Biotransformation of Androgenic Steroid Mesterolone with Cunninghamella blakesleeana and Macrophomina phaseolina.

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Biotransformation of androgenic steroid mesterolone with *Cunninghamella blakesleeana* and *Macrophomina phaseolina*

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ABSTRACT

Fermentation of mesterolone (**1**) with *Cunninghamella blakesleeana* yielded four new metabolites, 1α-methyl-1β,11β,17β-trihydroxy-5α-androstan-3-one (**2**), 1α-methyl-7α,11β,17β-trihydroxy-5α-androstan-3-one (**3**), 1α-methyl-1β,6α,17β-trihydroxy-5α-androstan-3-one (**4**) and 1α-methyl-1β,11α,17β-trihydroxy-5α-androstan-3-one (**5**), along with three known metabolites, 1α-methyl-11α,17β-dihydroxy-5α-androstan-3-one (**6**), 1α-methyl-6α,17β-dihydroxy-5α-androstan-3-one (**7**) and 1α-methyl-7α,17β-dihydroxy-5α-androstan-3-one (**8**). Biotransformation of **1** with *Macrophomina phaseolina* also yielded a new metabolite, 1α-methyl, 17β-hydroxy-5α-androstan-3,6-dione (**9**). The isolated metabolites were subjected to various *in vitro* biological assays, such as anti-cancer, inhibition of α-glucosidase, and phosphodiesterase-5 enzymes and oxidative brust. However, no significant results were observed. This is the first report of biotransformation of **1** with *C. blakesleeana* and *M. phaseolina*.

1. Introduction

Biocatalysis for stereoselective synthesis of bioactive compounds has attracted major scientific interest in last four decades. The main advantages of the use of biocatalysts are their environmental acceptability, need of mild conditions, and resulting high stereoselectivity. Although there are various methods of biotransformation, filamentous fungi are capable of catalyzing stereoselective hydroxylation more efficiently [1–12]. Cytochrome P450, enzymes, present in filamentous fungi, are responsible for stereoselective hydroxylation of the substrate [13–17].

Steroids are used for the treatment of various ailments related to hormonal, metabolic and skin disorders. They are being transformed by biocatalysis to obtain compounds with therapeutic value [18]. Mesterolone (1α-methyl-17β-dihydroxy-5α-androstan-3-one, **1**) is a synthetic androgenic steroid, clinically used for the treatment of hypogonadism, caused by the deficiency of endogeneous androgen formation [19]. It is also used for the treatment of male infertility [20]. During the current study, fermentation of **1** with *Cunninghamella blakesleeana* and *Macrophomina phaseolina* yielded five new (**2**–**5**, and **9**), and three known metabolites (**6**–**8**) (see Figs. 1 and 2). Objective of the present study was to obtain structurally diverse analogues of mesterolone (**1**) by microbial transformation, with potential biologically activity. However, evaluation of some of the products obtained with the available biological assays, such as anticancer, inhibition of α-glucosidase and phosphodiesterase-5 enzymes and oxidative burst did not show significant activity [21–24].

2. Experimental

2.1. General method

Mesterolone (**1**) was extracted with dichloromethane from the drug Proviron, marketed by Bayer Schering Pharma. The purity of extracted mesterolone was checked with TLC and ^{1}H-NMR spectroscopy. Melting points were determined on Buchi M-560 apparatus. Precoated TLC plates (silica gel, PF_{254}, Merck, Germany) were used for thin layer chromatography. Column chromatography was carried out on silica gel (E. Merck, Germany). Bruker Avance-NMR (300 and 500 MHz) spectrometers were used to record ^{1}H- and ^{13}C-NMR spectra. Molecular mass and HREI-MS of some compounds were detected with JEOL JMS-600H mass spectrometer (Japan). HRESI-MS were recorded on QSTAR XL mass

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spectrometer (Applied Biosystems, USA). Optical rotations were measured on JASCO P-2000 polarimeter. IR Spectra were recorded on Bruker Vector 22 FTIR spectrometer. Phosphomolybdic acid hydrate and ceric sulfate were used for visualizing compounds on TLC. All solvents used for chromatography were of analytical grade.

2.2. Fungal cultures and medium

Fungal cultures used in biotransformation were purchased from American Type Culture Collection (ATCC) and Karachi University Culture Collection (KUCC). Stock cultures of fungi were stored on Sabouraud dextrose agar (SDA) at 4 °C.

Following ingredients were used to prepare 4 L culture medium for *C. blakesleeana* (ATCC 8688A) and *M. phaseolina* (KUCC 730) in distilled H_2O: Glucose (40.0 g), peptone (20.0 g), KH_2PO_4 (20.0 g), yeast extract (20.0 g), NaCl (20.0 g) and glycerol (40.0 mL).

