IRSTI 31.23.99; 62.13.99 https://doi.org/10.26577/IJBCh2024v17i1-a12

M. Siddiqui[1], Atia-tul-Wahab[2], B.M. Kudaibergenova[3*], Zh.A. Abilov[3], M.I. Choudhary[1,2,3]

[1] H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan
[2] Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi, Karachi, Pakistan
[3] Al-Farabi Kazakh National University, Almaty, Kazakhstan
*e-mail: bateskudaibergenova@yahoo.com

*(Received 2 May 2024; received in revised form 13 May 2024; accepted 24 May 2024)*

# Biotransformation of anabolic drug Dianabol with *Rizhopus oryzae*

**Abstract.** Microbial biotransformation technique is an excellent approach for the synthesis of stereo-, enantio-, chemo-, and regio-selective/specific analogues of existing steroidal and non-steroidal drugs by using bacteria, fungi, algae, actinomycetes, yeast, and plants and animals cell cultures. This technique is effectively used to synthesize compounds whose structures resemble to the substrates (parent drugs) without using protecting/deprotecting steps. In the current study, an anabolic-androgenic steroid (AAS) based drug, methandienone (dianabol) (1) was incubated with the filamentous fungi *Rhizopus oryzae* ATCC 11145 for twelve days under ambient reaction conditions (at room temperature and neutral pH) by using aqueous media. Therefore, the present study has successfully helped to produce the structural analogues of inert steroidal anabolic drug dianabol (1) without using expensive and toxic chemicals. This yielded five known structural analogues of dianabol (1), *i.e*., 17*β*,11*β*-dihydroxy,17*α*-methyl-androsta-1,4-diene-3-one (2), 17*β*-hydroxy,17*α*-methyl-androsta-1,4-diene-3,11-dione (3), 17*β*,6*β*-dihydroxy,17*α*-methyl-androsta-1,4-diene-3-one (4), 17*β*,6*β*-dihydroxy,17*α*-methyl-androsta-4-ene-3-one (5), and 17*β*-hydroxy,17*α*-methyl-androsta-4,6-diene-3-one (6). Structures of transformed products 2-6 were determined through $^1$H-NMR, and FAB-MS spectroscopic techniques.

**Key words:** microbial biotransformation, fungi, *Rhizopus oryzae*, anabolic-androgenic drug, Dianabol.

## Introduction

Derivatization of organic compounds through conventional synthetic methodologies is a difficult task, as they typically involve the use of expensive and toxic chemicals, and extreme pressure, temperature and pH during the transformations, along with the protection/deprotection steps. In contrast, microbial biotransformation is a robust approach that can effectively modify the structures of almost all classes of organic compounds. This technique is generally performed at ambient temperature and pressure, and neutral pH by using aqueous media. Microbial biotransformation technique is therefore, an efficient way to synthesize regio-, chemo-, and stereo-specific/selective derivatives of existing drugs. The chemical conversion of substrates is catalyzed by various enzymes (biological catalysts) without using protection, deprotection, and functional group activation steps. At present microbial biotransformation is recognized as a green chemistry approach, because it generates less toxic wastes as compared to chemical syntheses. Currently the use of microbial biotransformation is increasing significantly in the production of pharmaceutical products, i.e., vitamins, hormones, enzymes, enzyme inhibitors, vaccines, *etc.* as it preserves the original skeleton of starting material during the formation of products, making it important for industries [1-7].

Methandrostenolone/methandienone (1) is an anabolic-androgenic steroidal drug, used to improve physical performances, and increase muscles without gaining fat. Previously, it was sold under the brand name of dianabol (1) in USA and Germany. Like other steroids, methandienone also has some side effects, including acne, increased hair growth on body, change in voice, estrogenic effects, and liver damage [8, 9]. It is therefore, important to synthesize its analogues by environmentally friendly methods.

In continuance of our biocatalytic studies of steroidal-based compounds/drugs [5-7, 10-13], we

report here biotransformation of dianabol (1) with *Rhizopus oryzae* under mild reaction conditions. This yielded five known transformed products (Figure-1), 17*β*, 11*β*-dihydroxy, 17*α*-methyl-androsta-1,4-diene-3-one (2), 17*β*-hydroxy,17*α*-methyl-androsta-1,4-diene-3,11-dione (3), 17*β*,6*β*-dihydroxy,17*α*-methyl-androsta-1,4-diene-3-one (4), 17*β*,6*β*-dihydroxy,17*α*-methyl-androsta-4-ene-3-one (5), and 17*β*-hydroxy,17*α*-methyl-androsta-4,6-diene-3-one (6). Structures of metabolites 2-6 were identified through $^1$H-NMR, and FAB-MS spectroscopic techniques.

