Steroids 115 (2016) 56–61

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Microbial transformation of contraceptive drug etonogestrel into new metabolites with *Cunninghamella blakesleeana* and *Cunninghamella echinulata*

Elias Baydoun [a,*], Atia-tul Wahab [b], Nayab Shoaib [c], Malik Shoaib Ahmad [c], Roula Abdel-Massih [d], Colin Smith [a], Nimra Naveed [c], M. Iqbal Choudhary [b,c,e,*]

[a] Department of Biology, American University of Beirut, Beirut 1107 2020, Lebanon
[b] Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
[c] H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
[d] Department of Biology, University of Balamand, Tripoli, Lebanon
[e] Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah 21412, Saudi Arabia

ARTICLE INFO

Article history:
Received 18 April 2016
Received in revised form 26 July 2016
Accepted 1 August 2016
Available online 5 August 2016

Keywords:
Etonogestrel
Contraceptive
Biotransformation
Cunninghamella blakesleeana
Cunninghamella echinulata
β-Glucuronidase inhibition
Colon cancer

ABSTRACT

Biotransformation of a steroidal contraceptive drug, etonogestrel (**1**), (13-ethyl-17β-hydroxy-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-3-one) was investigated with *Cunninghamella blakesleeana* and *C. echinulata*. Five metabolites **2–6** were obtained on incubation of **1** with *Cunninghamella blakesleeana*, and three metabolites, **2**, **4**, and **6** were isolated from the transformation of **1** with *C. echinulata*. Among them, metabolites **2–4** were identified as new compounds. Their structures were deduced as 6β-hydroxy-11,22-epoxy-etonogestrel (**2**), 11,22-epoxy-etonogestrel (**3**), 10β-hydroxy-etonogestrel (**4**), 6β-hydroxy-etonogestrel (**5**), and 14α-hydroxy-etonogestrel (**6**). Compounds **1–6** were evaluated for various biological activities. Interestingly, compound **5** was found to be active against β-glucuronidase enzyme with IC_{50} value of 13.97 ± 0.12 μM, in comparison to standard compound, D-saccharic acid 1,4-lactone (IC_{50} = 45.75 ± 2.16 μM). Intestinal bacteria produce β-glucuronidase. Increased activity of β-glucuronidase is responsible for the hydrolyses of glucuronic acid conjugates of estrogen and other toxic substances in the colon, which plays a key role in the etiology of colon cancer. Inhibition of β-glucoronidase enzyme therefore has a therapeutic significance. Compounds **1–6** were also found to be non cytotoxic against 3T3 mouse fibroblast cell lines.

1. Introduction

Biocatalysis is extensively used for the stereoselective synthesis of chiral molecules. The reactions catalyzed by biocatalysts are regio- and stereo-selective. Many of such reactions are not possible through conventional chemical methods [1–6]. Microbial cells are factories of enzymes that catalyze a number of chemical reactions, such as oxidation, reduction, hydroxylation, and epoxydation. Microorganisms, particularly fungi, contain cytochrome P450 monooxygenase systems, which are responsible for stereoselective hydroxylation at various sites of the steroidal skeleton. Fungi are also used as models for the study of drug metabolism [7–9].

Etonogestrel (**1**) is used as a hormonal contraceptive. It is a steroidal progestin available as subdermal implant. Nexplanon and implanon are two well known products of etonogestrel, marketed as subdermal contraceptive implants that provide effective contraception for several years [10,11]. They have excellent reversibility: fertility is restored within a month after removal. Implanon is also safe for breastfeeding mothers, has an estrogen-sparing effect, and does not affect the bone mineral density [12,13]. It is a drug of choice for adolescents and women with diabetes mellitus, systemic hypertension, endometriosis, and anemia [14–17]. In order to synthesize new derivatives of **1,** we subjected etonogestrel (**1**) to transformation with *Cunninghamella blakesleeana* and *C. echinulata.* This was in continuation of our research on biotransformation of bioactive compounds [18–20]. The biotransformation of etonogestrel (**1**) with these fungi has yielded three new (**2–4**), and two

* Corresponding authors at: Department of Biology, American University of Beirut, Beirut 1107 2020, Lebanon (E. Baydoun), H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan (M.I. Choudhary).

