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Original Article

Study on Anticancer Activity of 4, 4'-[1,4-phenylenebis(1,3,4-thiadiazole-5,2-diyl)] bis(azaneylylidene) bis(methaneylylidene) diphenolon Breast Cancer Cells

Saeed, B. M. S[1*], Al-Jadaan, S. A. N[2], Abbas, B. A[3]

1. University of Basrah, Al-Zahraa College of Medicine, Microbiology Department, Basrah, Iraq
2. University of Basrah, College of Pharmacy, Pharmaceutical Chemistry Department, Basrah, Iraq
3. Department of Microbiology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq

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Corresponding Author: ban.saeed@uobasrah.edu.iq

Abstract

New medicinal compounds are being evaluated due to the increasing prevalence of cancer in human societies and the necessity to produce new medications for treatment. The new Schiff base compound 4,4'-[1,4-phenylenebis(1,3,4-thiadiazole-5,2-diyl)] bis (azaneylylidene) bis (methaneylylidene) diphenol, which was previously produced from the reaction of 5,5' [(1,4-Phenelene) bis (1,3,4-thiadiazol-2-amine)] and the para-hydroxy ben aldehyde was synthesized and different concentrations (250 and 300 mg/mL) of this new compound were exposed to breast cancer (MCF-7) cells to examine its cytotoxicity effect. Cell line viability, acridine orange/propidium iodide staining, and DNA fragmentation were assessed in evaluating the antitumor effect of the new composition. Obtained data from cell viability assays demonstrated cytotoxic activity against MCF-7 breast cancer cell lines. No fragmentation was observed in DNA fragmentation of the novel compound base with MCF-7 and Vero cell line. The new Schiff base compound indicated well-defined anti-cancer activity when treated with breast cancer cells (MCF-7). The compound blocked the proliferation of cancer cells without apoptosis. As a consequence of the findings, it was recommended to use this compound in treating breast cancer.

Keywords: Breast Cancer, DNA Laddering Assay, Thiadiazole Compound

1. Introduction

Malignant neoplasmsare one of the leading causes of death after cardiovascular diseases according to the data provided by the World Health Organization (1, 2). Breast cancer is the most common cancer in women, and epidemiological studies have shown that it accounts for approximately one-third of all cancers that affect women. Breast cancer is the second leading cause of death after lung cancer and one of the leading causes of death in women between the ages of 40 and 55 (1-3). For decades, chemotherapy has been the most common type of anticancer medication (4).

Chemotherapy has greatly progressed and the effectiveness of many other medications has been investigated due to the high prevalence of cancer in recent years. Evaluation and optimization of medications prescribed are every important due to the Narrow Therapeutic Index drugs and possible side effects (5-7). In vitro condition was used to evaluate the efficacy of new compounds for the treatment of tumors cell lines.MCF-7 is a breast cancer cell line that was previously isolated from a69-year-old Caucasian woman in 1970 (8). It is a widely used cell line for

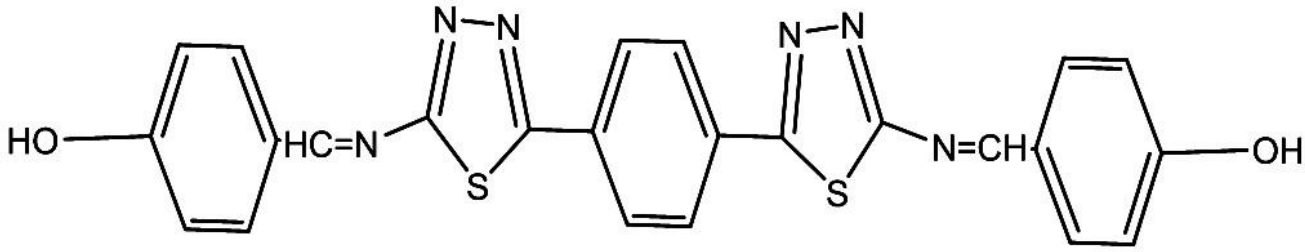
breast cancer that has been propagated by various groups for several years (9, 10).

Thiadiazole is a heterocyclic five-member compound consisting of two particles of nitrogen and one iota of sulfur. Four isoforms of these compounds are found in nature: 1,2,3-thiadiazole, 1,2,4-thiadiazole,1,2,5-thiadiazole, and 1,3,4-thiadiazole. Thiadiazole is a bioisostere of pyrimidine and oxadiazole. Nowadays, 1,3,4-thiadiazole ring compounds are used in skeletons of many pharmaceutical compounds. These compounds include a wide range of antiviral, antibacterial, antifungal, antiparasitic, anti-inflammatory, and anticancer effects (11). Thiadiazoles are prepared to cross cell membranes considering their mesoionic nature. Their incredible liposolubility is a direct result of the presence of the sulfur atom (12). One of the thiadiazole-derived compounds is Imatinib, which is a tyrosine-kinase inhibitor. The compound is used to treat chronic myelogenous leukemia, gastrointestinal stromal tumors, and many other malignancies. Also, doxorubicin are used to treat some cancers of the bladder, breast, stomach, lung, ovary, thyroid, soft tissue sarcoma, and multiple myeloma and leukemia (13-15). The present study aimed to investigate anticancer effects of the novel chemical compound 4,4'-[1,4-phenylenebis(1,3,4-thiadiazole-5,2-diyl)] bis (azaneylylidene) bis (methaneylylidene) diphenol, (14) against the MCF-7 cell line and its therapeutic effect in inhibiting the growth of breast tumors.