## Materials and methods

*Fungi.* Fungal cell culture *e.g*., *Rhizopus oryzae* ATCC 11145 was purchased from ATTCC (American Type Culture Collection, USA). Fungal cell culture was grown on SDA at 3-4 °C.

*Media.* Five liters of media for the growth of *R. oryzae* was prepared by using following ingredients: Glucose (50 g), NaCl (25 g), peptone (25 g), $KH_2PO_4$ (25 g), and glycerol (50 mL) in five liters of distilled water.

Ingredients for the preparation of media were acquired from Scharlau Chemicals and Reagents (Spain), VWR Chemicals (USA), Dae-Jung Chemicals and Metals Company Limited (Korea), Sigma-Aldrich (Germany), and Oxoid Limited (England).

*General.* Dianabol (**1**), (*m/z* = 300.4, $C_{20}H_{28}O_2$), was procured from the Shenzhen Simeiquan Biotechnology Company Limited, (China). Silica coated ($PF_{254}$) TLC plates (Merck KGaA, Germany) were used to determine the transformations, and purity of compounds. Fractions were obtained by performing silica gel (70–230 mesh) (E. Merck, Germany) column chromatography. Final purifications of fractions were performed *via* recycling reverse phase HPLC (LC–908, YMC L-80) using methanol/water. The $^1$H-NMR spectra of compounds 1-6 were run in $CDCl_3$ on the Bruker Avance-NMR (Bruker, Switzerland). FAB-MS of compounds 1-6 were performed on the Joel JMS H×110 mass spectrometer (Joel, Japan).

*Fermentation of Dianabol (1).* Media (5 L) was prepared by aforementioned recipe. Media (400 mL) was transferred to each 20 flasks of 1 L. Each flask was cotton plugged, and autoclaved. All the flasks were then cooled at room temperature. Media was inoculated with the culture of *R. oryzae* under sterilized conditions, and placed for four days on rotary shaker at 25 ºC. After maximum growth of *R. oryzae*, 2 g of dianabol (**1**) was dissolved in 20 mL of methanol, and fed 1 mL in each *R. oryzae*-containing flask. These flasks were again placed on rotary shaker at 25 ºC for twelve days.

*Extraction of Transformed Products.* After incubation, EtOAc (ethyl acetate) was added in each flask. Flasks were filtered, and rinsed with EtOAc. The filtrate was extracted three times with EtOAc, and biomasses were discarded. Sodium sulfate was added in each extracted flask to absorb water, and filtered. The extracted was then evaporated by using rotary evaporator. As a result, a brown color gummy crude extract was obtained.

*Purification of Transformed Products.* Crude extract was fractionated by silica-gel column chromatography using hexanes-acetone mixture (5-100%), passing 400 mL at each mixture concentration. TLCs were taken for each fractions. TLCs were stained by ceric sulfate spraying reagent. Fractions were purified through recycling RP-HPLC using methanol (70%) and water (30%) solvent system.

## Results and discussion

*R. oryzae*-mediated structural modification of dianabol (1) afforded five derivatives, presented on Figure 1.

Their structures were determined by comparing their $^1$H-NMR and FAB-MS data with the spectral data reported in the literature.

Metabolite 2 showed its $[M-H]^+$ at *m/z* 315.3 in the FAB-MS (-ve mode). Newly appeared H-11 of compound 2 was observed at $\delta$ 4.40, d ($J_{11,12/11,9}$ = 2.88 Hz). Compound 2 was previously reported by our research group [14].

Transformed product 3 showed its $[M+H]^+$ at *m/z* 315.1 in the FAB-MS (+ve). $^1$H-NMR spectrum of compound 3 was distinctly similar to compound 2. Signals for methylene protons ($H_2$-12) were found missing in the $^1$H-NMR spectrum of 3. Compound 3 was also previously reported by our research group [14].

$[M-H]^+$ of compound 4 was observed at *m/z* 315.2 in the FAB-MS (-ve mode). Newly appeared H-6 of compound 4 was observed at $\delta$ 4.53, d ($J_{6,7}$ = 2.18 Hz) in the $^1$H-NMR spectrum. Compound 4 was previously reported by our research group [14].

Derivative 5 displayed its $[M-H]^+$ at *m/z* 317.2 in the FAB-MS (-ve mode). Newly appeared H-6 of compound 5 was observed at at $\delta$ 4.53, d ($J_{6,7}$ = 2.22 Hz) in the $^1$H-NMR spectrum. Signals for olefinic protons H-1 and H-2 were found missing in the $^1$H-NMR spectrum of 5 [15].