E-mail addresses: eliasbay@aub.edu.lb (E. Baydoun), iqbal.choudhary@iccs.edu (M.I. Choudhary).

http://dx.doi.org/10.1016/j.steroids.2016.08.003
0039-128X/

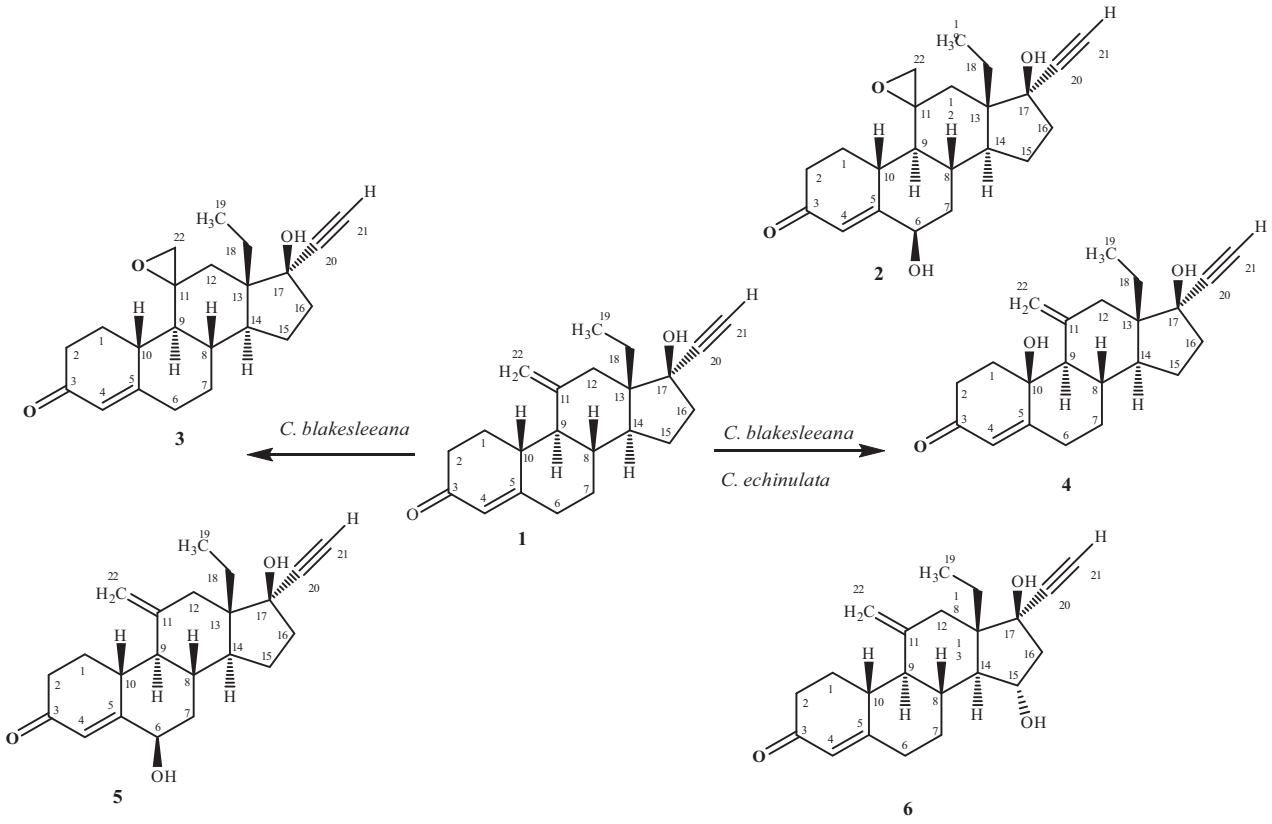

Fig. 1. Biotransformation of etonogestrel (**1**) with *Cunninghamella blakesleeana and C. echinulata.*

known (**5** and **6**) metabolites (Fig. 1). All isolated metabolites, and substrate **1**, were evaluated for β-glucuronidase inhibitory activity. Compound **5** was found to be active against β-glucuronidase enzyme.