2.3. General fermentation and extraction conditions

Culture medium (4 L) was prepared by dissolving the aforementioned chemicals in distilled water. It was transferred to 40 flasks of 250 mL in about equal volume, i.e., each flask containing 100 mL of solution. All flasks were autoclaved. Five seed flasks were inoculated with stock fungal culture slants and kept on rotatory shaker (100 rpm) at 22 °C for four days. When appropriate growth was observed in seed flasks, the spores were transferred to remaining flasks and placed again on shaker at 22 °C. After four days, further growth was observed. One gram of compound **1** was dissolved in 40 mL of acetone and dispersed to the culture flasks. They were place on a shaker table for nine days at 22 °C.

The culture media was filtered and extracted with CH_2Cl_2 and the solvent was evaporated under reduced pressure to obtain 2.5 g of a gum. The degree of transformation was checked by using comparative TLC with substrate, positive control (substrate + fungal medium) and negative control (fungal culture contents). The crude extract was fractionated with silica gel column chromatography using hexanes enriched with acetone. Further purification was carried out by column chromatography using hexanes and ethyl acetate and finally by reversed phase recycling HPLC.

2.4. Fermentation of mesterolone (**1**) with Cunninghamella blakesleeana

The crude material (2.5 g) was subjected to silica gel column chromatography. The mobile phase used was composed of 5% gradient of hexanes and acetone. The seven main fractions (CB-1 to CB-7) were obtained, which were further purified with column chromatography and HPLC. The fraction CB-1 was subjected to reverse phase recycling HPLC (methanol: water 70:30), which yielded metabolite **2** (5 mg, R_T = 32 min). Metabolite **3** (9 mg, R_T = 27 min) was obtained from fraction CB-2 on reverse phase recycling HPLC (methanol: water 70:30), while fraction CB-3 yielded metabolite **4** (4 mg, R_T = 23 min) when subjected to reverse phase recycling HPLC (methanol: water 75:25). Similarly fraction CB-4 yielded metabolite **5** (4 mg, R_T = 24 min) by reverse phase recycling HPLC (methanol: water 75:25). Fraction CB-5 yielded metabolite **6** (3 mg) on elution from silica gel column (hexanes: ethyl acetate = 65:35), whereas metabolite **7** (7 mg) was obtained when fraction CB-6 was subjected to silica gel column chromatography (hexanes: ethyl acetate = 63:37). Similarly fraction CB-7 provided metabolite **8** (10 mg) through silica gel column chromatography (hexanes: ethyl acetate = 61:39) (see Figs. 1 and 3).

2.4.1. 1α-Methyl-1β,11β,17β-trihydroxy-5α-androstan-3-one (**2**)

White crystalline; Melting point 194–196 °C; $[\alpha]_D^{25} = -21.2°$ (*c* 0.013, $CHCl_3$); IR ($CHCl_3$); υ_{max} 3346 (O—H stretching), 2924 (C—H stretching), 2854 (C—H stretching), 1707 (C = O stretching); HRES-I-MS *m/z* 337.2369 $[M + H]^+$ (mol. formula $C_{20}H_{33}O_4$, calc. for 336.2339): $[M + H—H_2O]^+ = m/z$ 319.2244; $[M + H—2H_2O]^+ = m/z$ 301.2146; ^{1}H-NMR (CD_3OD, 300 MHz): Table 1; ^{13}C-NMR (CD_3OD, 125 MHz): Table 2.

2.4.2. 1α-Methyl-7α,11β,17β-trihydroxy-5α-androstan-3-one (**3**)

White crystalline; Melting point 241–242 °C; $[\alpha]_D^{25} = -53°$ (*c* 0.024, $CHCl_3$); IR ($CHCl_3$); υ_{max} 3433 (O—H stretching), 2951 (C—H stretching), 2910 (C—H stretching), 1700 (C=O stretching); ; HRESI-MS *m/z* 337.2329 $[M + H]^+$ (mol. formula, $C_{20}H_{33}O_4$, calc. for 336.2339): $[M + H—H_2O]^+ = m/z$ 319.2165; $[M + H—2H_2O]^+ = m/z$ 301.2134; ^{1}H-NMR (CD_3OD, 300 MHz): Table 1; ^{13}C-NMR (CD_3OD, 125 MHz): Table 2.