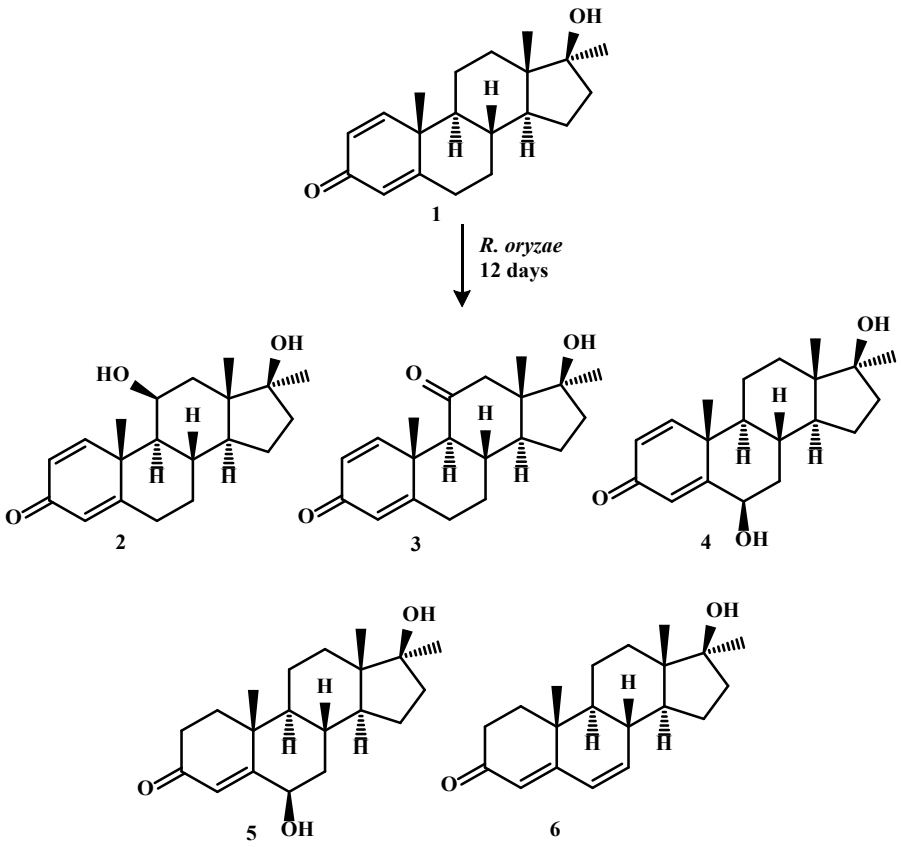


**Figure 1 –** Biotransformation of dianabol (1) with *R. oryzae*

Compound 6 showed its $[M+H]^+$ at 301.0 in the FAB-MS (+ve). Olefinic protons H-1 and H-2 were found missing while new downfield signals for olefinic protons H-6, and H-7 were observed at $\delta$ 6.09 s in the $^1$H-NMR spectrum of 6 [16].

## Conclusion

In the present study biotransformation of dianabol (1) was performed at ambient reaction conditions with *R. oryzae*. This afforded five known structural analogues of anabolic drug 1. The study indicates that *R. oryzae* was able to catalyze $\beta$ hydroxylation at C-6, and C-11 of drug 1. Dehydrogenation, reduction, and ketone formation was also observed during the transformation of dianabol (1). In future, transformed products 2-6 will be evaluated against various biological activities. These derivatives will also be studied at enzymatic levels for their mechanism of production.

## References

1. Adams, J. P., Brown, M. J., Diaz-Rodriguez, A., Lloyd, R. C., et al. (2019). Biocatalysis: A pharma perspective. *Adv Synth and Catal.*, 361 (11), 2421-2432. https://doi.org/10.1002/adsc.201900424

2. Alcántara, A. R. (2018). Biotransformations in drug synthesis: a green and powerful tool for medicinal chemistry. *J Med Chem and Drug Design*, 1 (1), 1-7. DOI: 10.16966/2578-9589.102

3. Atia-tul-Wahab, Siddiqui, M., Ibrahim, I., Hussain, A., et al. (2018). *Cunninghamella blakesleeana*-mediated biotransformation of a contraceptive drug, desogestrel, and anti-MDR-*Staphylococcus aureus* activity of its metabolites. *Bioorg Chem.* 77, 152-158. https://doi.org/10.1016/j.bioorg.2017.12.027

4. Bianchini, L. F., Arruda, M. F., Vieira, S. R., Campelo, P., et al. (2015). Microbial biotransformation to obtain new antifungals. *Front Microbiol*, 6, 1433. https://doi.org/10.3389/fmicb.2015.01433

5. Chegaing, S. P. F., Kengni, A. D. M., Siddiqui, M., Fowa, A. B., et al. (2020). Fungal transformation of norandrostenedione with *Cunninghamella blakesleeana* and anti-bacterial activity of the transformed products. *Steroids*, 162, 108679. https://doi.org/10.1016/j.steroids.2020.108679

