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Original paper

Investigating the Impact of a Bioactive Nitro Derivative of Pyrimidine on Human Breast Cancer (MCF-7) Cells through Flow Cytometry and Western Blot Analysis

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Abstract

Background and Aim: Breast cancer is a significant worldwide health issue, highlighting the need for innovative therapeutic approaches. This study aims to explore the impact of a bioactive nitro derivative of pyrimidine on human breast cancer cells (MCF-7) using flow cytometry and western blot analysis.

Materials and Methods: The structure and properties of 2,6-diamino-5-((5-methylisoxazole-3-yl) diazenyl) pyrimidin-4-ol (referred to as compound 3B) were confirmed through IR-FT, NMR-C, and NMR-H analyses. MCF-7 cells were treated with various concentrations of the compound, and its effects on cell viability, proliferation, and apoptosis were evaluated using flow cytometry. Additionally, western blot analysis was employed to assess changes in the expression of key proteins associated with cell survival, proliferation, and apoptosis.

Results: Compound 3B exhibited notable cytotoxic effects on MCF7 cells, leading to apoptosis as confirmed by flow cytometry analysis. The western blot results revealed a significant decrease in the levels of HSP70, P53, and Bcl-2 proteins, indicating their downregulation upon treatment with compound 3B. Conversely, there was an observed increase in the levels of Noxa, Bad, and Apaf-1 proteins, suggesting their upregulation in response to compound 3B treatment.

Conclusion: These findings highlight the impact of compound 3B on the regulation of key proteins involved in cell survival, apoptosis, and cellular pathways associated with cancer progression in MCF7 cells. Further studies are warranted to elucidate the underlying mechanisms and explore the therapeutic potential of compound 3B as a promising candidate for breast cancer treatment.

Keywords: *Nitro derivative of pyrimidine, MCF-7, Apoptosis, Flow cytometry, Western blot*

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Introduction

Breast cancer, a prevalent and multifaceted disease, holds a significant place in global health. Its epidemiology reflects its widespread impact, making it the most common cancer among women. A complex interplay of genetic predisposition, hormonal influences, and environmental factors contributes to its etiology. Despite advancements, the disease's complications encompass not only physical but also emotional and psychological dimensions, affecting patients and their families. In response, treatment strategies have evolved, ranging from surgery and radiation to targeted therapies and emerging immunotherapies, emphasizing personalized approaches for enhanced outcomes [1], [2], [3].

Nitro derivatives of pyrimidine constitute a class of organic compounds that bear significant importance in various fields, spanning medicinal chemistry, agriculture, and materials science. These derivatives are characterized by the presence of a nitro group (-NO2) attached to the pyrimidine ring, imparting distinct physicochemical properties and biological activities. Due to their versatile nature, nitro pyrimidines have garnered attention for their potential as pharmacologically active agents, including antimicrobial, anticancer, and antiviral agents. Their involvement in diverse biochemical pathways and interactions with biological macromolecules make them intriguing targets for drug development. Furthermore, the incorporation of nitro groups in pyrimidine scaffolds can also influence the reactivity and electrochemical behavior of these compounds, leading to applications in materials science and catalysis [4], [5], [6].

The incorporation of a nitro group (-NO2) onto the pyrimidine scaffold introduces unique chemical properties that could be harnessed for targeted interventions in cancer therapy. These derivatives have demonstrated versatile modes of action, including interference with critical cellular processes and pathways that are dysregulated in cancer cells. By exploring the interactions between nitro pyrimidines and key molecular targets implicated in cancer progression, there is a promising avenue to uncover novel therapeutic strategies with the ultimate goal of improving cancer treatment outcomes [7]. Nitro derivatives of pyrimidine compounds offer a compelling avenue for potential advancements in breast cancer research and therapy. Their distinct chemical structure, characterized by the incorporation of a nitro group (-NO2) onto the pyrimidine framework, holds the promise of targeted interventions against breast cancer [8].