2.4.3. 1α-Methyl-1β,6α,17β-trihydroxy-5α-androstan-3-one (**4**)

White crystalline; Melting point 168–169 °C; $[\alpha]_D^{25} = 26.6°$ (*c* 0.05, MeOH); IR ($CHCl_3$); υ_{max} 3402 (O—H stretching), 2957 (C—H stretching), 1669 (C=O stretching); HRESI-MS *m/z* 337.2325 $[M + H]^+$ (mol. formula, $C_{20}H_{33}O_4$, calc. for 336.2339); $[M + H—H_2O]^+ = m/z$ 319.2165; $[M + H—2H_2O]^+ = m/z$ 301.2134; ^{1}H-NMR (CD_3OD, 300 MHz): Table 1; ^{13}C-NMR (CD_3OD, 125 MHz): Table 2.

2.4.4. 1α-Methyl-1β,11α,17β-trihydroxy-5α-androstan-3-one (**5**)

White crystalline; Melting point 165–166 °C; $[\alpha]_D^{25} = 53.8°$ (*c* 0.02, MeOH); IR ($CHCl_3$); υ_{max} 3433 (O—H stretching), 2951 (C—H stretching), 2910 (C—H stretching), 1700 (C=O stretching); HRES-I-MS *m/z* 337.2394 $[M + H]^+$ (mol. formula $C_{20}H_{33}O_4$, calc for 336.2378); $[M + H—H_2O]^+ = m/z$ 319.2106; $[M + H—H_2O]^+ = m/z$ 301.2039; $[M + H—2H_2O]^+ = m/z$ 283.1964; ^{1}H-NMR (CD_3OD, 300 MHz): Table 1; ^{13}C-NMR (CD_3OD, 75 MHz): Table 2.

2.5. Biotransformation of mesterolone with M. phaseolina

Incubation of **1** (1 g/10 mL) acetone) with three-day old culture of *M. phaseolina* for nine days yielded a new metabolite **9** (4 mg) after purification by silica gel column chromatography (hexanes: ethyl acetate = 67:33) (see Figs. 2 and 3).

2.5.1. 1α-Methyl, 17β-hydroxy-5α-androstan-3,6-dione (**9**)

Colorless crystalline; Melting point: 226.6–228.7 °C; $[\alpha]_D^{25} = 13°$ (*c* 0.01, MeOH); IR ($CHCl_3$); υ_{max} 3434 (O—H stretching), 2952 (C—H stretching), 2910 (C—H stretching), 1710 (C=O stretching), 1242; EI-MS: *m/z* 318 (100), 303.1 (87), 275.1 (60), 259 (41), 248.1 (47), 233.1 (44), 222.1 (40), 147 (51), 133 (50), 123 (62), 107 (67), 95 (74), 81 (98), 69.1 (98), 55 (100), 41 (99); HREI-MS: *m/z* 318.2164 $[M^+]$, (mol. formula $C_{20}H_{30}O_3$, calc for 318.2189); ^{1}H-NMR (CD_3OD, 300 MHz): Table 1; ^{13}C-NMR (CD_3OD, 125 MHz): Table 2.

2.6. Biological studies

Most of the compounds isolated by the microbial fermentation of mesterolone (**1**) were evaluated for anti-cancer, inhibition of α-glucosidase and phosphodiesterase-5 enzymes and oxidative brust activities, where they showed no significant activity. These assays were performed according to standard protocol, reported in literature.

Compounds **3**, **4** and **7** were evaluated for anticancer activity employing using cell viability assay against two cancer cell lines, i.e., HeLa and PC3. No significant results were observed. Whereas, the evaluation of α-glucosidase inhibitory activity of compounds **2**, **3**, **6**, **7** and **9** also did not show any significant results. Similarly compounds **5**–**7** and **9** were inactive in phosphodiestease-5 enzyme inhibition assay, and compounds **2**–**4**, **6** and **7** were also inactive in oxidative brust assay.

Table 1
^{1}H-NMR (300 MHz, CD_3OD) chemical shift assignments of **1**–**5** and **9** compounds (*J* in Hz).

