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Cunninghamella blakesleeana-mediated biotransformation of a contraceptive drug, desogestrel, and anti-MDR-*Staphylococcus aureus* activity of its metabolites

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ABSTRACT

Staphylococcus aureus is one of the most infectious agents among staphylococcal bacteria. Currently many strains of *S. aureus* have developed resistance against available antibiotics. Therefore, the treatment of infections caused by them is a major challenge. During current study, desogestrel (**1**), a contraceptive drug, was found to be a potent growth inhibitor of drug resistant strains of *S. aureus*. Therefore, in search of new and effective agents against multi-drug resistant *S. aureus* strains, whole-cell bio-catalytic conversion of desogestrel (**1**) by *Cunninghamella blakesleeana* ATCC 8688A at pH 7.0 and 25 °C was carried out, yielding three new metabolites, 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-6β,15β,17β-triol (**2**), 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-3β,6β,17β-triol (**3**), and 13-ethyl-11-methylene-18,19-dinor-17α-pregn-20-yn-3α,5α,6β,17β-tetraol (**4**), along with a known metabolite, 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-6β,17β-dihydroxy-3-one (**5**). Among them, compounds **1**–**2** showed a potent activity against *S. aureus* EMRSA-17, *S. aureus* NCTC 13277 (MRSA-252), and *S. aureus* NCTC 13143, and clinically isolated Pakistani strain of *S. aureus* in an *in vitro* Microplate Alamar Blue Assay (MABA). Vancomycin was used as the standard drug in this assay. In addition, compound **1** also showed a significant activity against vancomycin-resistant *S. aureus* (VRSA) ATCC 700699. Compounds **1**–**5** were also evaluated against 3T3 normal cell line (mouse fibroblast) where they all were identified as non-cytotoxic. The present study thus provides new leads for the development of anti-bacterial drugs against MDR *S. aureus*.

1. Introduction

Due to the emergence of resistance against available antibiotics, and rapid pathogenic mutation rate, the treatment of bacterial infections have become a global health challenge [1,2]. *S. aureus,* gram positive bacteria, is an etiological agent of many infections, ranging from mild to life threatening infections, *e.g.*, skin and soft-tissue infections, blood stream infections, pneumonia, *etc*. It is the most common causative agent of blood stream infections in the Canada, United States, and Latin America. Each year, around 50,000 patients die in the USA alone due to *S. aureus* infections [3–7]. Asia is also a region with the highest prevalence of drug resistant *S. aureus* infections worldwide. The dissemination of anti-biotic resistant *S. aureus* infections are serious public health problems in many countries of Asia [8].

Natural products, such as steroids, terpenes, and flavonoids have been the most effective compounds for the treatment of many diseases, including cancer, immune system disorders, cardiovascular diseases, and bacterial, viral and fungal infections since several decades. Due to the presence of many asymmetric centers and inert hydrocarbon skeleton, derivatization of natural products is often a challenging task through conventional methodologies. However, biotransformation by microorganisms, *i.e.*, bacteria or fungi, and plant cell cultures and pure enzymes is an effective and efficient

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approach for the synthesis of structural analogues of natural products. Resulting products are regio-, stereo-, and chemo-selective due to the catalytic action of a range of enzymes. At present, biotransformation techniques are successfully used in various fields, such as medicines, agriculture, polymer, and food industry, *etc.* due to selective nature of biological catalysts (enzymes) [9–13].

Desogestrel (13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol) (**1**) is an orally active third generation contraceptive steroidal drug. It is a synthetic progestin, used successfully in hormonal contraceptives with low or no androgenic activity. The rational to develop this synthetic progestin was to replace the use of classical oral progestogens that possess androgenic effects, specifically harmful effects on plasma lipids. Like traditional progestogen-only pills (POPs) containing 30 μg levonorgestrel, and 75 μg desogestrel also have low effects on the metabolism of lipids. Therefore, the POPs have been marketed for over three decades. However, due to lower contraceptive efficacy, and random bleeding pattern, POPs have been less accepted than the combined oral contraceptive pills (COCPs) by the prescribers, and consumers [14–16].

In continuation of our work on fungal-mediated synthesis of bioactive steroids [17–20], including 17α-ethynyl substituted steroidal drugs [21–23], an oral contraceptive drug, desogestrel (**1**) ($C_{22}H_{30}O$), was incubated with *C. blakesleeana*, yielding metabolites **2**–**5** (Fig. 1). Interestingly, substrate **1** and its metabolite **2** showed a potent anti-bacterial activity against resistant strains of *S. aureus,* while metabolites **3**–**5** showed no growth inhibition of these bacterial strains.

2. Materials and methods

2.1. Fungal cell culture and media

The fungal cell culture of *C. blakesleeana* (ATCC 8688A) was used for biotransformational study. *C. blakesleeana* was grown on SDA slants (Sabouraud Dextrose Agar), and maintained at 4 °C.