These derivatives exhibit a range of bioactive properties that could be harnessed to target specific molecular pathways involved in breast cancer development and progression. By exploring the interactions between nitro pyrimidines and key biomolecules associated with breast cancer, there lies a valuable opportunity to uncover novel approaches for diagnosing, treating, and managing this complex disease, thereby contributing to the ongoing efforts to improve breast cancer outcomes.

Material and Methods

Confirmation of Compound Structure and Properties

The structure and properties of compound 2,6-diamino-5-((5-methylisoxazole-3-yl) diazenyl) pyrimidin-4-ol (referred to as compound 3B) were verified through analyses of IR-FT, NMR-C, and NMR-H.

Cell Culture and Treatment

MCF-7 breast cancer cells were cultured in appropriate growth media under standard conditions, followed by treatment with various concentrations of compound 3B for defined durations.

Cell Viability, Proliferation, and Apoptosis Assessment

Cell viability, proliferation, and apoptosis were assessed using flow cytometry after treatment of

MCF-7 cells with different concentrations of compound 3B. Cell viability was determined through fluorescence-based viability assays, cell proliferation was evaluated using thymidine analog incorporation assays, and apoptosis was analyzed using Annexin V/propidium iodide staining.

Western Blot Analysis

Cellular proteins were extracted from treated MCF-7 cells, and their concentrations were quantified using a protein assay. The proteins were separated by SDS-PAGE, transferred to a membrane, and subsequently probed with primary antibodies targeting proteins associated with cell survival, proliferation, and apoptosis. Secondary antibodies linked to enzymes or fluorophores were applied, and protein bands were visualized and quantified using appropriate detection methods.

Results

Remarkable cytotoxic effects were evident in MCF7 cells upon exposure to compound 3B, inducing apoptosis, as verified through meticulous flow cytometry analysis (Figure 1 and 2).

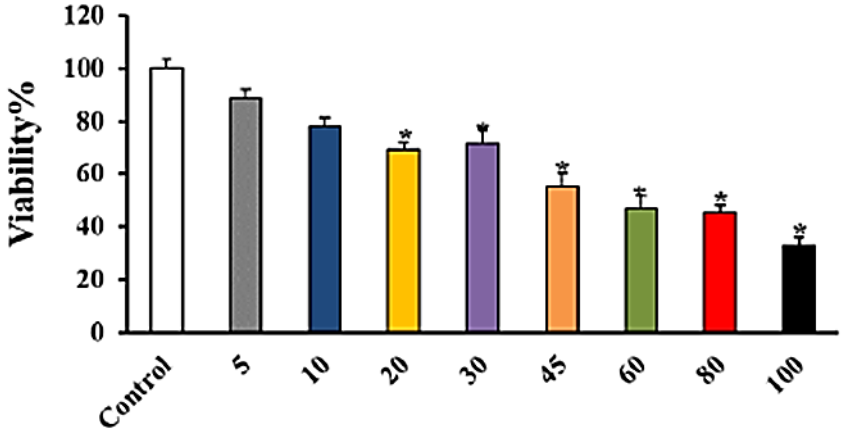

Figure 1. Viability of MCF7 cell treated with various concentrations of 3B.

