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Microbial transformation of danazol with *Cunninghamella blakesleeana* and anti-cancer activity of danazol and its transformed products

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ABSTRACT

Biotransformation of danazol (**1**) (17β-hydroxy-17α-pregna-2,4-dien-20-yno-[2,3-d]-isoxazole) with *Cunninghamella blakesleeana* yielded three new metabolites **2–4** and a known metabolite **5**. These metabolites were identified as 14β,17β-dihydroxy-2-(hydroxymethyl)-17α-pregn-4-en-20-yn-3-one (**2**), 1α,17β-dihydroxy-17α-pregna-2,4-dien-20-yno-[2,3-d]-isoxazole (**3**), 6β,17β-dihydroxy-17α-pregna-2,4-dien-20-yno-[2,3-d]-isoxazole (**4**), and 17β-hydroxy-2-(hydroxymethyl)-17α-pregn-1,4-dien-20-yn-3-one (**5**). Danazol (**1**) and its derivatives were evaluated against cervical cancer cell line (HeLa). Compound **1** showed a potent cytotoxicity with IC_{50} = 0.283 ± 0.013 μM, as compared to doxorubicin (IC_{50} = 0.506 ± 0.015 μM), where compound **3** was also found to be significantly active with IC_{50} = 13.427 ± 0.819 μM.

1. Introduction

Biotransformation is an important tool for the synthesis of medicinally important organic compounds. The use of biocatalysis for the stereoselective synthesis of chiral molecules has several advantages over classical chemical synthesis. Biocatalyzed reactions are generally regio- and stereo-selective, cost effective and environmental friendly [1–6]. Microbial cultures are frequently used for biocatalysis. Microorganisms contain a large variety of enzymes which catalyze many chemical reactions, including oxidation, hydroxylation, and reduction. Fungi can also serve as microbial models of mammalian steroid drug metabolism. Microbial cytochrome P450 monooxygenase system facilitates stereoselective hydroxylation at multiple sites of steroidal skeleton. Stereoselective hydroxylation of steroids at non activated position have been achieved by using various fungi [7–9].

Danazol (**1**) is a heterocyclic steroidal drug in which an isoxazole ring is fused with ring-A of a steroidal nucleus. It is a synthetic analogue of 17α-ethinyltestosterone. Danazol (**1**) inhibits endometrial tumor cell migration and invasive activity [10]. Compound **1** is orally effective, and used for the treatment of endometriosis, and for the inhibition of pituitary gonadotropin. It is also used in the treatment of precocious puberty, and benign fibrocystic mastitis [11–14].

Cancer is a major cause of human mortality. Many current chemotherapic agents against cancers, such as doxorubicin, function by highly toxic, untargeted DNA damage mechanisms. Their efficacy is now seriously challenged by emerging multidrug-resistant in cancer cells [15–17]. However, hormonal therapies targeting cancer do not rely on DNA damage. The HeLa cell line is derived from a human cervical cancer and is a common model to study preliminary anti-cancer potential of chemical compounds [18]. Initially, we evaluated danazol (**1**) against the HeLa cell line, where it showed an excellent cytotoxic effect. Therefore, we decided to evaluate the transformed products of **1** against this cell line. Interestingly, only compound **3** showed some level of cytotoxicity against HeLa cell line.

This manuscript is in the continuation of our studies on the biotransformation of bioactive steroidal compounds [19–23]. We subjected danazol (**1**) to biotransformation with *C. blakesleeana*, which yielded three new metabolites **2–4**, and a known metabolite **5** (Fig. 1).