Position	1	2	3	4	5	9
1	2.15 m	–	2.38 m,	–	–	2.24 m
2	2.81 dd (*J* = 14.7, 6.3), 1.98 m	2.89 d, 2.18 d (*J* = 13.6)	2.83 dd (*J* = 14.4, 5.7), 1.39 m	2.87 d (*J* = 13.5), 2.13 d (*J* = 13.8)	2.86 d, 2.14 d (*J* = 14.1)	2.25 m, 2.09 m
3	–	–	–	–	–	–
4	2.33 m, 1.97 t (*J* = 14.1)	2.35 m, 1.97 m	1.89 m, 2.31 m	2.61 m, 2.30 m	2.40 t (*J* = 13.8), 1.98 m	2.62 m, 2.11 m
5	1.76 m	1.66 m	2.33 m	1.38 m, 3.49 m	1.72 m	2.95 dd (*J* = 12.9, 4.5)
6	1.44 m, 1.35 m	1.95 m, 1.43 m	1.67 m, 1.43 m	1.98 m, 0.82 m	1.72 m 1.27 m	–
7	0.88 m, 1.70 m	1.82 m, 0.92 m	3.8 m ($W_{1/2}$ = 8)	1.57 m	1.45 m, 1.27 m	2.81 dd (*J* = 15.0, 6.0), 1.93 m
8	1.53 m	1.87 m	1.91 m	1.57 m	1.53 m	1.88 m
9	0.99 m	1.17 dd (*J* = 10.3, 3.9)	1.55 dd (*J* = 11.4, 3.3)	1.14 m	1.38 m	1.57 m
10	–	–	–	–	–	–
11	1.40 m, 1.51 m	4.51 m ($W_{1/2}$ = 7)	4.25 m ($W_{1/2}$ = 9)	2.23 m, 1.41 m	3.94 td (*J* = 10.2, 5.2)	1.64 m, 1.48 m
12	1.05 m, 1.83 m	2.06 dd (*J* = 14.1, 24.0), 1.32 m	1.22 m, 1.98 dd (*J* = 13.8, 3.0)	1.80 m, 1.03 m	2.18 m, 1.18 m	1.88 m, 1.17 m
13	–	–	–	–	–	–
14	0.98 m	0.96 m	1.41 m	1.01 m	1.08 m	1.28 m
15	1.25 m, 1.57 m	1.62 m, 1.31 m	1.33 m, 1.72 m	1.62 m, 1.30 m	1.59 m, 1.27 m	1.56 m, 1.28 m
16	1.43 m, 1.95 m	1.81 m, 1.28 m	1.45 m, 1.96 m	1.98 m, 1.43 m	1.98 m, 1.46 m	1.99 m, 1.48 m
17	3.56 t (*J* = 8.4)	3.54 t (*J* = 9.0)	3.57 t (*J* = 8.4)	3.58 t (*J* = 8.4)	3.51 t (*J* = 8.7)	3.62 t (*J* = 8.7)
18	0.7 s	0.72 s	0.99 s	0.74 s	0.75 s	0.77 s
19	1.16 s	1.38 s	1.36 s	1.18 s	1.25 s	1.06 s
20	0.85 d (*J* = 7.2)	1.22 s	0.90 d (*J* = 7.2)	1.17 s	1.30 s	0.97 d (*J* = 7.2)

Table 2
^{13}C-NMR (Compounds **1** at 75 MHz and **2**–**5** and **9** at 125 MHz in CD_3OD) chemical shift assignments.

Carbon	1	2	3	4	5	9
1	40.9	78.7	40.7	79.0	78.3	40.4
2	46.8	55.9	46.8	56.4	54.9	46.5
3	216.2	211.5	215.6	211.6	211.5	212.4
4	45.8	45.2	44.9	40.8	46.1	38.1
5	41.5	44.0	35.0	50.2	43.4	52.9
6	29.9	29.9	37.5	70.9	32.3	214.5
7	32.3	32.7	67.7	41.8	30.8	46.0
8	36.9	34.1	37.9	35.9	36.8	39.4
9	50.1	54.4	45.4	50.4	56.4	49.5
10	39.1	45.5	39.7	43.5	47.6	44.7
11	21.3	69.7	68.4	23.9	67.8	21.6
12	37.9	44.9	46.6	37.9	47.7	37.7
13	44.2	42.6	43.2	38.0	43.9	44.5
14	52.3	52.9	46.9	52.3	51.2	52.5
15	24.3	24.7	23.8	24.6	24.6	24.1
16	30.7	30.2	30.3	30.4	30.7	30.5
17	82.5	83.0	83.1	82.5	82.1	82.1
18	11.7	13.1	13.8	11.6	12.5	11.6
19	15.1	12.4	17.4	10.1	9.4	15.4
20	14.7	23.0	15.4	23.4	23.7	15.0

3. Results and discussions

We report here the biotransformation of mesterolone (**1**) ($C_{20}H_{32}O_2$, *m/z* 304.4) with *C. blakesleeana* and *M. phaseolina* for the first time. Fermentation of **1** with *C. blakesleeana* afforded seven metabolites **2**–**8**, out of which four **2**–**5** were characterized as new metabolites, while three **6**–**8** were found to be known. Biotransformation of **1** with *M. phaseolina* yielded a new metabolite **9**. The structures of new metabolites **2**–**5** and **9** were evaluated through spectroscopic studies.