The media (1 L) was prepared by adding 10 g glucose, 5 g peptone, 5 g NaCl, 5 g KH_2PO_4, and 10.0 mL glycerol in 1 L distilled water for the maximum growth of *C. blakesleeana.*

2.2. Bacterial strains and media

Drug resistant *S. aureus* strains, *S. aureus* EMRSA-17, *S. aureus* NCTC 13277, *S. aureus* NCTC 13143, clinically isolated *S. aureus,* and vancomycin-resistant *S. aureus* ATCC 70-069 were targeted

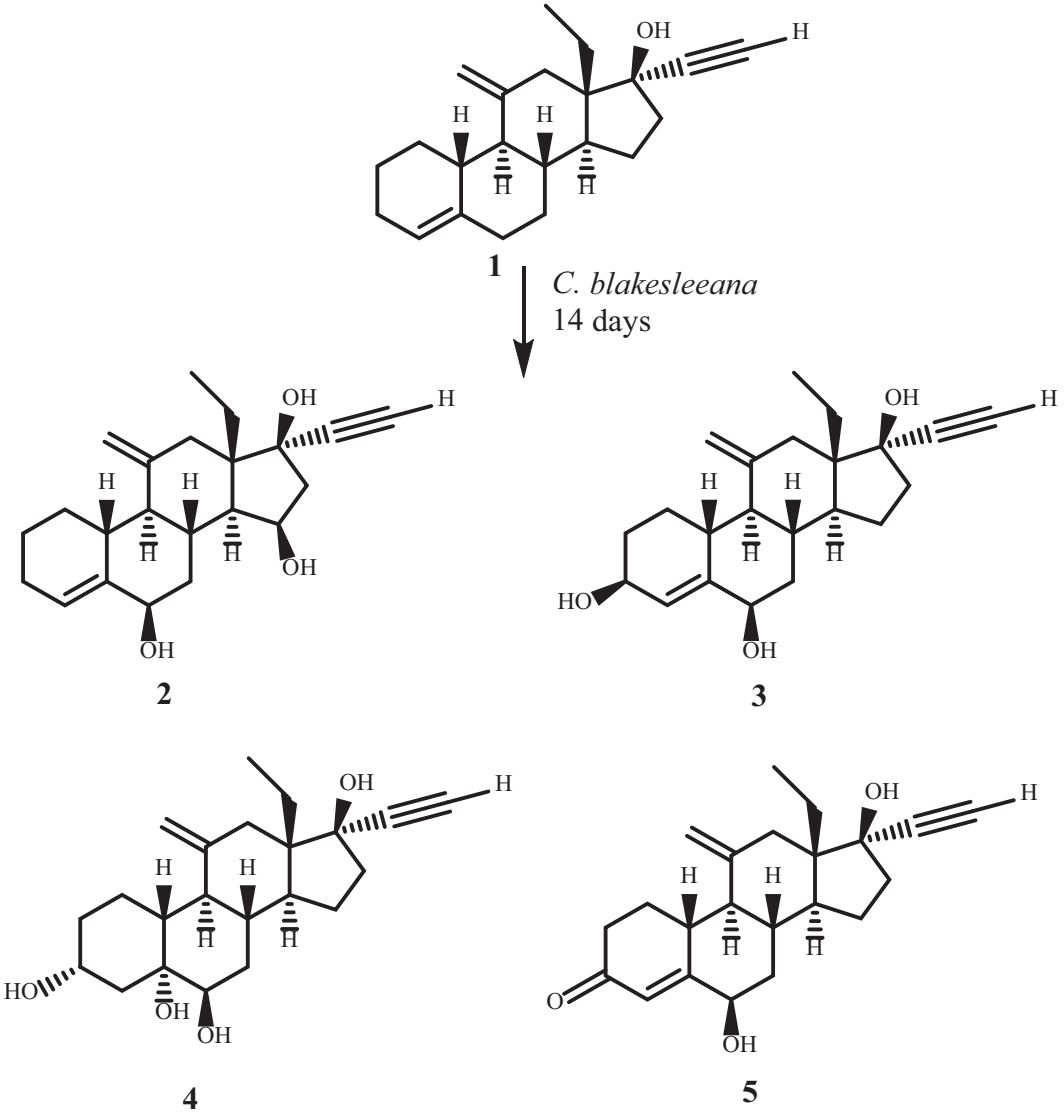

Fig. 1. Biotransformation of desogestrel (1) with *Cunninghamella blakesleeana.*

Table 1
^{1}H-NMR chemical shift data (J and $W_{½}$ in Hz) of compounds **1**–**5** (δ ppm).

