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Growth Inhibition of Norethisterone, Dydrogesterone, and their Biotransformed Products against Human Breast Cancer (MCF-7) Cells

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Abstract

*The in vitro growth inhibitory effects of norethisterone (**1**), dydrogesterone (**2**) and their biotransformed products 17a-ethinyl estradiol (**3**) and progesterone (**4**), respectively on a human breast cancer (MCF-7) cell line were investigated. All compounds showed weak activity, but compounds **1** and **3** strongly suppressed the growth of the breast cancer (MCF-7) cell lines (GI_{50} : 88±1.01 and 87±3.52, respectively) at 100 µM.*

Keywords: Biotransformation, Norethisterone, Dydrogesterone, Anti-cancer activity, Breast cancer(MCF-7) cell line, Sulforhodamine B assay

1. INTRODUCTION

Biotransformation is the process of bringing structural changes to organic compounds through the use of variety of enzymes found in the fungus, bacteria, algae, plants, and mammals.[1,2] Biotransformation is useful method for synthesizing aroma compounds, fragrances, flavors, potent biologically active analogues of organic compounds, through numerous biocatalytic reactions such as aromatization, reduction, oxidation, hydroxylation, double bond formation, acetylation, deacetylation, isomerization etc.[3-7] Generally, biotransformations are being used to synthesize organic molecules that are

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difficult to achieve with traditional synthetic methods. Compared to traditional chemical synthesis, biotransformation reactions are economical, environmentally benign, and regio- and stereo-selective.[8-10]

Cancer is one of the complicated diseases in which the abnormal cells development to occur into the body regions. The signs and symptoms of cancer depend on its type and grade. Cancer is caused due to inherited mutations, infections, air pollution, tobacco, alcohol.[11-14] Common signs of cancer include fatigue, fever, weight loss, irregular bleeding, skin changes, lump, and tissue mass.[15] There are numerous common chemotherapeutic medications available to treat cancer. The numerous negative consequences of these have limited their use and patient acceptance.[16,17] Cancer is a leading cause of human mortality. Globally, breast cancer is one of the main causes of cancer-related death and morbidity in women. All across the world, women can get breast cancer at any age after puberty, although the incidence rises with age.[18] The statistics expressed that breast cancer has become more common than lung cancer. There are approximately 2.3 million new instances of the breast cancer are reported, accounting for 11.7% among all malignancies worldwide.[19] Asia's highest regional rate of breast cancer is found in Pakistan.[20] In Pakistan, the prevalence of breast cacner has increased, and currently, one in nine women are at risk of developing the disease during their lifetime.[21]

The biotransformation of antifertility agent, norethisterone (**1**) by using *Paecilomyces variotii* yielded 17α-ethynylestradiol (**3**) through ring-A aromatization (Figure 1),[22] while swine wastewater bacteria converted fertility agent, dydrogesterone (**2**) into progesterone (**4**) through isomerization and hydrogenation (Figure 2).[23] The current work is conducted as a follow-up to our investigation into the biological activities of steroids and their biotransformed products.[22,24-26] Using the SRB cytotoxicity test, we are presenting the growth inhibition of substrates **1** and **2** and their biotransformed products **3** and **4** against the human breast cancer (MCF-7) cell line at different concentrations.

2. MATERIALS AND METHODS

2.1 General Experimental Conditions

The American Type Culture Collection (ATCC) provided the human breast cancer cell lines. Tris base, trichloroacetic acid (TCA), sulforhodamine B (SRB), acetic acid, dimethyl sulfoxide (DMSO), and Roswell Park Memorial Institute (RPMI) 1640 medium were bought from Sigma-Aldrich (USA) whereas doxorubicin were obtained from ICN, USA.

2.2 Sulforhodamine B Assay

Using the sulforhodamine B (SRB) cytotoxicity assay,[27] the growth inhibition of **1**-**4** was assessed against the human breast cancer (MCF-7) cell line. 96-well plates were utilized to culture breast cancer cells, with each well containing 7500 cells in 100 µL of culture media. The plates were then placed in a humidified 5% CO_2 incubator and incubated at 37 ºC for 24 hours. Compounds **1**-**4** were introduced in a volume of 100 µL, and the resulting mixture was subjected to an incubation period of 48 hours at a temperature of 37 ºC in a 5% CO_2 incubator with controlled humidity. Subsequently, 50 µL of 50% cold TCA was added and left at room temperature (28±2 ºC) for duration of 30 minutes. Subsequently to this step, all the plates underwent through washing with

distilled water and were left to air dry overnight. After the addition of 100 µL of SRB solution (0.4% w/v in 1% acetic acid) to each well, the excess SRB was removed by washing with 1% acetic acid and then left to dry at room temperature (28±2 ºC) for half an hour. Subsequently, the protein-bound dye dissolved using 10 mMtris base (pH=10.2) and the absorbance was determined at 515 nm using a synergy multi-mode microplate reader.