The metabolite **2** was found more polar on TLC as compared to substrate **1**. The HRESI-MS of metabolite **2** showed the $[M + H]^+$ at *m/z* 337.2369 ($C_{20}H_{33}O_4$, calc. 336.2339), which indicates the presence of two more oxygen functionalities as compared to **1** (*m/z* = 304.24). The IR absorbance showed the presence of hydroxyl (3346 cm^{-1}), and carbonyl functionality (1707 cm^{-1}). The methyl proton at C-20 appeared as singlet (δ 1.22) indicating substitution at C-1 position. An additional methine proton signal resonating at δ 4.51 ($W_{1/2}$ = 7 Hz) in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum further confirmed the presence of two additional hydroxyl group through downfield signals, one at δ 78.7 (C-1) and second at δ 69.7 (C-11). The protons at C-19 (δ 1.38) and C-20 (δ 1.22) showed HMBC interactions with C-1 (δ 78.7), indicating hydroxylation at C-1. The methine proton at δ 4.51 showed HMBC interactions with C-9 (δ 54.9) and C-12 (δ 44.9), indicating the second hydroxylation at C-11. The protons at C-20 (see Fig. 3) showed NOESY correlations with H-5, indicated a *β* hydroxylation at C-1 (Fig. 4). The NOESY correlation of H-11 with H-9 indicated the *β* hydroxylation at C-11. The metabolite **2** was identified as 1α-methyl-1*β*,11*β*,17*β*-trihydroxy-5α-androstan-3-one.

The HRESI-MS of metabolite **3** showed the $[M + H]^+$ at *m/z* 337.2329 ($C_{20}H_{33}O_4$, calc. 336.2339), which showed the addition of two more oxygens in contrast to compound **1**. The IR spectra showed absorbances at 3433 and 1700 cm^{-1}, due to the presence of hydroxyl and carbonyl groups, respectively. The appearance of two new downfield methine protons at δ 3.84 ($W_{1/2}$ = 8 Hz) and δ 4.27 ($W_{1/2}$ = 9 Hz) in ^{1}H-NMR spectrum also indicated the addition of two hydroxyl groups. The ^{13}C-NMR spectrum further showed two new downfield methine carbon signals at δ 67.7 and 68.4 indicated two hydroxyl groups in **3**. In COSY-45°, the new downfield methine proton at δ 3.84 showed coupling with C-6 methylene protons (δ 1.67, 1.43) and C-8 methine proton (δ 1.91), indicating hydroxylation at C-7 in ring B. Similarly the methine proton at δ 4.27 showed COSY correlations with C-9 methine proton and C-12 methylene protons, suggesting the second hydroxylation at C-11. The C-7 methine proton (δ 3.84) showed the HMBC interactions with C-5 (δ 35.0) and C-9 (δ 45.4). Similarly, the C-11 methine proton (δ 4.27) showed HMBC interactions with C-8 (δ 37.8) and C-13 (δ 43.2), further supporting the position of second hydroxylation at C-11 (see Fig. 4). H-7 (δ 3.84) showed NOESY correlations with H-6 and H-8, suggesting α hydroxylation at C-7. The NOESY correlation of H-11 (δ 4.27) with H-9 indicated the α orientation of H-11. The metabolite **3** was deduced as 1α-methyl-7α,11*β*,17*β*-trihydroxy-5α-androstan-3-one.