Position	**1**[a]	**2**[b]	**3**[b]	**4**[a]	**5**[a]
1	2.23, m; 1.13, m	1.63, overlap; 1.55, m	2.39, overlap; 1.05, overlap	1.23, overlap, 2(H)	1.94, m; 1.28, m
2	1.62, overlap 2[H]	2.31, overlap; 1.13, overlap	1.91, overlap; 1.38, overlap	1.91, overlap; 1.27, overlap	2.54, m; 1.59, m
3	1.92, overlap 2[H]	1.91, overlap 2[H]	4.10, overlap ($W_{½}$ = 18.9)	3.91, br. d ($J_{e/e}$ = 2.5, $W_{½}$ = 9.6)	–
4	5.45, br. s	5.71, br. s	5.51, br. s	1.90, overlap; 1.64, overlap	5.92, br. s
5	–	–	–	–	–
6	2.18, m; 1.94, overlap	4.18, overlap ($W_{½}$ = 6.4)	4.15, br. s ($W_{½}$ = 6.3)	3.41, br. t ($J_{6e/7e}$ = 2.5, $W_{½}$ = 6.7)	4.31, br. t ($J_{6e/7e}$ = 2.8, $W_{½}$ = 8.1)
7	1.68, m; 0.88, m	2.13, overlap; 1.21, overlap	1.89, overlap; 1.12, overlap	1.92, overlap; 1.52, overlap	1.94, m; 1.28, m
8	2.25, m	2.14, overlap	2.67, overlap	1.89, overlap	2.04, m
9	1.81, m	1.34, m	1.25, overlap	1.25, overlap	1.52, overlap
10	1.29, m	2.66, overlap	1.85, m	1.88, overlap	3.03, m
11	–	–	–	–	–
12	2.64, m; 2.26, m	2.68, overlap; 2.22, overlap	2.65, overlap 2.26, m	2.64, overlap; 2.27, overlap	2.71, overlap; 2.32, overlap
13	–	–	–	–	–
14	1.28, m	1.66, overlap	1.79, overlap	2.61, overlap	1.87, m
15	1.63, m; 1.39, m	4.16, overlap ($W_{½}$ = 5.1)	1.51, overlap; 1.33, overlap	1.71, overlap; 1.34, overlap	1.63, m; 1.37, m
16	2.26, m; 2.05, m	2.71, overlap; 2.12, overlap	2.26, overlap; 2.06, overlap	2.28, overlap; 2.06, overlap	2.31, overlap; 2.10, overlap
17	–	–	–	–	–
18	1.41, m; 1.01, m	1.55, overlap; 1.49, overlap	1.49, m; 1.00, m	1.62, overlap; 1.38, overlap	1.48, m 2[H]
19	1.02, t ($J_{19/18}$ = 7.8)	1.03, t ($J_{19/18}$ = 7.8)	1.01, t ($J_{19/18}$ = 7.5)	1.02, t ($J_{19/18}$ = 7.5)	1.05, t ($J_{19/18}$ = 7.5)
20	4.95, s; 4.75, s	5.01, s; 4.81, s	4.99, s; 4.81, s	4.98, s; 4.76, s	5.05, s; 4.90, s
21	–	–	–	–	–
22	2.90, s	2.91, s	2.91, s	2.92, s	2.91, s

[a] 500 MHz.
[b] 600 MHz.

in this study. All the bacterial strains were grown on SDA slants, and maintained at 4 °C.

2.3. General experimental conditions

Desogestrel (**1**) ($C_{22}H_{30}O$) was acquired from Haihang Industry Co., Ltd. (China). Ingredients for media preparation were purchased from Sigma-Aldrich (Germany), Oxoid Ltd. (England), and Daejung Chemicals and Metals Co., Ltd. (Korea). The degree of transformations and purity of compounds were analyzed by using silica coated thin layer chromatographic plates (PF_{254}, Merck KGaA, Germany). Fractionations of gummy crude extract was carried out by column chromatography (CC) (Silica gel, 70–230 mesh, E. Merck, Germany). All the fractions were finally purified through RP-HPLC (LC–908, Japan), equipped with YMC M-80 (20–250 mm i.d. 4–5 μm). JASCO P–2000 polarimeter (Japan), Bruker Vector 22 spectrophotometer (France), and Buchi 560 device (Switzerland) were used for the measurements of optical rotations, IR absorbances, and melting points of all purified compounds, respectively. ^{1}H- (500, and 600 MHz), ^{13}C- (125 MHz) and 2D-NMR spectra were recorded in CD_3OD on Bruker Avance-NMR (Switzerland). EI- and HREI-MS of all compounds were determined on Jeol JMS-600H mass spectrometer (Japan), while Evolution 300 UV–visible spectrophotometer (England) was used for recording of UV absorbances. Analytical grade solvents were used in this research work.

2.4. Fermentation and extraction protocol of desogestrel (*1*) with C. blakesleeana

Based on small scale screening results, 4 L media for the growth of *C. blakesleeana* was prepared by mixing aforementioned media ingredients in 4 L distilled water, and distributed into 250 mL of 40 Erlenmeyer flasks (100 mL in each), cotton plugged, and autoclaved at 121 °C. This sterilized media was cooled at room temperature, and inoculated with seed flasks of *C. blakesleeana* under sterilized conditions. The flasks were kept for 3–4 days on a shaker (121 rpm) at 25 °C for growth of fungi. After mature growth of *C. blakesleeana* in each flask, 1 *g* of desogestrel (**1**) ($C_{22}H_{30}O$) was dissolved in 20 mL of methanol, dispensed (0.5 mL) in each flask, and again placed on a shaker (121 rpm) at 25 °C for fourteen days.