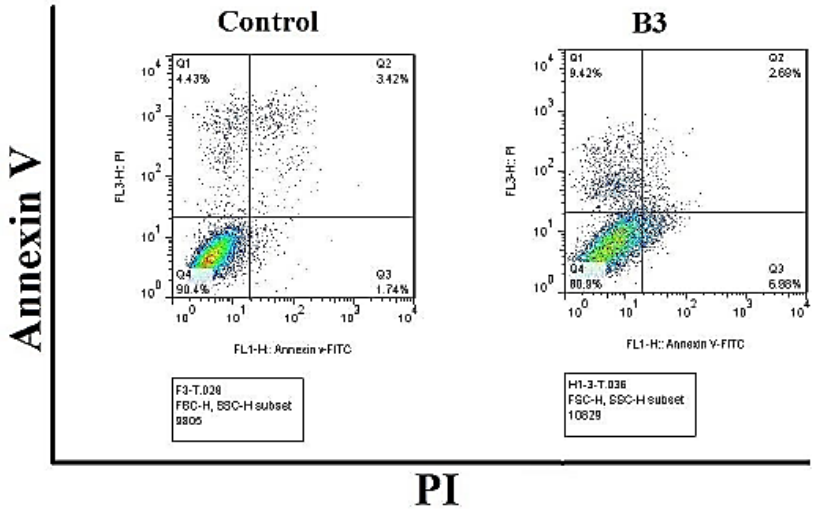

Figure 2. Flow cytometry analysis of MCF7 cells treated with compound 3B. Subpopulations defined by quadrants: Q1 (Necrotic Cells), Q2 (Apoptotic Cells), Q3 (Preapoptotic Cells), and Q4 (Normal Cells).

The comprehensive insights provided by western blot outcomes showcased intricate protein dynamics. A significant reduction in the protein expression levels of HSP70, P53, and Bcl-2 was evident when MCF7 cells were treated with 30 µg/ml of B3. In contrast, an observable elevation surfaced in the levels of Noxa, Bad, and Apaf-1 proteins was observed in MCF7 cells treated with 30 µg/ml of B3 (Figure 3).