2. Material and Methods

2.1. Cell Line Viability

The new Schiff base mentioned below (Figure 1) was previously prepared by Saeed, Al-jadaan (14). The new compound 4,4'-[1,4-phenylenebis(1,3,4-thiadiazole-5,2-diyl)] bis (azaneylylidene) bis (methaneylylidene) diphenol which was previously prepared from the reaction of the 5,5' [(1,4-Phenelene) bis (1,3,4-thiadiazol-2-amine)] and the para-hydroxybenzaldehyde using the microwave method.



4,4'-((1*E*,1'*E*)-((1,4-phenylenebis(1,3,4-thiadiazole-5,2-diyl))bis(azaneylylidene))bis(methaneylylidene))diphenol

Figure 1. Structure of the new Schiff base

The cell line examination was acquired from the Iraqi Center for Cancer and Medical Genetics Research (ICCMGR), which includes malignancies in the human breast (MCF-7).

Cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum at 37ºC, 5% CO_2, and humidity of 95%. At 1 * 10^4 other compounds containing thiadiazole rings such as cells/well, cells were seeded in96-well plates and allowed to adhere. The cells were treated with different concentrations of the chemical compound after 24 h. From the stock solution (10.00 µg/ml), different concentrations of a novel compound base were used and resuspended in DMSO. Triplicate concentrations of 250 and 300 mg/ ml were used to treat the cells. After 24, 48, and 72 h of treatment, 20 ml of the solvent solution (DMSO) was applied to each well and

incubated at 37°C. The compound-untreated cells were considered as control cells (16, 17). An ELISA reader was used to calculate the number of viable cells. The value for cell exposure to the new Schiff base was expressed as a percentage of the value for cell viability and that of the control after 48 h. The absorbance for each well was measured at 540 nm in a microplate reader and the percentage of cell viability (CV) was manually determined using the formula (18):

CV= (Average abs of drug wells / Average abs of control wells) * 100

2.2. Acridine Orange/Propidium Iodide (AO/PI) Assay

Acridine orange (AO)/propidium iodide (PI) staining was used to evaluate cell apoptosis at different concentrations of the chemical compound (19, 20). The untreated MCF-7 cells served as the control. An aliquot of 1 µl of 0.5 mg/ml acridine orange/propidium iodide (AO/PI) reagent was added to each well containing different concentrations of a novel compound base composition and incubated for 10 min at room temperature according to the previous step. Dual fluorescence was measured using a multi-detection microplate reader with an excitation wavelength of 460 nm and an emission wavelength of 650 nm for AO and an excitation wavelength of 525 nm and an emission wavelength of 595 nm for PI (16). Green and red cells represent living and apoptotic cells, respectively.

2.3. DNA Laddering Assay

In 25 cm cell culture flasks, 0.5 mL of cell suspension of MCF-7 line of human cancer cells was treated with the new Schiff base centrifuged at 2000 rpm at 4°C for 10 min. Then 0.5 mL of TES lysis buffer and vortex, as well as 20 µL of RNase were added and mixed well and incubated for 30-120 minutes at 37°C. Afterward, 20 µL of proteinase K was added and mixed by flipping the tip of the tube and incubating at 50°C for at least 90 minutes. After mixing the DNA samples with the loading buffer, samples loaded 10-20 µL of DNA samples into each well of a standard 1% agarose gel containing 0.5 µg/mL ethidium bromide (16).

3. Results

3.1. Cytotoxicity Assay

4,4'-[1,4-phenylenebis (1,3,4-thiadiazole-5,2-diyl)] bis (azaneylylidene) bis (methaneylylidene) diphenol was obtained according to the procedure described by (21). Evaluation of cytotoxicity and viability of breast cancer cells exposed to the new compound base was calculated in MCF-7 cell culture and Vero cells were used as a control group. Two concentrations of 250 and 300 µg/ml were used for the cell line and growth inhibition in the MCF-7 cell line showed the effectiveness of the product. The percentage of cell viability in the culture medium of the MCF-7 cell 72 hours after exposure to different concentrations of new Schiff is shown in table 1. Evidence of DNA fragmentation was observed after staining using ethidium bromide in agarose gel. The present study indicated inhibitor activity of viability of MCF-7cells after being treated with new Schiff. This emphasizes that growth inhibition occurs in MCF-7 cells. Apoptosis was also determined using AO/PI staining and DNA fragmentation test. The expansion halted when the cell line had a fixation equivalent to 250µg/ml after 72 h of incubation (Figure 2).