After fourteen days incubation period, the reaction was stopped by adding dichloromethane (DCM) in each flasks, and filtered in order to separate fungal mass. The filtrate (organic + aqueous phase) was separated by extracting with 12 L DCM. The organic phase was made moisture free by adding anhydrous sodium sulfate (Na_2SO_4), filtered, and then concentrated under reduced pressure. This yielded 1.5 g brownish gummy crude which was fractionated by column chromatography (CC) with a mobile phase of hexanes-acetone. By increasing 5–100% gradients of acetone, the polarity of mobile phase was increased. As a result, four main fractions (1–4) were obtained which were analyzed by thin layer chromatography (TLCs). The fractions were purified through recycling RP-HPLC. Compounds **2** (methanol–water; 7/3, R_T = 29 min), **3** (methanol–water; 6/4, R_T = 26 min), **4** (methanol–water; 6/4, R_T = 22 min), and **5** (methanol–water; 7/3, R_T = 27 min) were purified from fractions 1–4, respectively, through recycling RP-HPLC.

2.4.1. 13-Ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-3β-15β-17β-triol (*2*)

White solid; m. p. 246–248 °C; $[\alpha]_D^{25}$ = −310 (*c* 0.001, MeOH); IR (CH_3Cl): υ_{max} (cm^{-1}) 3393, 3303 (OH), 2926 (CH), 1644 (C=C); HREI-MS *m*/*z* 342.2179 [M^+] ($C_{22}H_{30}O_3$, calcd. 342.2195); EI-MS

Table 2
^{13}C-NMR chemical shift data of compounds **1–5** (δ ppm) at 125 MHz NMR spectrometer.

Carbons	1	2	3	4	5
1	30.5	30.1	27.9	29.5	37.5
2	22.8	22.7	32.3	25.2	29.0
3	26.7	26.5	67.8	69.6	203.3
4	122.2	126.5	129.7	40.3	126.7
5	149.3	148.4	144.0	76.1	168.4
6	36.6	74.2	73.3	74.4	72.2
7	33.2	38.1	38.8	29.0	38.2
8	37.9	33.6	33.5	38.7	35.9
9	53.7	56.0	56.1	47.5	54.9
10	44.2	33.1	36.4	43.7	34.8
11	141.0	142.1	148.5	149.5	148.2
12	41.8	41.8	41.7	41.6	41.6
13	51.6	51.5	49.8	51.4	51.7
14	56.4	58.2	53.6	56.1	53.4
15	23.0	68.6	22.6	21.8	22.6
16	40.5	52.3	40.4	40.5	40.5
17	81.4	81.3	81.3	81.4	81.3
18	20.9	22.9	20.9	20.8	20.9
19	9.6	9.7	9.5	9.7	9.54
20	108.8	109.1	109.2	109.3	109.3
21	89.1	88.7	89.0	89.1	89.0
22	74.8	74.0	74.8	74.8	74.9

m/z (%): 342.1 [M$^+$] (2.2), 324.2 (4.8), 306.1 (6.9), 295.2 (2.7), 256.1 (7.0), 227.1 (100.0); ^{1}H NMR (CD_3OD, 600 MHz) Table 1; ^{13}C NMR (CD_3OD, 125 MHz); Table 2.

2.4.2. 13-Ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-6β-15β-17β-triol (**3**)

White solid; m. p. 241–243 °C; $[\alpha]_D^{25}$ = −299 (*c* 0.001, MeOH); IR ($CHCl_3$): υ_{max} (cm^{-1}) 3388, 3294, (OH), 2935 (CH), 1641 (C=C); HREI-MS *m/z* 342.2185 [M$^+$] ($C_{22}H_{30}O_3$, calcd. 342.2195); EI-MS *m/z* (%): 342.3 [M$^+$] (41), 324.2 (61), 277.2 (78), 241.2 (69), 133.0 (100); ^{1}H NMR (CD_3OD, 600 MHz) Table 1; ^{13}C NMR (CD_3OD, 125 MHz) Table 2.

2.4.3. 13-Ethyl-11-methylene-18,19-dinor-17α-pregn-20-yn-3α-5α-6β-17β-tetraol (**4**)

White solid; m. p. 239–242 °C; $[\alpha]_D^{25}$ = −282 (*c* 0.001, MeOH); IR ($CHCl_3$): υ_{max} (cm^{-1}) 3379, 3255 (OH), 2929 (CH), 1637 (C=C); HREI-MS *m/z* 360.2305 [M$^+$] ($C_{22}H_{30}O_4$, calcd. 360.2301); EI-MS *m/z* (%): 360.2 [M$^+$] (09), 342.3 (17), 277.2 (37), 133.1 (48), 91.1 (65); ^{1}H NMR (CD_3OD, 500 MHz) Table 1; ^{13}C NMR (CD_3OD, 125 MHz) Table 2.