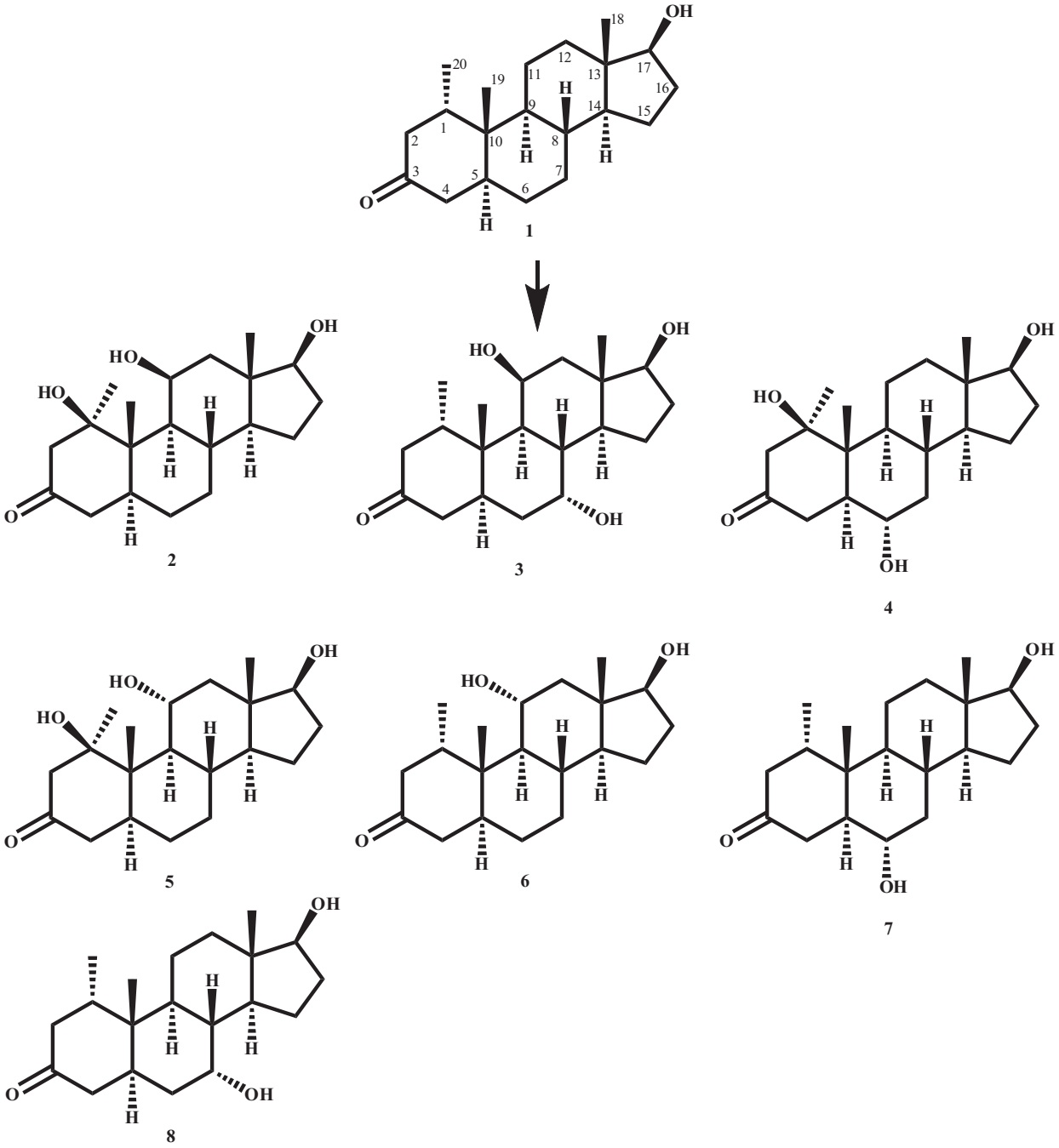

Fig. 1. Biotransformation of mesterolone (**1**) with *Cunnighamella blackesleena*.

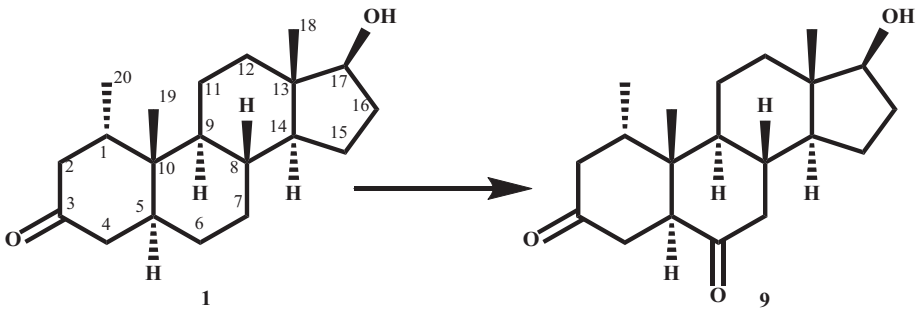

Fig. 2. Biotransformation of **1** with *Macrophomina phaseolina*.