6. Siddiqui, M., Atia-tul-Wahab, Jabeen, A., Wang, Y., et al. (2020). Whole-cell fungal-mediated structural transformation of anabolic drug metenolone acetate into potent anti-inflammatory metabolites. *J Adv Res.,* 24, 69-78. https://doi.org/10.1016/j.jare.2020.02.009

7. Siddiqui, M., Ahmad, M. S., Atia-tul-Wahab, Yousuf, S., et al. (2017). Biotransformation of a potent anabolic steroid, mibolerone, with *Cunninghamella blakesleeana*, *C. echinulata*, and *Macrophomina phaseolina*, and biological activity evaluation of its metabolites. *PloS one*, 12 (2), e0171476. https://doi.org/10.1371/journal.pone.0171476

8. Dürbeck, H. W., and Büker, I. (1980). Studies on anabolic steroids. The mass spectra of 17*α*-methyl-17*β*-hydroxy-1,4-androstadien-3-one (dianabol) and its metabolites. *Biomed Mass Spectrom.*, 7 (10), 437-445. https://doi.org/10.1002/bms.1200071007

9. Van der Kuy, P. H., Hooymans, P. M., Stegeman, A., and Looij jr, B. J. (1997). Falsification of Thai dianabol. *Pharm World Sci.*, 19 (4), 208-209. https://doi.org/10.1023/A:1008669917252

10. Ahmad, M. S., Yousuf, S., Atia-tul-Wahab, Jabeen, A., et al. (2017). Biotransformation of anabolic compound methasterone with *Macrophomina phaseolina*, *Cunninghamella blakesleeana*, and *Fusarium lini*, and TNF-*α* inhibitory effect of transformed products. *Steroids*, 128, 75-84. https://doi.org/10.1016/j.steroids.2017.04.001

11. Bano, S., Atia-tul-Wahab, Yousuf, S., Jabeen, A., et al. (2016). New anti-inflammatory metabolites by microbial transformation of medrysone. *PloS One*, 11 (4), e0153951. https://doi.org/10.1371/journal.pone.0153951

12. Choudhary, M. I., Siddiqui, M., Atia-tul-Wahab, Yousuf, S., et al. (2017). Bio-catalytic structural transformation of anti-cancer steroid, drostanolone enanthate with *Cephalosporium aphidicola* and *Fusarium lini*, and cytotoxic potential evaluation of its metabolites against certain cancer cell lines. *Front Pharmacol.,* 8, 900. https://doi.org/10.3389/fphar.2017.00900

13. Farooq, R., Hussain, N., Yousuf, S., Atia-tul-Wahab, et al. (2018). Microbial transformation of mestanolone by *Macrophomina phaseolina* and *Cunninghamella blakesleeana* and anticancer activities of the transformed products. *RSC Adv.*, 8 (39), 21985-21992. DOI: 10.1039/C8RA01309H

14. Khan, N. T., Zafar, S., Noreen, S., Al Majid, A. M., et al. (2014). Biotransformation of dianabol with the filamentous fungi and *β*-glucuronidase inhibitory activity of resulting metabolites. *Steroids*, 85, 65-72. https://doi.org/10.1016/j.steroids.2014.04.004

15. Kolet, S. P., Niloferjahan, S., Haldar, S., Gonnade, R., et al. (2013). Biocatalyst mediated production of 6*β*,11*α*-dihydroxy derivatives of 4-ene-3-one steroids. *Steroids*, 78 (11), 1152-1158. https://doi.org/10.1016/j.steroids.2013.08.004

16. Cooper, E. R., McGrath, K. C., Li, X., Akram, O., et al. (2017). The use of tandem yeast and mammalian cell *in vitro* androgen bioassays to detect androgens in internet-sourced sport supplements. *Drug Test Anal.*, 9 (4), 545-552. https://doi.org/10.1002/dta.2000

***Information about authors:***

*Mahwish Siddiqui – PhD at H.E.J. Research Institute of Chemistry (Karachi, Pakistan, e-mail: mahwish.siddiqui88@gmail.com)*

*Atia-Tul- Wahab – Professor at ICCBS, University of Karachi (Karachi, Pakistan, e-mail: tulwahab@yahoo.com)*

*Kudaibergenova Bates – PhD, Acc.professor at Al-Farabi Kazakh National University (Almaty, Kazakhstan, e-mail: bateskudaibergenova@yahoo.com)*

*Zharylkasyn Abilov – Professor at Al-Farabi Kazakh National University (Almaty, Kazakhstan, e-mail: abilov229@mail.ru)*

*Choudhary, Mohammed Iqbal – Director and Professor of Bioorganic and Natural Product Chemistry at the International Center for Chemical and Biological Sciences (H. E. J. Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research), University of Karachi (Karachi, Pakistan, e-mail: iqbal.choudhary@iccs.edu)*