2.4.4. 13-Ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-6β-17β-dihydroxy-3-one (**5**)

White solid; m. p. 234–236 °C; IR ($CHCl_3$): υ_{max} (cm^{-1}) 3379, 3255 (OH), 2929 (CH), 1637 (C=C); EI-MS *m/z* (%): 340.3 [M$^+$] (56), 322.3 (62), 254.2 (77), 133.0 (100), 90.9 (70); ^{1}H NMR (CD_3OD, 500 MHz) Table 1; ^{13}C NMR (CD_3OD, 125 MHz) Table 2.

2.5. Anti-bacterial activity evaluation

Compound **1**, and its metabolites **2–5**, were screened against *S. aureus* EMRSA-17, *S. aureus* NCTC 13277, *S. aureus* NCTC 13143, clinically isolated *S. aureus*, and vancomycin-resistant *S. aureus* ATCC 700699 by using *in vitro* assay, MABA (Microplate Alamar Blue Assay). Whereas vancomycin was used as standard drug.

In this assay, bacterial cells were grown in Muller Hinton medium and inoculums were adjusted to 0.5 McFarland turbidity index. Stock solutions of all test compounds **1–5** were prepared in DMSO and dispensed in 96-well plates up to the volume of 200 μL. 5x10^6 bacterial cells were added in all wells, negative control, and test compounds. The plates were sealed with parafilm and incubated for 18–20 h. After incubation, alamar blue dye was dispensed in each well, followed by shaken at 80 RPM in a shaking incubator for 2–3 h. The change in a color of alamar blue dye from blue to pink, indicated the growth of bacterial strains. The absorbances were recorded at 570, and 600 nm by using ELISA reader. The percent growth inhibition results of test compounds are presented in Table 3.

$$\%\ Inhibition = \frac{(OX)\ \lambda 2A\ \lambda 1\ -\ (OX)\ \lambda 1A\ \lambda 2}{(RED)\ \lambda 1A\ \lambda 2\ -\ (RED)\ \lambda 2A'\ \lambda 1} \times 100$$

Whereas;

OX = Molar Extinction coefficient of Alamar Blue oxidized form (Blue)
RED = Molar Extinction coefficient of Alamar Blue reduced form (Pink)
A = Absorbance of test wells
A′ = Absorbance of negative control well
λ1 = 570 nm
λ2 = 600 nm

3. Results and discussion

Fermentation of desogestrel (**1**), ($C_{22}H_{30}O$, *m/z* 310.2), with *C. blakesleeana* yielded three new metabolites **2–4**, along with a known compound **5**.

The HREI-MS of compound **2** showed the [M$^+$] at *m/z* 342.2179 ($C_{22}H_{30}O_3$, calcd. 342.2195), indicating addition of two oxygen atoms as hydroxyl groups in substrate **1** (*m/z* 310). The IR absorbances at 3872, 3393, 3303, and 1644 cm^{-1} were due to the presence of OH, and C=C groups, respectively. The ^{13}C-NMR spectrum of compound **2** showed additional signals for two new methine carbons at δ 74.2 and 68.6 as compared to substrate **1**, indicating the hydroxylation of substrate **1** (Table 2). Similarly, two new signals for methine protons at δ 4.18, and 4.16 further supported the presence of hydroxyl containing methine groups in substrate **1.** The signals for methylene protons of C-6 and C-15 were also found missing in the ^{1}H-NMR spectrum (Table 1) of metabolite **2**. The first OH group was placed at C-6 (δ 74.2), based on HMBC correlations of H-8 (δ 2.14, overlap), H-4 (δ 5.71, br. s) and H_2-7 (δ 2.13, overlap; 1.21, overlap) with C-6 (δ 74.2) (Fig. 2). This was further supported by COSY-dfqf correlations of H_2-7 (δ 2.13, overlap;

Table 3
Anti-bacterial activity of compounds **1–5** against *Staphylococcus aureus.*

Compounds [20 μg/mL]	*S. aureus* EMRSA-17 (% inhibition)	*S. aureus* NCTC 13277 (% inhibition)	*S. aureus* NCTC 13143 (% inhibition)	*S. aureus* clinical (% inhibition)	*S. aureus* (VRSA) ATCC 700699 (% inhibition)
1	92	82	88	89	74
2	84	86	94	87	–
3	27	–	26	–	–
4	–	–	–	–	–
5	25	–	30	–	24
Standard drug, vancomycin	90	88	92	91	65

