, J =8.5, H-7); ¹³C NMR (125 MHz, CDCl₃): 66.47 (C-3), 79.42 (C-8), 82.15 (C-5), 130.74 (C-7), 132.30 (C-23), 135.20 (C-6), 135.41 (C-22).

Compound 2 White solid, ESIMS: m/z 449 [M+Na]⁺; ¹H NMR (500 MHz, CDCl₃): 0.74 (3H, s, H-18), 1.09 (3H, s, H-19), 0.83 (3H, d, J =6.0, H-26), 0.84 (3H, d, J =6.0, H-27), 0.92 (3H, d, J =6.5, H-28), 1.00 (3H, d, J =6.5, H-21), 3.98 (1H, m, H-3), 5.16 (1H, dd, J =8.5, 17.0), 5.26 (1H, dd, J =8.0, 17.0) (H-22, 23), 5.43 (1H, m, H-11), 6.30 (1H, d, J =8.5, H-6), 6.60 (1H, d, J =8.5, H-7); ¹³C NMR (125 MHz, CDCl₃): 66.47 (C-3), 78.35 (C-8), 82.71 (C-5), 119.74 (C-11), 130.75 (C-7), 132.43 (C-23), 135.20 (C-6), 135.46 (C-22), 142.53 (C-9).

Compound 3 White solid, ESIMS: m/z 421 [M+Na]⁺; ¹H NMR (500MHz, CDCl₃): 0.55 (3H, s H-18), 0.80 (3H, s, H-19), 0.82 (3H, d, J =7, H-26), 0.83 (3H, d, J =7.5, H-27), 0.91 (3H, d, J =6.5, H-28), 1.01 (3H, d, J =6.5, H-21), 3.59 (1H, m, H-3), 5.19 (3H, m, H-7, 22 and 23).

Compound 4 White solid, ESIMS: m/z 419 [M+Na]⁺; ¹H NMR (500 MHz, CDCl₃): 0.57 (3H, s, H-18), 0.99 (3H, s, H-19), 0.82 (3H, d, J =7.0, H-26), 0.83 (3H, d, J =7.5, H-27), 0.92 (3H, d, J =7.0, H-28), 1.01 (3H, d, J =7.5, H-21), 5.20 (3H, m, H-7, 22, 23).

Compound 5 White solid, ESIMS: m/z 453 [M+Na]⁺; ¹H NMR (500 MHz, CDCl₃): 0.60 (3H, s, H-18), 0.82 (3H, d, J =7, H-26), 0.84 (3H, d, J =7.5, H-27), 1.09 (3H, s, H-19), 0.91 (3H, d, J =7, H-28), 1.03 (3H, d, J =6.5, H-21), 4.08 (1H, m, H-3), 3.63 (1H, s, H-6), 5.18 (1H, dd, J =8.0, 16.0) and 5.21 (1H, dd, J =7.5, 16.0) (H-22, 23), 5.36 (1H, d, J =2.5, H-7); ¹³C NMR (125 MHz, C₅D₅N), 67.60 (C-3), 76.14 (C-5), 74.28 (C-6), 120.48 (C-7), 141.58 (C-8), 136.20 (C-22), 132.13 (C-23).

Compound 6 Brown solid, high-resolution electrospray mass spectrum (HRESIMS) m/z 164.0343 [M-H]⁻, m/z 166.0504 [M+H]⁺, (calcd. for C₈H₇NO₃); ¹H NMR (500MHz, CD₃OD): 4.25 (2H, s, H-3), 6.48 (1H, d, J =2.0, H-5), 6.67 (1H, d, J =2.0, H-7); ¹³C NMR (125MHz, CD₃OD) : 42.87(C-3), 100.13(C-7), 106.06(C-5), 121.85(C-3a), 133.92(C-7a), 153.63(C-4), 159.05(C-6), 172.78(C-1).