β-Glucuronidase is an exoglycosidase enzyme. Its increased levels create problems in detoxification of various toxic substances in body. Increased activity of β-glucuronidase is also associated with colon cancer. A number of carcinogenic substances along with endogenously produced toxic metabolites, such as steroids, undergo metabolism in the liver. These substances are then conjugated with glucuronic acid before their excretion into the small intestine *via* the bile. These conjugates undergo hydrolyses in the colon, catalyzed by β-glucuronidase enzyme produced by intestinal bacteria. Therefore, β-glucuronidase plays a key role in the etiology of colon cancer [21–25]. The inhibition of β-glucuronidase enzyme could help to overcome such types of complications. Interestingly, compound **5** was found to be a potent inhibitor of β-glucuronidase with IC_{50} value of 13.97 ± 0.12 μM, whereas metabolite **3** showed a weak activity (IC_{50} = 222.80 ± 5.60 μM), in comparison to standard compound D-saccharic acid 1,4-lactone (IC_{50} = 45.75 ± 2.16 μM).

2. Experimental

2.1. General experimental conditions

Etonogestrel (**1**), $C_{22}H_{25}O_2$, was purchased from Haihang Industry Co., Ltd. (Jinan, China). Silica gel, TLC plates (PF_{254}; 20 × 20, 0.25 mm) were acquired from Merck (Darmstadt, Germany). Ceric sulfate was used to visualize compounds on TLC. Column chromatography was carried with silica gel (70–230 mesh, Merck, Darmstadt, Germany). A recycling preparative HPLC (JAI LC-908W, Japan), equipped with YMC M-80 (4–5 μm, 20 × 250 mm i.d.), was used to purify etonogestrel metabolites. A JEOL JMS-600H (Japan) mass spectrometer was used to record EI-MS and high HREI-MS. Bruker Avance NMR spectrometers were used to record ^1H and ^{13}C NMR spectra (Bruker, Wissembourg, France). UV absorptions were recorded on Evolution 300 spectrophotometer (Thermo Scientific, England). Melting points were observed using a Buchi-560 apparatus (Japan). Optical rotations were measured in methanol with a JASCO P-2000 polarimeter (Japan). IR spectra (cm^{-1}) were recorded on Vector 22 spectrophotometer (Bruker, France).

2.2. Fungal cultures and media

Fungal cultures of *Cunninghamella blakesleeana* (ATCC 8688A), and *Cunninghamella echinulata* (ATCC 9244) were obtained from ATCC, grown on Sabouraud dextrose agar, and stored at 4 °C. Culture media were prepared by dissolving following ingredients in 1 L of solvent; glucose (10.0 g), peptone (5.0 g), KH_2PO_4 (5.0 g), NaCl (5.0 g), and glycerol (10.0 mL) for each liter.

2.3. Biotransformation etonogestrel (*1*) with Cunninghamella blakesleeana and Cunninghamella echinulata

Growth media (6 L for each fungus) was prepared in distilled H_2O by using above chemicals, transferred into 60 conical flasks of 250 mL equally (each containing 100 mL) and autoclaved at 121 °C. One seed flask for each fungus was inoculated, and incubated for 3 days with shaking (128 rpm) at 26 °C. Once grown, the seed cultures were distributed to the remaining flasks, and incubation was continued with shaking at 26 °C for four days. Compound **1** (0.500 g) was dissolved in 60 mL of methanol, and evenly dispensed to 60 flasks containing mature growths of *Cunninghamella blakesleeana*. The cultures were incubated with