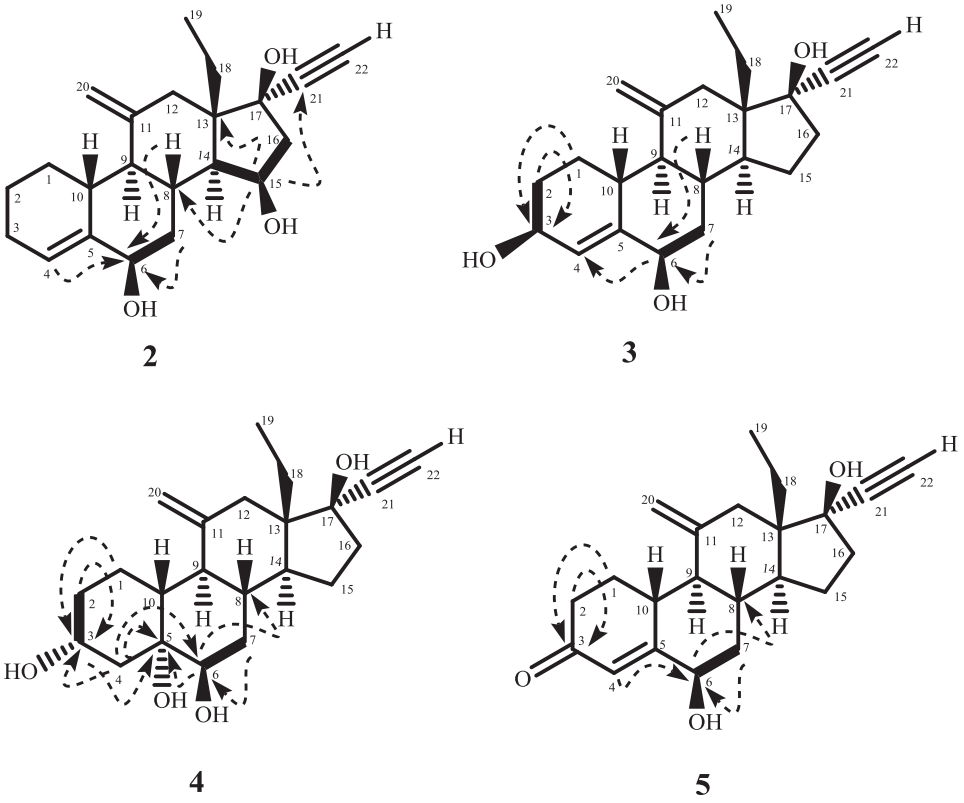

Fig. 2. Key HMBC (- - - - ➤), and COSY (▬) correlations in metabolites 2–5.

1.21, overlap) with H-6 (δ 4.18, overlap) (Fig. 2). The OH group at C-6 (δ 74.2) was deduced to be β-oriented (*axial*), based on NOESY correlations of *equatorially*-oriented H-6 (δ 4.18, overlap) with vinylic H-4 (δ 5.71, br. s) and H_2-7 (δ 2.13, overlap; 1.21, overlap). This can only be possible when H-6 is *equatorial* (Fig. 3). The half-width of *equatorially*-oriented H-6 ($W_{1/2}$ = 6.4 Hz) also a β-orientation (*axial*) of geminal OH group at C-6. The second OH group was placed at C-15 (δ 68.6), based on HMBC correlations of H-15 (δ 4.16, overlap) with C-8 (δ 33.6), C-13 (δ 51.5) and C-21 (δ 88.7) (Fig. 2). This was further supported by COSY-dfqf correlations of H-15 (δ 4.16, overlap) with H_2-16 (δ 2.71, overlap; 2.12, overlap) and H-14 (δ 1.66, overlap) (Fig. 2). The OH group at C-15 (δ 68.6) was deduced to be β-oriented, based on NOESY correlations of H-15 (δ 4.16, overlap) with *axially*-oriented H-14 (δ 1.66, overlap) (Fig. 3). Thus the structure of new metabolite **2** was deduced as 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-6β,15β,17β-triol.

The HREI-MS of metabolite **3** showed the [M^+] at *m*/*z* 342.2185 (calcd. 342.2195, $C_{22}H_{30}O_3$), suggesting addition of two hydroxyl groups in compound **1** (*m*/*z* 310). The IR absorbances at 3388, 3294, and 1641 cm^{-1} were due to the presence of OH, and C=C groups, respectively. The ^{13}C-NMR spectrum showed signals for two new methine carbons at δ 67.8 and 73.3, indicating addition of two hydroxyl groups in substrate **1** (Table 2). Similarly, in the ^{1}H-NMR spectrum the appearance of methine proton signals at δ 4.10, and 4.15 also indicated dihydroxylation of substrate **1** (Table 1). The first OH group was placed at C-3 (δ 67.8), based on HMBC correlations of H_2-1 (δ 2.39, overlap; 1.05, overlap) and H_2-2 (δ 1.91, overlap; 1.38, overlap) with C-3 (δ 67.8). This was further supported by key COSY-dfqf correlations of H-3 (δ 4.10, overlap) with H_2-2 (δ 1.91, overlap; 1.38, overlap) (Fig. 2). The OH group at C-3 (δ 67.8) was deduced to be β-oriented (*equatorial*), based on NOESY correlations of *axially*-oriented H-3 (δ 4.10, overlap) with H-2 (δ 1.91, overlap) and H-1 (δ 1.05, overlap). This was further supported by NOESY correlations of H-2 (δ 1.38, overlap) with H-10 (δ 1.85, m) (Fig. 3). The half-width of *axially*-oriented H-3 ($W_{1/2}$ = 18.9 Hz) also indicated β-orientation (*equatorial*) of geminal OH group at C-3. The second OH group was placed at C-6 (δ 73.3), based on HMBC correlations of H-6 (δ 4.15, br. s) with C-4 (δ 129.7), and H-8 (δ 2.67, overlap) with C-6 (δ 73.3). This was further supported by COSY-dfqf correlations of H-6 (δ 4.15, br. s) with H_2-7 (δ 1.89, overlap; 1.12, overlap) (Fig. 2). The OH group at C-6 (δ 73.3) was deduced to be β-oriented (*axial*), based on NOESY correlations of *equatorially*-oriented geminal H-6 (δ 4.15, br. s) with H_2-7 (δ 1.89, overlap; 1.12, overlap) and vinylic H-4 (δ 5.51, br. s) (Fig. 3). This can only be possible when H-6 is *equatorial* (Fig. 3). The half-width of *equatorially*-oriented broad singlet of H-6 ($W_{1/2}$ = 6.3 Hz) also indicated β-orientation (*axial*) of geminal OH group at C-6. Thus the structure of new metabolite **3** was deduced as 13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-3β,6β,17β-triol.

