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Synthesis, pharmacological evaluation and docking studies of progesterone and testosterone derivatives as anticancer agents

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ABSTRACT

Steroidal hormones progesterone and testosterone play a vital role in breast and prostate cancers. In this research, we have synthesized and characterized a total of thirty-one (**31**) new nitrogenous derivatives of progesterone and testosterone. The synthesized derivatives (**1–31**) were screened for their anti-cancer potential against MCF-7 and PC-3 cell lines of breast using MTT assay. The compounds **1-31**exhibited significant inhibitory potentials against MCF-7 and PC-3 cell lines. In MCF-7 assay, compound **17** displayed IC_{50} value of 04 ± 0.02 μM while compound **18** was leading in PC-3 assay with IC_{50} of 03.14 ± 0.4 μM. Tamoxifen was used as positive control which exhibited an $IC_{50}$of 0.12 ± 0.03 and 0.26 ± 0.01 μM against MCF-7 and PC-3 respectively. The compounds also showed good anti-inflammatory activity according to oxidative burst inhibition by chemiluminescence technique where ibuprofen was used as positive control with 73.2 ± 1.4% ROS inhibition. The compounds showed the percent ROS inhibition between 23.2 ± 0.2 and −3.2 ± 4.1. The results of the compounds were compared with the positive control ibuprofen. Molecular docking correlations suggest that the compounds exerted their inhibitory activity by binding to the active of the enzyme.

1. Introduction

Cancer, being the foremost cause of mortality worldwide is a key target for the upcoming researchers. In North America and Europe, cancer has been reported as the second leading cause of death. The pharmaceutical companies and various governmental and non-governmental organizations including National Cancer Institute (NCI) USA, European Organization for Research and Treatment of Cancer (EORTC) and the British Cancer Research Campaign (CRC) have been persistently focusing on the diagnosis, prevention and treatment of cancer. Various etiological factors have been previously described in different reports. One of the most important factors has been identified as the hormonal fluctuation [1]. Breast cancer is one of the leading diseases arisen from the hormonal fluctuations. Numerous therapeutic measures have been followed for the management of breast cancer. The chemotherapy has been considered as an effective therapeutic approach for the management of cancer [2]. But due to various adverse effects associated with existing drugs in the market, the researchers are in continuous efforts to explore novel sources of natural and synthetic drugs. The derivatization of existing natural and synthetic compounds gives rise to novel and effective compounds which lead to novel drug development [3].

Derivatization of steroids has gain attention of the drug designing scientists for the treatment of cancer [4]. Yahya et al. synthesized several heterocyclic derivatives of progesterone and evaluate their effect on the apoptotic and angiogenic pathway in MCF-7 breast cancer cell line. Similarly, megestrol, α-hydroxyprogesterone and medroxyprogesterone, heterocyclic steroids and curcumin derivatives are used a starting point for anticancer research and showed excellent anticancer potential [5,6]. Thiosemicarbazides and hydrazides are the class of compounds that have a been evaluated for their potential as anticancer activity. Many anticancer agents in clinical trials such as triapine A and 3-aminpyridine thiosemicarbazones are the examples [7].

Based on the clinical importance steroidal drugs in the context of cancer therapy, the current project has been designed to synthesize

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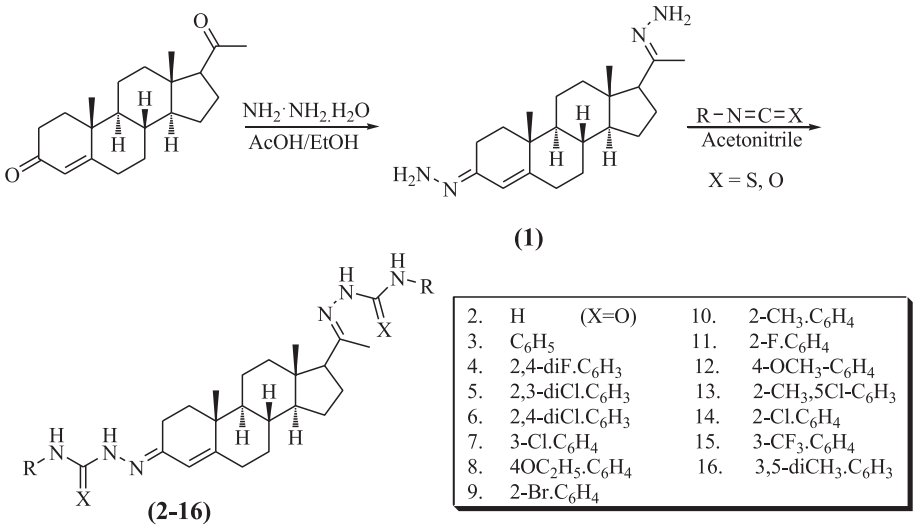


Scheme 1. Synthesis of semicarbazone (**2**) and thiosemicarbazone derivatives (**3–16**) of progesterone.

novel derivatives of progesterone and testosterone to evaluate them against various types of cell lines including MCF-7 and PC-3.