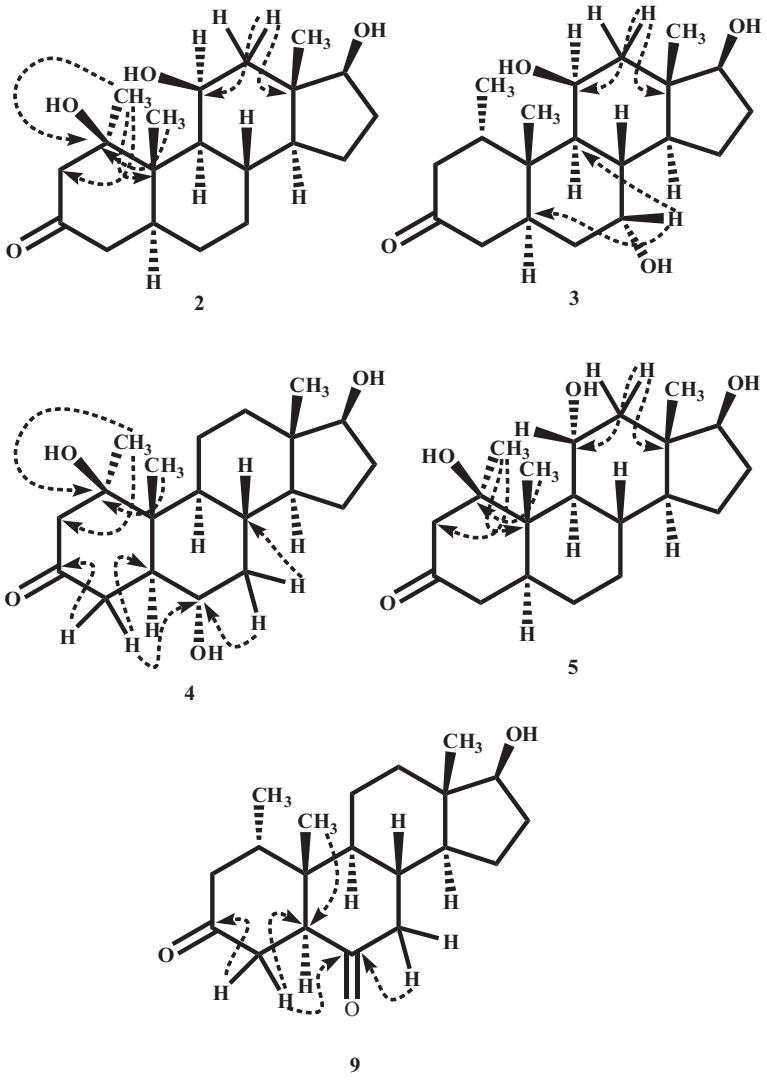

Fig. 3. Key HMBC correlations in new compounds **2**–**5** and **9**.

The metabolite **4** showed the molecular ion peak $[M + H]^+$ in HRESI-MS at *m/z* 337.2325 ($C_{20}H_{33}O_4$, calc. 336.2339), which indicated dihydroxylation in substrate **1**. IR Spectrum showed absorptions at 3402 (hydroxyl) and 1669 cm^{-1} (ketonic carbonyl). In ^{1}H-NMR spectrum, H-20 (δ 1.17) appeared as a singlet which indicated a vicinal hydroxylation at C-1. A downfield methine proton at δ 3.49 indicated the presence of another hydroxyl group. In ^{13}C-NMR spectrum, the presence of two new signals, quaternary carbon (δ 79.0) and methine carbon (δ 70.9) inferred dihydroxylation. The COSY-45° spectrum showed couplings between new methine proton (δ 3.49) and C-7 methylene protons (δ 1.89, 0.82). This indicated OH at C-6. The H_3-19 (δ 1.17) and H_3-20 (δ 1.18) showed HMBC with C-1 (δ 79.0), which indicated the hydroxylation at C-1. H_2-4 (δ 2.61, 2.30) and H_2-7 (δ 2.87, 0.82) showed HMBC correlations with the new methine carbon (δ 70.9). This further indicated second hydroxylation C-6 position (see Fig. 3). The H-20 (δ 1.18) showed the NOESY correlation with H-5 (δ 1.39), thus indicating *β* hydroxylation at C-1. The stereochemistry of H-6 was assigned *β* (*axial*) by its NOESY correlation with H_3-19 (δ 1.17) (see Fig. 4). Thus the structure of compound **4** was deduced as 1α-methyl-1*β*,6α,17*β*-trihydroxy-5α-androstan-3-one.

The molecular composition of polar metabolite **5** [$[M + H]^+$ at *m/z* 337.2394 ($C_{20}H_{33}O_4$, calc. 336.2378)] was similar to metabolite **2** as deduced from HRESI-MS. The spectral data of the compound **5** was similar to **2**. The only difference was in stereochemistry of the hydroxyl group at C-11, which was α in case of **5**. The methine proton (δ 3.94) showed NOESY across peaks with H-19 and H-18 indicating an α hydroxylation at C-11. The compound **5** was thus identified as 1α-methyl-1*β*,11α,17*β*-trihydroxy-5α-androstan-3-one.

The HREI-MS of new compound **9** ($C_{20}H_{30}O_3$) showed the M^+ at *m/z* 318.2164 (calc. 318.2189), 16 amu higher than **1**. This suggested the addition of an oxygen moiety in substrate **1**. The IR absorbances at 3434 and 1710 cm^{-1} indicated the presence of hydroxyl and carbonyl functionalities, respectively. The ^{13}C-NMR spectrum of **9** showed the presence of another ketonic carbonyl group by characteristic signal at δ 214.5. The H_2-4 (δ 2.62, 2.11), H_2-7 (δ 2.81, 1.92) and H-8 (δ 2.24) showed HMBC correlations with C-6 (δ 214.5) (see Fig. 3). The metabolite **9** was identified as 1α-methyl-17*β*-hydroxy-5α-androstan-3,6-dione.