The HREI-MS of compound **4** showed the [M^+] at *m*/*z* 360.2305 ($C_{24}H_{30}O_4$, calcd. 360.2301), indicating an addition of three hydroxyl groups in substrate **1** (*m*/*z* 310). The overlapped IR absorbances at 3379, and 3255 cm^{-1} were due to the presence of hydroxyl groups. The ^{13}C-NMR spectrum showed a signal for new quaternary carbon at δ 76.1, along with two new methine signals at δ 69.6, and 74.4, suggesting the presence of hydroxyl groups in substrate **1** (Table 2). Similarly, new signals for methine protons at δ 3.91, and 3.41 further supported the hydroxylation of substrate **1**. The signals for methylene protons of C-3 and C-6 were also found missing in the ^{1}H NMR spectrum. The signals for vinylic protons were also found missing in the ^{1}H-NMR spectrum of metabolite **4** (Table 1). The first OH group was placed at C-3 (δ 69.6), based

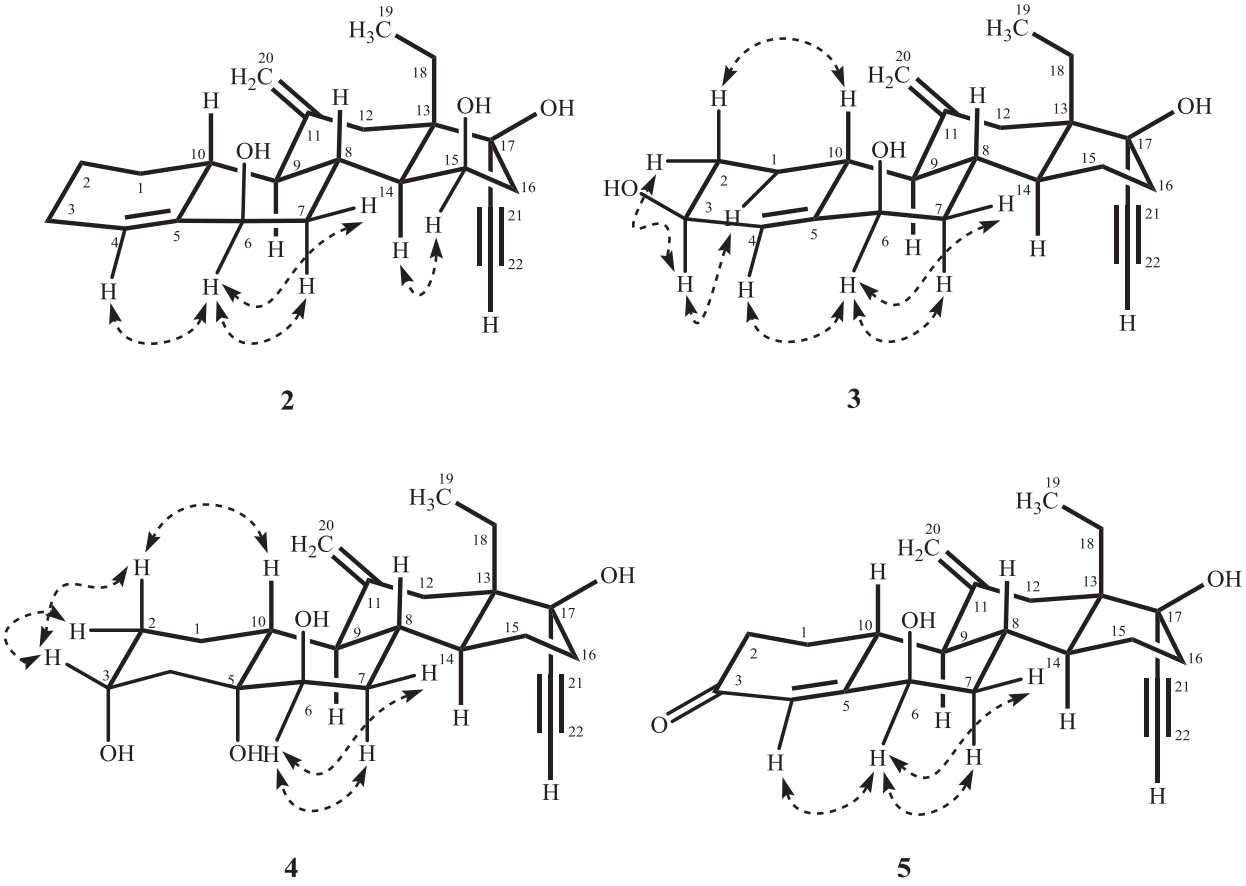

Fig. 3. Key NOESY correlations in metabolites 2–5.

on key HMBC correlations of H_2-1 (δ 1.23, overlap) and H-4 (δ 1.64, overlap) with C-3 (δ 69.6). This was further inferred from key COSY-dfqf correlations of H-3 (δ 3.91, br. d, $J_{e/e}$ = 2.5 Hz) with H_2-2 (δ 1.91, overlap; 1.27, overlap) and H_2-4 (δ 1.90, overlap; 1.64, overlap) (Fig. 2). The OH group at C-3 (δ 69.6) was deduced to be α-oriented (*axial*), based on NOESY correlations of *equatorially*-oriented H-3 (δ 3.91, br. d, $J_{e/e}$ = 2.5 Hz) with H_2-2 (δ 1.91, overlap; 1.27, overlap) and H_2-4 (δ 1.90, overlap; 1.64, overlap). This was further supported by NOESY correlations of H-2 (δ 1.27, overlap) with H-10 (δ 1.88, overlap). The half-width of *equatorially*-oriented broad doublet of H-3 ($W_{1/2}$ = 9.6 Hz) also indicated α-orientation (*axial*) of geminal OH group at C-3 (Fig. 3). The second OH group was placed at C-5 (δ 76.1), based on key HMBC correlations of H-3 (δ 3.91, br. d), H_2-4 (δ 1.90, overlap; 1.64, overlap) and H-6 (δ 3.41, br. t) with C-5 (δ 76.1). The OH group at C-5 was deduced to be α-oriented (*axial*), based on NOESY correlations of *axially*-oriented OH-3 (δ 4.62, m) with *axially*-oriented OH-5 (δ 2.81, overlap) (acetone-d_6) (Fig. 3). The third OH group was placed at C-6 (δ 74.4), based on key HMBC correlations of H-6 (δ 3.41, br. t) with C-5 (δ 