2. Results and discussion

2.1. Chemistry

Progesterone (Scheme 1) was reacted with hydrazine hydrate in the presence of acetic acid in ethanol heated at reflux for 30 min. The resulting hydrazone (**1**) was further reacted with various phenyl isothiocyanates to obtain progesterone semicarbazone derivatives **2–16**.

In Scheme 2, Schiff base derivatives **17–21** were synthesized in good to excellent yield (71–88%) by the reaction of progesterone with substituted anilines in the presence of ethanol as solvent and acetic acid as catalyst.

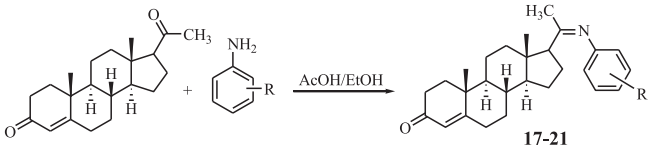
In Scheme 3, the progesterone was heated to reflux with substituted hydrazides to get hydrazones in the presence of ethanol and acetic acid 2–3 drop as catalyst.

Subsequently, we tried testosterone was reached with hydroxylamine and acetic acid was used as catalyst and further product was treated with phenyl isothiocynate as shown in Scheme 1.

2.2. Cell viability assay against MCF-7 and PC-3 cell line

A cell viability assay was performed using tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT).The synthesized derivatives of progesterone and testosterone (**1–31**) were screened against MCF-7 and PC-3 cell lines. The anticancer potential of all the synthesized compounds is tabulated in Tables 1 and 2. The viability was calculated by the following formula.

$$(A_s - A_b)/(A_n - A_b) \times 100$$



Scheme 2. Synthesis of Schiff base derivative (**17–21**) of progesterone.

where A_s = absorbance of sample; A_b = absorbance of blank; A_n = absorbance of control.

2.2.1. MTT results on PC-3

All the synthesized derivatives (**1–31**) were screened against PC-3 cell line as shown in Table 1. Table 1 revealed that all the compounds have the significant potential to act as anticancer agents. The compound **18** was highly active with IC_{50}value of 3.14 ± 0.4 μM. We can speculate that progesterone with 2,6 dimethyl group aniline substitution is more potent than progesterone and make it cytotoxic agent due to increase of hydrophobic nature. Similarly, hydroxylamine substitution with testosterone (as in compound **25**) also makes it more active with IC_{50} of 4.03 ± 0.07 μM. Compounds **17**, **26**, and **29** were also active with IC_{50} values of 5.24 ± 0.07, 5.00 ± 0.01 and 5.02 ± 0.01 μM respectively. Compound **22** also exhibited IC_{50}value of 06.02 ± 0.09 μM. The compound **24** and **28** showed a similar inhibition with IC_{50} values of 08.07 ± 0.05 and 8.31 ± 0.01 μM respectively. Compounds **6**, **23**, and **31** were almost similar in exhibiting anticancer potentials attaining IC_{50} values of10.09 ± 0.21, 10.43 ± 0.71 and 10.01 ± 0.13 μM respectively. In comparison, the positive control tamoxifen exhibited IC_{50} value of 0.26 ± 0.01 μM.

2.2.2. MTT results on MCF-7

All the synthesized newderivatives were tested against MCF-7 cell line of breast cancer as shown in Table 2. Actually breast cancer is also steriod dependent cancer in which sex setiods play an important role. In this analysis tamoxifen was used as positive control with IC_{50} of 0.12 ± 0.03 μM. As comparison to standard drug used, compounds **6**, **17** and **29** exhibited IC_{50} values of 4.78 ± 0.42, 04.00 ± 0.02 and 04.80 ± 1.47 μM respectively. As our synthesized derivatives exhibited overwhelming anticancer activities, therefore, can play a possible role in the drug discovery.

2.3. Anti-inflammatory activity

2.3.1. Phagocytes oxidative burst inhibition by chemiluminescence technique

The synthesized nitrogenous derivatives were screened for phagocytes oxidative burst inhibition by chemiluminescence technique to represent their anti-inflammatory behavior. However, our compounds did not show any promising anti-inflammatory activity at 25 μg/mL. Ibuprofen was used as positive control, which showed the inhibition of 73.2%. Slightly active compounds were compounds **4** and **24** with % inhibition of 23.20 ± 0.2 and 23.40 ± 3.6 μg/mL respectively. Rest of