3.2. DNA Laddering Assay

Electrophoresis and 1.5% agarose gel were used to evaluate the DNA of MCF-7 cells and Vero cells exposed to the new Schiff and investigate DNA fragmentation. Apoptosis by the occurrence of ladders for treated MCF-7 cells is demonstrated in figure 3 which showed no fragmentation in DNA fragmentation of the novel compound base with MCF-7 and Vero cell line.

Table 1. Percentage of breast cancer cell lines that remain viable after being treated with the novel compound

Compounds concentrations	viable cells % 1	viable cells % 2	viable cells % 3	viable cells % (mean)
250(µM)	56.6	58.3	61.3	58.7
300(µM)	63.6	69.5	75.5	69.5
Control	100	100	100	100

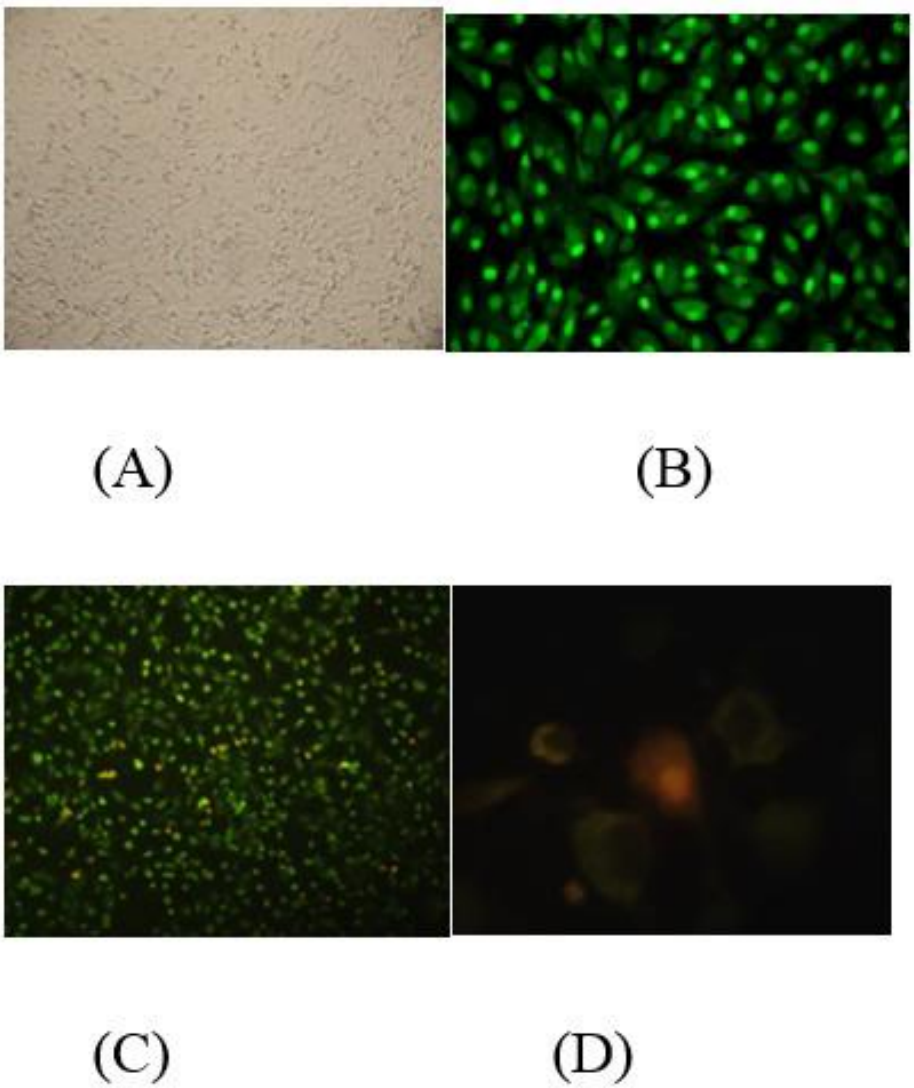


Figure 2. A) Under the light microscope, Vero cell line as control (at 10x); **B)** cancer cell (MCF-7) not treated with a novel compound, stained with the AO/PI (at 10x); **C)** (MCF-7) treated with the novel compound, stained with the AO/PI (at 10x); **D)** (MCF-7)treated with the novel compound, stained with the AO/PI (at 100×).

