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The cytotoxic effects of a bioactive nitro derivative of pyrimidine to human breast cancer (MCF-7) cells

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Abstract

Breast cancer is one of the leading causes of mortality among women worldwide mostly due to resistance to existing chemotherapeutic agents. In this study, a bioactive nitro derivative of pyrimidine compound 2,6-Diamino-5-((5-methylisoxazole-3-yl) diazenyl) pyrimidin-4-0l was synthesized by the condensation reaction under reflux conditions. Characteristics and structure of synthesized compound was confirmed by the FT-IR,C-NMR and H-NMR analyzes. The cytotoxic effect of the compound was measured by MTT assay at various concentrations (5-100 µg/ml) on breast cancer MCF-7 cells. The MTT results indicated that the synthesized compound had a significant cytotoxic effects on MCF-7 cells in a dose-dependent manner. Our findings indicated that bioactive nitro derivative of pyrimidine compound has a potential to be applied as anticancer agent against breast cancer cells.

Keywords: Pyrimidine nitro derivative, Cytotoxicity, MCF-7

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1 Introduction

Cancer is one of the major health problems in today's society and many extensive efforts are being made to combat it. However, in many cases, cancer cells can eventually cope with the therapies offered. Cancer is the second leading cause of death in humans. According to the American Health Association, 7.6 million people died of cancer in 2007 (Krapf et al., 2017). According to the statistics published by the Cancer Research, Treatment and Education Institute, based on the total number of men and women in 2008, skin cancer with 13.08%, breast cancer with 11.31% and gastric cancer with 10.17% were the most common cases (Wei and Malhotra, 2012). Worldwide, breast cancer is the most important type of cancer in women and accounts for 25% of all cancers (Silbermann et al., 2020). Breast cancer occurs in two different ways: (1) invasive breast cancer cells cross the barriers of normal breast tissue and spread through the bloodstream and lymph nodes to other parts of the body (Chikhale et al., 2018), and (2) non-invasive cancer cells remain at a specific point in the breast, without spreading to surrounding tissues. Although the exact cause of breast cancer is not known, it can be caused by factors such as obesity, lack of exercise, drinking alcohol, hormone replacement therapy during menopause, and radiation. Age and family history can also be a risk factor for breast cancer (He et al., 2020).

Pyrimidines are hexagonal rings composed of 4 carbon and 2 nitrogen atoms in the meta position relative to each other. Pyrimidines are the structural units of many compounds, including antibiotics, vitamins, and polysaccharides. The most important compounds with pyrimidine structure are cytosine, guanine and uracil, which are involved in the structure of RNA and DNA. Pyrimidines have received a great deal of attention due to their high variability and presence in DNA structure, and much research has been done on their pharmacological effects (Wang et al., 2018). Pyrimidine-containing compounds have anti-metabolic properties and are used in the treatment of various cancers (Chiacchio et al., 2019). Synthetic pyrimidine-based derivatives containing active nitro groups can be used as a heat shock protein (Hsp70) inhibitor and apoptosis inducer as an adjunct to chemotherapy drugs. Heat shock proteins have a wide range of functions in normal cells and are also active in the apoptotic pathway (Kandeel et al., 2015). Recent researches have shown that the use of Hsp70 inhibitors reduces both the invasiveness and toxicity of breast cancer cells (Taglieri et al., 2020; Farghaly et al., 2021). The pyrimidine-based bioactive compounds have been reported to act as Hsp70 inhibitors and inducers of apoptosis, and hence, can be used as adjunctive chemotherapy drugs (Laurenzana et al., 2016). It has been reported that new bioactive compounds based on nitro-containing pyrimidine are able to control and treat breast cancer as the most common cancer (Yellapu et al., 2014). The aim of this study was to investigate the cytotoxic effects of a bioactive nitro derivative of pyrimidine on human breast cancer (MCF-7) cells.

2 Materials and Methods

2. 1. Compound and cells

MCF7 cancer cells were purchased from Pasteur Institute of Iran. The cells were delivered frozen in vials placed by an expert in a nitrogen tank to protect the cells. In this study, a bioactive nitro derivative of pyrimidine compound 2,6-Diamino-5-((5-methylisoxazole-3-yl) diazenyl) pyrimidin-4-Ol was synthesized by the condensation reaction under reflux conditions.