The structures of three known metabolites **6–8** were identified by comparing their spectroscopic data with those reported in literature. The molecular mass of compounds **6**–**8** was 16 amu higher than **1** which showed mono-hydroxylation. Compounds **6**–**8** were identified as 1α-methyl-11α,17*β*-dihydroxy-5α-androstan-3-one (**6**), 1α-methyl-6α,17*β*-dihydroxy-5α-androstan-3-one (**7**) and 1α-methyl-7α,17*β*-dihydroxy-5α-androstan-3-one (**8**). The metabolite **6** was previously obtained by the microbial fermentation of **1** with

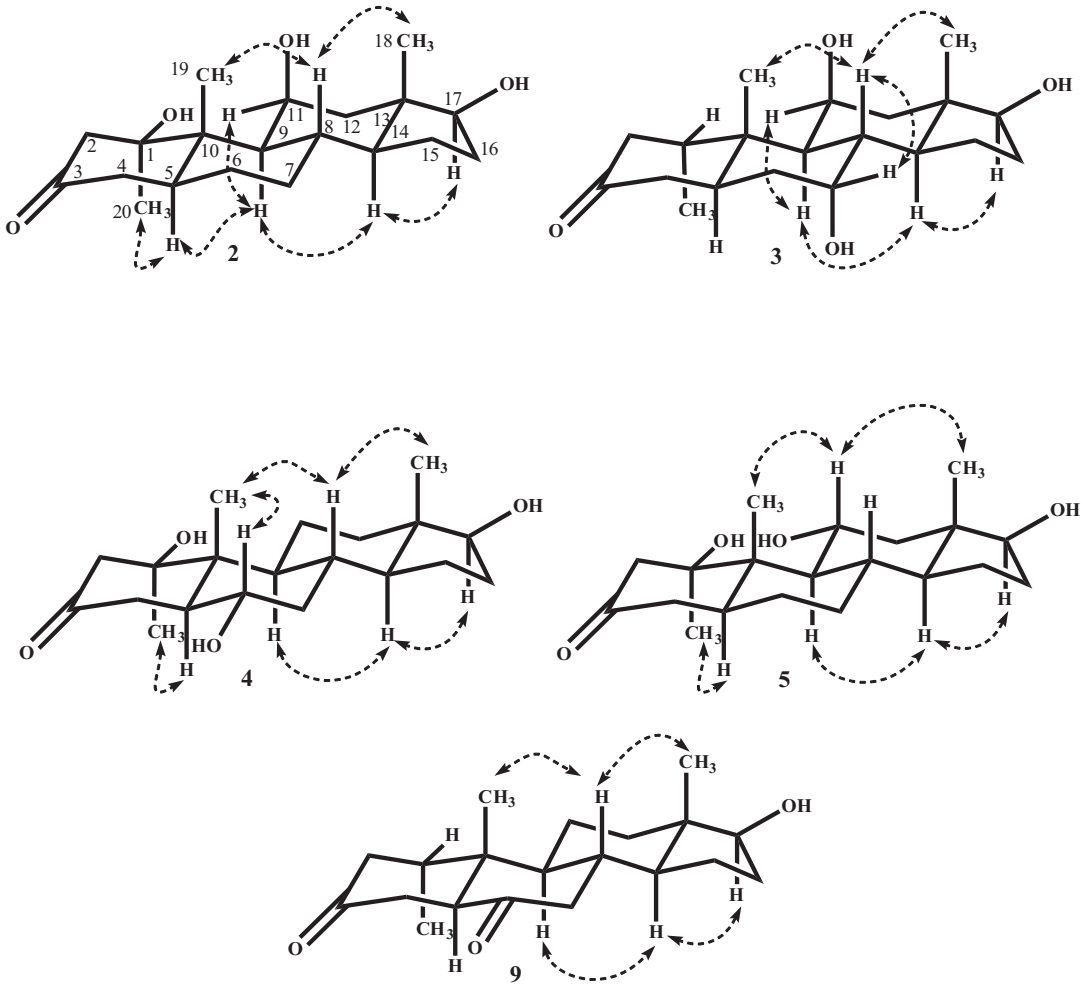

Fig. 4. NOESY correlations in compounds **2–5** and **9**.

Rhizopus stolonifer and *Fusarium lini* where as **7** and **8** were obtained from the fermentation of **1** with *Rhizopus stolonifer* [25].

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