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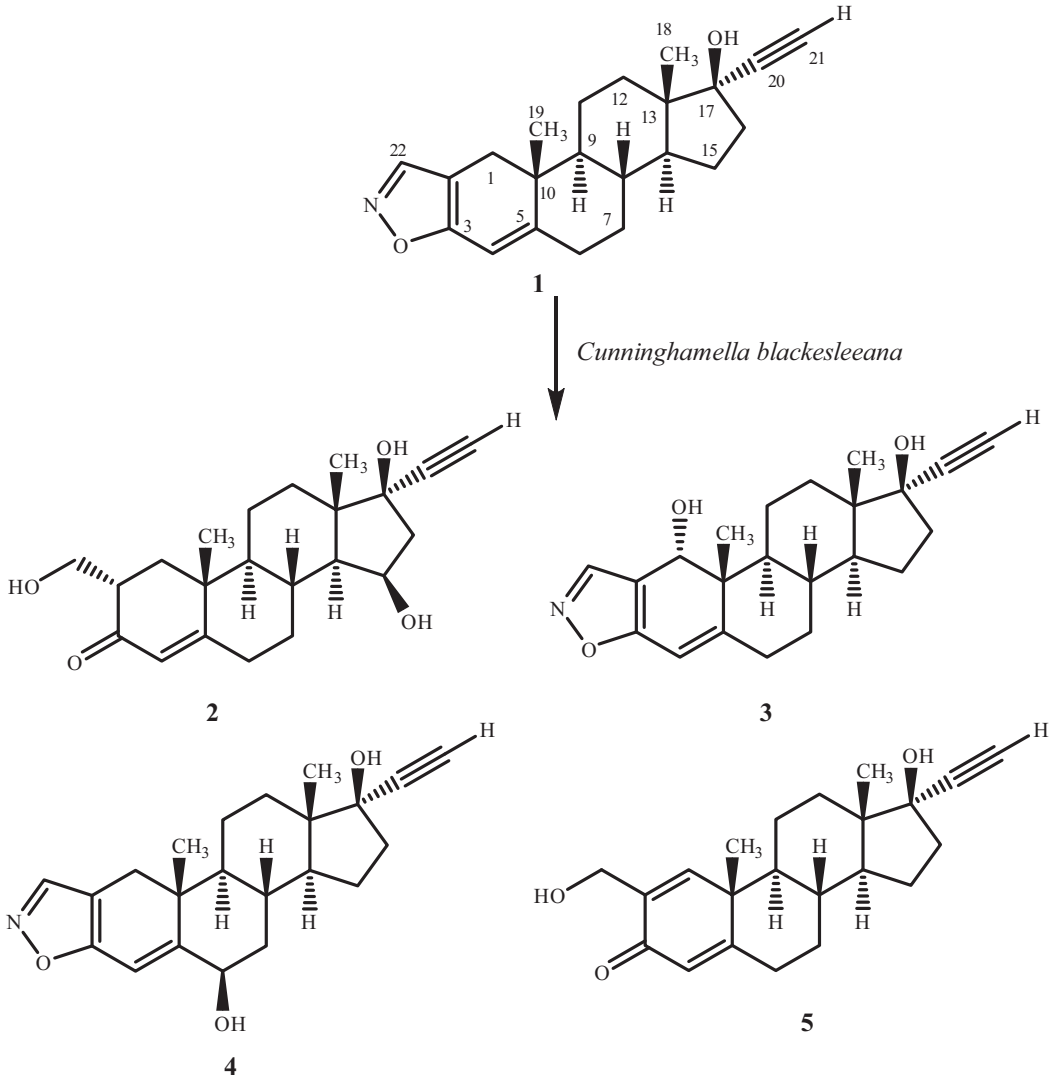

Fig. 1. Biotransformation of danazol (**1**) with *Cunninghamella blackesleeana*.

2. Experimental

2.1. General experimental conditions

Danazol (**1**) was isolated from a drug Danocrine (brand name), purchased from local market. The purity of compound was checked through TLC and ^{1}H NMR spectroscopy. Precoated TLC plates were used (Merck KGaA, PF_{254}, Germany). Silica gel (E. Merck, Germany) was used in column chromatography. Isolated compounds were purified on reverse phase recycling HPLC-LC-908. The HPLC was equipped with JAIGEL-ODS-L-80 column (L = 250 mm, I.D. = 20 mm). Buchi M-560 apparatus was used for determining melting points. Optical rotations of isolated compounds were measured on JASCO P-2000 polarimeter. ^{1}H and ^{13}C NMR experiments were performed on Bruker Avance spectrometers. UV (in nm) absorbance were recorded in methanol on Shimadzu UV 240 spectrophotometer (Shimadzu Corporation, Tokyo, Japan). Infrared (IR) spectra (in cm^{-1}) were recorded with an FT-IR-8900 spectrophotometer. EI-MS and HREI-MS of isolated compounds were recorded on Jeol JMS-600H mass spectrometer (Japan).

2.2. Fungal culture and medium

The fungal culture of *Cunninghamella blakesleeana* (ATCC 8688A) was acquired from ATCC (American Type Culture Collection). *C. blakesleeana* strains were grown on Sabouraud dextrose agar, and stored at 4 °C.

Following ingredients were used for one liter of medium for *C. blakesleeana* (ATCC 8688A) culture; KH_2PO_4 (5.0 g), glucose (10.0 g), peptone (5.0 g), NaCl (5.0 g), and glycerol (10.0 mL). All the ingredients were mixed in distilled H_2O.

2.3. Fermentation and extraction conditions of danazol (*1*) with C. blakesleeana

The experiment was performed in two stages. First small-scale screening was carried out, followed by preparative scale fermentation. During small-scale screening, 600 mL culture medium was prepared by using above mentioned ingredients and transferred to six flasks of 250 mL (each flasks contain 100 mL of medium). Among them, four flasks were used as test flasks and two were used as control flasks. The test flask contained fungal culture,