2. 2. Cell Culture

MCF-7 cells were evaluated for morphology and the possibility of infection and were seeded in T75 cell culture flasks at 1×10^6 cells/flask in low glucose Dulbecco's modified Eagle's medium (DMEM) containing 10-15% fetal bovine serum (FBS), and supplemented by 1-2 mM glutamine. Cells were cultured at 37°C in presence of 5% CO2. As MCF-7 cells reached approximately 85-90% confluency, the media was removed and cells were rinsed twice with 1x PBS. 2-3 mL of Trypsin was added to cells. Once the cell layer was detached, Trypsin was neutralized by adding 10mL of complete growth medium to the flask. Cells were suspended and centrifuged for 5 minutes at 125 x g to pellet cells. Trypsin/growth medium suspension was aspirated from tube. The cells were plated into a new flask containing complete growth medium and incubated at 37°C in humidified 5% CO2 atmosphere.

2. 3. MTT Assay

The cell viability assay was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye reduction assay which was performed to determine the cytotoxic effect of the synthesized compound at various concentrations. Briefly, the MCF-7 cells were plated onto 96-well flat bottom culture plates with various concentrations of synthesized compound. All cultures were incubated for 24 h at 37°C in a humidified incubator. After 24 h of incubation (37°C, 5% CO2 in a humid atmosphere), 10 µL of MTT (5 mg/mL in PBS) was added to each well, and the plate was incubated for a further 4 h at 37°C. The resulting formazan was dissolved in 100 µL of DMSO with gentle shaking at 37°C, and absorbance was measured at 570 nm with an ELISA reader. Concentrations of synthesized compound showing a 50% reduction in cell viability (i.e., IC50 values) were then calculated.

2. 4. Statistical analysis

The tests were repeated three times and the results were reported as Mean ± SD. The obtained data were statistically analyzed using Graphpad InStat software V. 8 (La Jolla, CA, USA). One-Way ANOVA was used for statistical analysis. $p < 0.05$ was considered as a significant level.

3 Results and Discussions

Cell viability significantly decreased in MCF7 cells treated with synthesized compound compared to control group 24 hours after treatment (Figures 1 and 2).

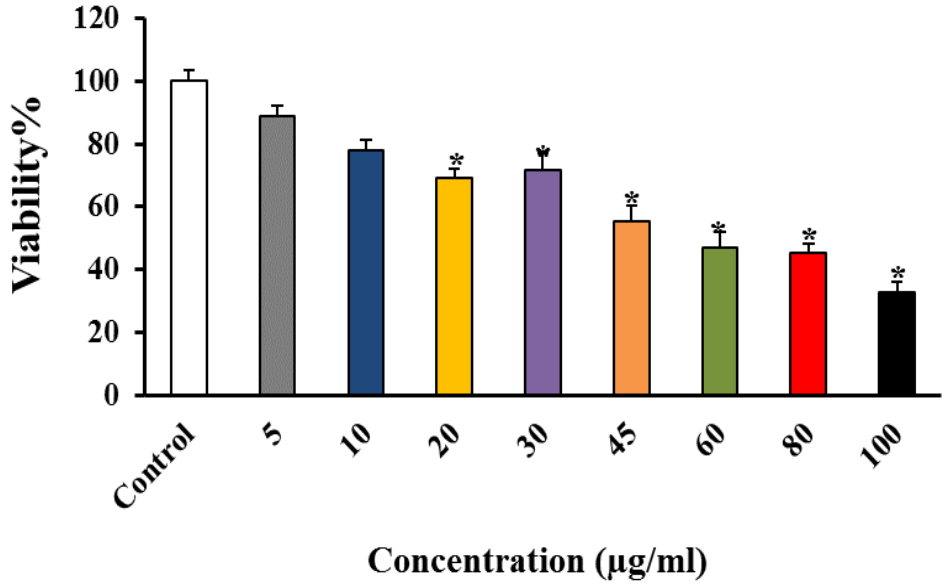

Figure 1. Viability of MCF7 cells treated with different concentrations of 2,6-Diamino-5- ((5-methylisoxazole-3-yl)-diazenyl) pyrimidin-4-0l 24 hours after treatment.