76.1), and C-8 (δ 38.7). This was further supported by key COSY-dfqf correlations of H-6 (δ 3.41, br. t, $J_{6e/7e}$ = 2.5 Hz) with H_2-7 (δ 1.92, overlap; 1.52, overlap) (Fig. 2). The OH group at C-6 (δ 74.4) was deduced to be β-oriented (*axial*) based on NOESY correlations of H-6 (δ 3.41, br. t, $J_{6e/7e}$ = 2.5 Hz) with H_2-7 (δ 1.92, overlap; 1.52, overlap) (Fig. 3). The half-width of *equatorially*-oriented broad triplet of H-6 ($W_{1/2}$ = 6.7 Hz) also indicated β-orientation (*axial*) of geminal OH group at C-6. Thus the structure of the new metabolite **4** was deduced as 13-ethyl-11-methylene-18,19-dino r-17α-pregn-20-yn-3α,5α,6 β,17β-tetraol.

Compound **5** was identified as 13-ethyl-11-methylene-18,19-d inor-17α-pregn-4-en-20-yn-6β-17β-dihydroxy-3-one which was previously reported by Verhoeven et al. through metabolism in animals body [24].

Placement of OH groups in desogestrel (**1**) is affecting its anti-bacterial activity. Apparently, the presence of OH groups at C-6 and C-15 in compound **2** increases its anti-bacterial activity only against *S. aureus* NCTC 13143 in comparison to substrate **1** and standard drug, vancomycin. Presence of OH groups at C-3 and C-6 in compound **3** diminished its bactericidal activity against clinically isolated *S. aureus*, and *S. aureus* NCTC 13277. Presence of OH groups at C-3, C-5, and C-6 in compound **4**, completely diminished its anti-bacterial activity. Presence of carbonyl ketone at C-3, and OH group at C-6 in compound **5** also diminished its anti-bacterial activity against clinically isolated *S. aureus,* and *S. aureus* NCTC 13277.

4. Conclusion

In conclusion, microbial transformations of desogestrel (**1**) with *C. blakesleeana* afforded four new metabolites **2**–**4**, along with a known metabolite **5**. Compounds **1**–**2** showed a potent growth inhibition against drug resistant strains of *S. aureus*. Compounds **1**–**5** were found to be non-cytotoxic against 3T3 normal cell line. The presented study indicated that compounds **1**–**2** can be further studied for their therapeutic potential against infections, caused by MDR *S. aureus*.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bioorg.2017.12.027.

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