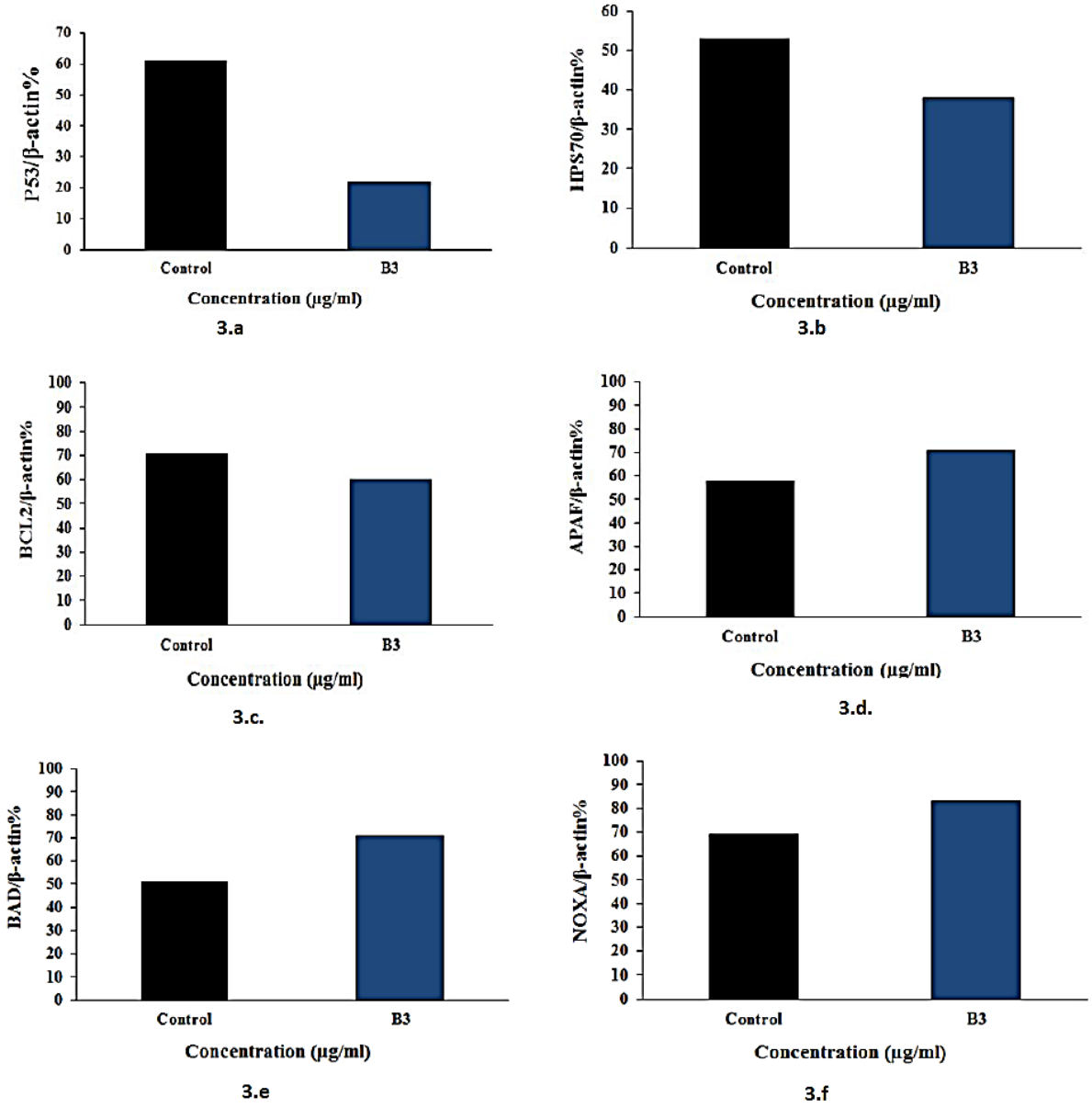

Figure 3. Western blot analysis. 3.a: P53 expression level, 3.b: HSP70 expression level, 3.c: Bcl-2 expression level, 3.d: Apaf-1 expression level, 3.e: Bad expression level, 3.f: Noxa expression level.

Discussion

The findings from this study demonstrate the substantial impact of nitro derivative of pyrimidine (Compound 3B) on MCF7 cells, underscoring its potential as a cytotoxic agent with apoptosis-inducing properties. The confirmation of apoptosis through flow cytometry analysis adds a vital

layer of evidence to this observation, highlighting the physiological consequence of Compound 3B treatment. The western blot results contribute valuable mechanistic insights into the observed cytotoxic effects. The significant reduction in HSP70, P53, and Bcl-2 protein levels aligns with the downregulation of key proteins associated with cell survival and anti-apoptotic pathways. This suggests that Compound 3B may exert its cytotoxic effects by interfering with these vital cellular components, potentially disrupting cell homeostasis and apoptotic regulation. In line with our findings a study highlights the synthesis and anticancer potential of specific pyrido[2,3-d]pyrimidine derivatives, which demonstrate efficacy as both inducers of apoptosis and inhibitors of cyclin-dependent kinases (CDKs) [6].

Another research study has reported the synthesis of thienopyrimidine urea derivatives, showcasing their potential as compounds with cytotoxic and pro-apoptotic properties against a breast cancer cell line [9]. Furthermore, a demonstrated instance elucidates the anticancer activity and mechanism of a dimetallic Ru(II)(η6-p-cymene) complex based on bis-pyrimidine. This complex exhibits its effect in human non-small cell lung cancer through a p53-dependent pathway [10]. Evidence highlights the potent induction of apoptosis by 4-anilino-N-methylthieno[3,2-d]pyrimidines and 4-anilino-N-methylthieno[2,3-d]pyrimidines [11]. A reported study encompasses the design, synthesis, anticancer evaluation, molecular docking, and cell cycle analysis of 3-methyl-4,7-dihydropyrazolo[1,5-a]pyrimidine derivatives, showcasing their potential as potent inhibitors of histone lysine demethylases (KDMs) and as inducers of apoptosis [12]. Additionally, research has demonstrated the antitumor activity of a novel triazolo[1,5-a]pyrimidine compound on cancer cells. This effect is achieved through the induction of cellular apoptosis and the inhibition of the epithelial-to-mesenchymal transition process [13].

Conclusion

In conclusion, the significant impact of compound 3B on the modulation of key proteins involved in cell survival, apoptosis, and various cellular pathways associated with cancer progression in MCF7 cells opens up a promising avenue for breast cancer research and potential therapeutic interventions. These findings underscore the importance of exploring compound 3B's mechanisms of action in greater detail to gain deeper insights into how it exerts its effects on cancer-related processes. Understanding these mechanisms could pave the way for the development of more targeted and effective treatments for breast cancer. Moreover, the potential therapeutic value of compound 3B in breast cancer treatment warrants comprehensive preclinical and clinical investigations. Assessing its safety profile, efficacy, and potential synergistic effects with existing treatments is essential for its translation into clinical practice.

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Conflict of interests

The authors declare that there are no competing interests.

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