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Biotransformation and molecular docking studies of aromatase inhibitors

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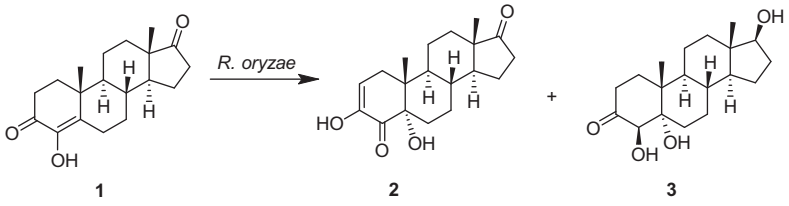
ABSTRACT

Bioconversion of the aromatase inhibitor formestane (4-hydroxyandrost-4-ene-3,17-dione) (**1**) by the fungus *Rhizopus oryzae* ATCC 11145 resulted in a new minor metabolite 3,5α-dihydroxyandrost-2-ene-4,17-dione (**2**) and the known 4β,5α-dihydroxyandrostane-4,17-dione (**3**) as the major product. The structural elucidation and bioactivities of these metabolites are reported herein. Molecular modeling studies of the interactions between these metabolites and the aromatase protein indicated that acidic (D309), basic (R115), polar (T310), aromatic (F134, F221, and W224), and non-polar (I133, I305, A306, V369, V370, L372, V373, M374, and L477) amino acid residues contribute important interactions with the steroidal substrates. These combined experimental and theoretical studies provide fresh insights for the further development of more potent aromatase inhibitors.



1. Introduction

Aromatase (EC 1.14.14.14) is an enzyme that converts androgens to estrogens [1]. In the conversion of androstenedione to estrone, for example, ring A of androstenedione is modified via a three-step sequence of C19 hydroxylations, C2–C3 enolization and finally, aromatization by the aromatase enzyme [2]. These estrogens, like estrone, 17β-estradiol and 17β,16α-estriol, are implicated in the proliferation of estrogen-dependent breast tumors in postmenopausal women. By inhibiting the aromatase it is possible to treat this and other types of cancers [3,4]. Current treatment of breast and ovarian cancers in postmenopausal women includes the use of third generation aromatase inhibitors such as the azole compounds anastrozole and letrozole. They compete with the substrate for binding to the aromatase enzyme active site [5,6]. Another clinically used drug is the steroid exemestane [7] which binds irreversibly to the enzyme [8,9].

In our search for new steroidal aromatase inhibitors (AIs) with potentially enhanced biological efficacy and fewer side effects, we recently reported the isolation of one new [4,11α,17β-trihydroxyandrost-4-en-3-one (**6**)] and three known metabolites from the bioconversion of the second-generation aromatase inhibitor formestane (**1**) by the prolific steroid transformers *Beauveria bassiana* ATCC 7159 and *Rhizopus oryzae* ATCC 11145 [10]. Bioactivity

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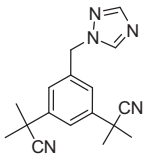
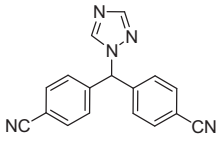
data studies showed that these metabolites were more potent aromatase inhibitors than formestane itself. This may be attributed to a more favorable substrate-enzyme interaction within the aromatase's active site.

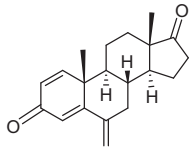
In continuation of this study, the extract from a large scale fermentation of formestane with *R. oryzae* was reinvestigated to identify the minor metabolites therein. The isolation, structural elucidation and biological studies of a new metabolite are reported in this paper. In addition, molecular docking studies were conducted to elucidate the binding modes of the aromatase and the potential steroidal aromatase inhibitors. The information obtained from these combined experimental and computational studies provide new insights for the design of better AI agents.