The RPMI medium by itself served as the blank while the reaction mixture without the sample served as the control. On the other hand, the RPMI medium containing sample served as the sample blank. Different dilutions ranging from 10 to 100 µM of **1**-**4** were employed for the initial screening. The extent of growth inhibition is quantified as GI_{50}, which denotes the concentration of compounds that result in a 50% reduction in cell growth.

2.3 Statistical Analysis

All samples were analyzed in three replicates for SRB cytotoxicity assay. Results were expressed as mean ± S.E.M. An excel software developed by the National Cancer Institute (NCI), USA was used for calculation of GI_{50}.

3. RESULTS AND DISCUSSION

After lung cancer, breast cancer is at second number among female causes of mortality. Chemotherapy, radiotherapy and occasionally surgery are the usual treatments for breast cancer, and come with a number of adverse effects. For numerous years, the primary approach to endocrine therapy in breast cancer has revolved around the use of antiestrogens, which effectively inhibit the estrogen receptor. The administration of tamoxifen, an antiestrogen, to countless women diagnosed with breast cancer has demonstrated a significant advantage. It has resulted in approximately 30-35% of patients experiencing relief from disease-related symptoms and a notable decrease in mortality rates by 20-25%.[28]

Norethisterone (**1**) is a potent synthetic sex steroidal hormone having extremely weak androgenic and estrogenic effect whereas 17α-ethynylestradiol (**3**) is a synthetic hormone possessing weak estrogenic activity. Dydrogesterone (**2**) is a strong synthetic steroidal sex hormone that lacks androgenic and estrogenic characteristics however progesterone (**4**) is an endogenous natural sex steroidal hormone, which is associated with pregnancy and menstrual cycle.

The *in vitro* growth inhibitory effects of **1**-**4** on a human breast cancer (MCF-7) cell line were investigated. Compounds **1** and **3** significantly inhibited the growth of human breast cancer (MCF-7) cell line (GI_{50} : 88±1.01 and 87±3.52, respectively) at 100 µM concentration whereas compounds **2** and **4** could not notably inhibited the growth of human breast cancer (MCF-7) cell line at similar concentration (GI_{50} : 50±1.22 and 66±1.51, respectively) (Table 1). The significant growth inhibition observed in compounds **1** and **3** can be attributed to their capacity to bind to estrogen receptor whereas the limited growth inhibition seen in compounds **2** and **4** is a result of their inability to bind to estrogen receptors.

4. CONCLUSION

In this study, we documented the growth-suppressing impacts of **1-4** on a human breast cancer (MCF-7) cell culture. The outcomes indicated that compounds **1** and **3** significantly suppressed the proliferation of the breast cancer (MCF-7) cell culture in comparison to compounds **2** and **4**. The results of this study support the use of steroidal compounds to develop new drugs for the treatment of human breast cancer.

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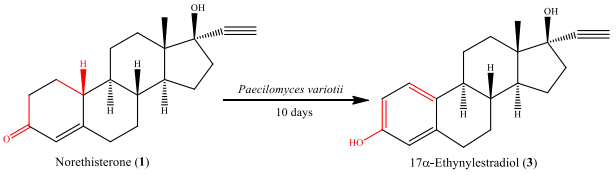

Figure 1. Biotransformation of norethisterone (**1**) with *P. variotii*

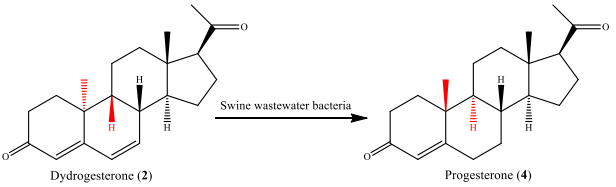

Figure 2. Biotransformation of dydrogesterone (**2**) with swine wastewater bacteria

Table 1. Growth inhibition of Compounds **1-4** against human breast cancer (MCF-7) cell lines

Conc. of Drugs	**% Growth Inhibition (GI_{50}) of Compounds and SEM**			
µM	**1**	**2**	**3**	**4**
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
10	29 ± 0.78	13 ± 1.65	19 ± 2.30	19 ± 0.99
25	56 ± 3.52	21 ± 1.65	50.8 ± 4.46	34 ± 0.87
50	63 ± 0.7	45 ± 1.42	57 ± 1.25	59 ± 1.74
100	88 ± 1.01	50 ± 1.22	87 ± 3.52	66 ± 1.51

Each value represents % mean ± standard error of mean of three independent experiments as compare to control.
GI_{50} = concentration of drug causing 50% growth inhibition of cells.
Doxorubicin (standard) GI_{50} = 78 % at 0.5 µM