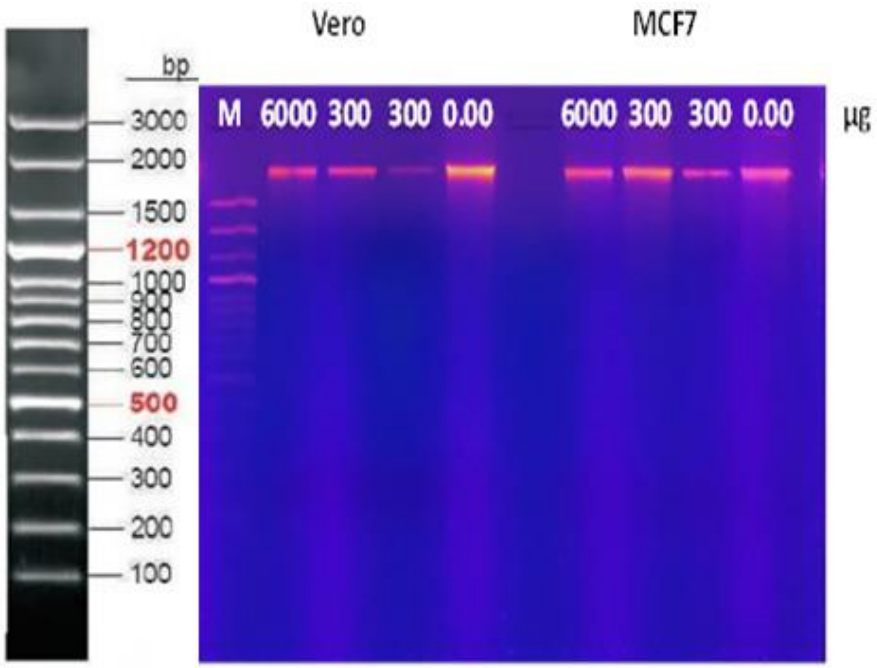


Figure 3. Genomic DNA isolated from MCF-7 cellline and Vero cell after being treated with the novel compound

4. Discussion

Breast cancer is one of the most malignant tumors in the female population worldwide due to its histological features and problematic treatment. Nowadays, extensive medical research focuses on new anticancer medications to reduce cancer problems. The use of new medications and compounds is always considered by researchers due to the recurrence of the disease, high treatment costs, drug resistance, and side effects of chemotherapy (22, 23).

Recently, several pharmacophores containing1, 3, 4-thiadiazole rings have been reported with potential anticancer activity. 4-Thiadiazole which has an amino group has inhibition activities against many tumors. MTT assays have investigated new thiadiazoles with thiazolidin-4-one moieties and in vitro antiproliferative activity in human breast adenocarcinoma cells (MCF-7) (24). The results of this study investigated the activity of new compound4,4'-[1,4-phenylenebis(1,3,4-thiadiazole-5,2-diyl)] bis(azaneylylidene) bis (methaneylylidene) diphenol which was previously prepared from the reaction of the 5,5'[(1,4-Phenelene) bis (1,3,4-thiadiazol-2-amine)] and the para-hydroxybenzaldehyde was used against the breast cancer MCF-7 cells due to the presence of amino-group in thiadiazole compounds. In the present study, no DNA fracture occurred which is consistent with the results of other researchers (16, 25, 26). The deficiency of caspase-3 in apoptosis of MCF-7 cell lines was observed without proof of DNA laddering (16). Song, Shao (27) reported anticancer evaluation of new fluorinated pyrazolo [3, 4-d] pyrimidine with a 1, 3, 4-thiadiazole against HL-60 (human leukemia cancer cell) using MTT assay. Wang et al. (2019) indicated 3,6-disubstituted 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole effect of antiproliferative activities in vitro against human hepatocarcinoma (SMMC-7721), HeLa, human lung carcinoma (A549), and mouse fibroblasts (L929) cell lines using CCK-8 assay. Rahman and Mohamed (17) reported the novel 1, 3, 4-thiadiazole analogues with expected anticancer activity against A549 (human lung carcinoma) cell lines using sulforhodamine B assay.

The results revealed that 4,4'-[1,4-phenylenebis(1,3,4-thiadiazole-5,2-diyl)] bis(azaneylylidene) bis(methaneylylidene) diphenol were found to be highly active compounds with antitumor activity. The present compound can be used as a new approach and developing strategies for treating breast cancer.

Authors' Contribution

Study concept and design: B. M. S. S.
Acquisition of data: B. M. S. S. and S. A. N. A.
Analysis and interpretation of data: B. M. S. S. and B. A. A.
Drafting of the manuscript: S. A. N. A.
Critical revision of the manuscript for important intellectual content: B. M. S. S.
Statistical analysis: B. A. A.
Administrative, technical, and material support: B. M. S. S.

Conflict of Interest

The authors declare that they have no conflict of interest.

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