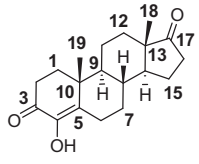
anastrazole



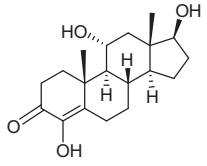
letrozole



exemestane



formestane (**1**)



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2. Experimental

2.1. General procedures

Melting points were determined on a Digimelt melting point apparatus and are uncorrected. Infrared spectra were recorded on a Thermo Fisher Nicolet iS10 FT-IR spectrometer. Optical rotations were performed on a Rudolph Research Analytical Autopol IV Automatic Polarimeter. Ultraviolet–Visible measurements were conducted on an Agilent Technologies Cary 60 UV–vis instrument. ^{1}H and ^{13}C NMR data were obtained on Avance-II-600 and Avance-III-600 MHz NMR instruments. Deuterated chloroform ($CDCl_3$) was used as solvent with tetramethylsilane (TMS) as internal standard. LRMS data was acquired on a Thermo Fisher LTQ-XL and HRMS data was obtained on an Agilent 6210 TOF-MS. Column chromatography was performed on silica gel (37–63 μm dia.). Detection of the compounds on thin layer chromatography (TLC) was achieved by spraying the plates with phosphomolybdic acid solution followed by heating until the color developed. *Rhizopus oryzae* ATCC 11145 was obtained from the American Type Culture Collection (ATCC), Rockville, MD, USA.

2.2. Microorganism and culture medium

Rhizopus oryzae ATCC 11145 was maintained on potato dextrose agar slants at 28 °C for two weeks. For each fungus, 10 slants (4 days old) were used to inoculate ten 500 mL Erlenmeyer flasks, each containing 250 mL of liquid culture medium. The liquid medium (2.5 L) for *R. oryzae* contained glucose (20 g/L), yeast extract (5 g/L), sodium chloride (5 g/L), and dipotassium hydrogen phosphate (5 g/L).

2.3. Fermentation and extraction

The flasks were shaken at 180 rpm at 27 °C. Formestane (1 g), dissolved in acetone, was pulse fed to the growing fungus (nine flasks) in portions of 10, 20, 30 and 40% at 24, 36, 48 and 60 h respectively after inoculation. One flask served as the control in which no substrate was added. The fermentation was allowed to proceed for five days. The fermentation beer was pooled and the fungal cells were separated from the broth via vacuum filtration. The broth was extracted with ethyl acetate (2 × 750 mL) and the fungal cells were homogenized and extracted in warm ethyl acetate (1000 mL). The organic solutions from the broth and fungal cells were dried separately with anhydrous sodium sulfate, filtered, concentrated *in vacuo* to afford crude extracts.

2.4. Bioconversion with R. oryzae

Formestane (1 g) was pulse fed to *R. oryzae*. Workup yielded broth and mycelial extracts which were pooled (1.2 g) and purified

Table 1
^{1}H (600 MHz) and ^{13}C (151 MHz) NMR assignments of compound **2** in $CDCl_3$.

Position	δ_C, type	δ_H, mult (*J* in Hz)	HMBC (H → C)[a]
1	29.5, CH_2	2.56 dd (18.7, 2.6), 2.25 dd (18.7, 7.2)	2, 3, 5, 9, 10
2	115.1, CH	5.96, dd (6.8, 2.9)	3, 4, 10
3	144.7, C	5.8, br s (OH)	2, 3, 4
4	194.3, C		
5	77.0, C		
6	28.5, CH_2	2.31 dt (12.5, 3.5), 1.64 td (13.2, 4.8)	4, 5, 7
7	27.1, CH_2	1.76 m, 0.86 m	6, 8
8	33.9, CH	1.60 m	9, 14
9	43.6, CH	1.23, m	8
10	43.5, C		
11	21.1, CH_2	1.55 m, 1.42 m	9, 13
12	31.1, CH_2	1.84 dt (12.6, 2.9), 1.21 m	18
13	47.8, C		
14	51.1, CH	1.22, m	8, 17, 18
15	21.8, CH_2	1.93 m, 1.56 m	13, 14, 17
16	35.9, CH_2	2.08 dt (19.4, 9.2), 2.46 dd (18.7, 7.8)	14, 17
17	220.6, C		
18	13.9, CH_3	0.89, s	12, 14, 17
19	16.5, CH_3	1.12, s	1, 5, 9, 10

[a] HMBC correlations are from proton(s) stated to the indicated carbon(s).