Cell culture

Hela (human cervical cancer cells), A431 (human skin cancer cells), GES-1

(human gastric mucosal epithelium cells), HBE (human bronchial epithelium cells) were maintained in DMEM (Hyclone); MGC-803 (human gastric cancer cells), HUVEC (human umbilical vein endothelial cells) were maintained in PRMI 1640 (Hyclone). All of the mediums contained 10% fetal bovine serum (Hyclone), and 100 U/ml of penicillin and streptomycin. The cells were cultured at 37 °C with 5% CO₂ in a humidified incubator. All of the cells were provided by Professor Zheng, Cancer Research Center, Liaoning Medical University.

Cytotoxicity assay

Cells were seeded in 96-well plates at about 5000 cells per well. After the cells attached, the pure compounds were added and the mixture was incubated for 72 hours. Twenty μ l of MTT (5 mg/ml) was added to each well and the plates were further incubated for 4 hours. After the medium was removed and 150 μ l DMSO was added to each well, the absorbance at 570 nm was measured, and the cytotoxicity was calculated by comparison of the absorbance of the sample wells with that of the control wells. (Table S1, Figure S2)

Detection of intracellular paclitaxel content

The cells were seeded in 6-well plates. After growing to 90% confluence, the cells were treated with PTX (10 μ g/ml) and EP (10 μ g/ml) or PTX (10 μ g/ml) alone for 2.5 hours. The mediums were removed and cells were washed with PBS twice. After trypsin digestion, the cells were harvested into centrifuge tubes, centrifuged and the supernatants were discarded, the cells were re-suspended in H₂O and the mixture was centrifuged again, then the supernatants were removed. To the cells in each tube was added 500 μ l MeOH and the mixture was sonicated to have the cells disrupted. Then, the mixtures were centrifuged at 12000 g for 20 min. The resulting supernatants were passed through an ODS cartridge followed by filtration with 0.22 μ m filters. The filtrates were analysis by UHPLC-MS.

Statistical analysis

Bioassays and quantifications were carried out in triplicates. Statistical analysis and significances were calculated using Microsoft Office Excel 2010 (Microsoft Corporation, Redmond, WA 98052 USA).

Table S1. IC₅₀ $\pm$ SD (μ g/ml) on cancer and normal cells of compounds from *L. fenzlii*

	MGC-803 ^a	A549 ^b	Hela ^c	GES-1 ^d
EP (1)	15.2 $\pm$ 0.88	17.2 $\pm$ 0.41	13.6 $\pm$ 0.31	>100.0 $\pm$ 0.00
9(11)-DHEP (2)	7.8 $\pm$ 0.80	9.7 $\pm$ 0.53	7.6 $\pm$ 0.42	8.7 $\pm$ 0.18
Compound 3	51.6 $\pm$ 1.34	>100.0 $\pm$ 0.00	>100.0 $\pm$ 0.00	>100.0 $\pm$ 0.00
Compound 4	>100.0 $\pm$ 0.00	>100.0 $\pm$ 0.00	>100.0 $\pm$ 0.00	>100.0 $\pm$ 0.00
Compound 5	>100.0 $\pm$ 0.00	>100.0 $\pm$ 0.00	>100.0 $\pm$ 0.00	>100.0 $\pm$ 0.00
Compound 6	>100.0 $\pm$ 0.00	>100.0 $\pm$ 0.00	>100.0 $\pm$ 0.00	>100.0 $\pm$ 0.00
Paclitaxel ^e	0.15 $\pm$ 0.01	0.21 $\pm$ 0.02	0.12 $\pm$ 0.01	0.38 $\pm$ 0.01

^ahuman gastric cancer cell line; ^bhuman lung cancer cell line; ^chuman cervical cancer cell line; ^dhuman gastric mucosa cell line; ^ePaclitaxel was used as positive control.

Figure captions

Figure S1. Chemical structures of compounds isolated from *Lasiosphaera fenzlii*

Figure S2. The cytotoxicity of EP on different cancer and normal cells after treatment for 72 hours; X axis is the EP concentrations in $\mu\text{g/ml}$. GES-1: human gastric mucosal epithelium cells; HBE: human bronchial epithelium cells; HUVEC: human umbilical vein endothelial cells; Hela: human cervical cancer cells; MGC-803: human gastric cancer cells; A431: human skin cancer cells.

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