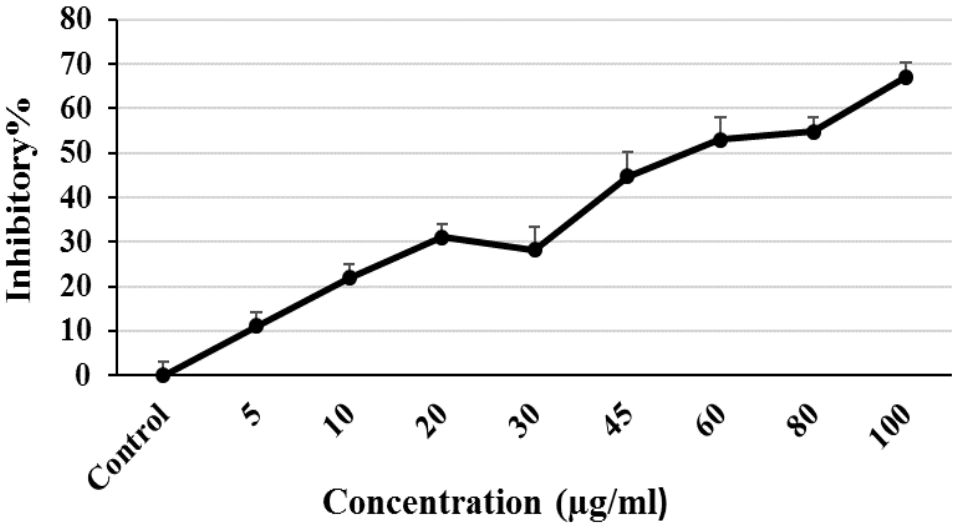

Figure 2. MCF7 cells treated with different concentrations of 2,6-Diamino-5-((5-methylisoxazole-3-yl)diazenyl)pyrimidin-4-0l 24 hours after treatment.

The results of this study showed that the IC50 value was 45 µgr/ml for synthesized compound 24 hours after treatment.

Cancer is a major problem worldwide. Among the various types of malignant tumors, breast cancer is the second leading cause of death in women. Although current advances in early breast cancer can play an important role in the recovery of these patients, resistance to treatment and recurrent conditions have led to continued efforts to find new treatments. One of the most important mechanisms that causes cancer cells to become resistant to drugs is the escape of these cells from the process of apoptosis and consequently their immortality (Kilic – Kurt et al., 2020). The results of the present study showed that, depending on the dose used, the

synthesized primidine compound 2,6-diamino-5 - ((5-methyl isooxazole-3-yl) diazenyl) pyrimidine-4-ol decreased viability of MCF-7 breast cancer cells. At a dose of 45 micrograms per milliliter, about 50% of the cells were not alive and at a dose of 100 µgr/ml more than 60% of the cells were killed. Due to their known anti-cancer activities, pyrimidine derivatives have attracted much attention from pharmacological and organic chemists, and numerous analogues are being developed or approved for the treatment of cancers (Nemr and AboulMagd, 2020). The results of previous studies have shown that nitrogen-based heterocyclic derivatives, including aminopyrimidine derivatives, are biologically important because they exhibit high bioactive activities and can have significat anti-cancer and anti-proliferative effects. Taglieri et al. found that treatment with nitrogen-containing pyrimidine compounds was able to kill colon, breast and brain cancer cells (Taglieri et al., 2020). It has recently been discovered that the anti-cancer activities of pyrimidine and pyridone derivatives are involved in inhibiting cellular signal transduction pathways that regulate a variety of cellular functions such as proliferation, differentiation, apoptosis, and cell migration (Safari et al., 2020). Deregulation of signal transduction pathways has been identified as a key factor in the development of many types of cancer. Thus, these pyrimidine derivatives specifically target abnormal pathways, a new targeted molecular therapy in cancer treatment with less reliance on non-discriminatory killing of tumor and host cells (Prajapti et al., 2016). In a study by Kemnitzer et al., two compounds, 4-anilino-N-methylthieno [3,2-d] pyrimidines and 4-anilino-N-methylthieno [2,3-d] pyrimidines, caused the death of T47D breast cancer cells (Kemnitzer et al., 2009). Also in the study it has been shown that thienopyrimidine combination treatment in MCF-7 breast cancer cell line caused cell death and reduced their survival (Abdelhaleem et al., 2018).

4 Conclusion

The results of this study revealed that 2,6-Diamino-5-((5-methylisoxazole-3-yl)diazenyl) pyrimidin-4-0l has significant cytotoxic effects on breast cancer (MCF7) cells *in vitro* and the treatment duration plays a role to increase the cytotoxic effect of the drug on breast cancer cells.

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Conflict of interests

The authors state that there are no conflicts of interests regarding the publication of this article.

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