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

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Abstract

Due to the increasing prevalence of cancer in human societies and the necessity for new drugs for treatment, new compounds are being evaluated. The new Schiff base compound 4,4’-[1,4-phenylenebis(1,3,4-thiadiazole-5,2-diyl)] bis(azaneylylidene) bis(methaneylylidene) diphenol, which was prepared previously from the reaction of the 5,5’[(1,4-Phenelene) bis (1,3,4-thiadiazol-2-amine)] and the para-hydroxybenzaldehydewere synthesized andthis new compound was exposed to breast cancer (MCF-7) cell to examine its cytotoxic effect using different concentrations (250 and 300 mg/ml). Cell line viability, acridine orange/propidium iodide staining and DNA fracture examines in evaluating the anti-tumor efficacy of the new composition was assessed. According to the obtained data from cell line viability assay, demonstrated a cytotoxic activity against MCF-7 breast cancer cell line, DNA fragmentation of novel compound base with MCF-7 and Vero cell line showed no fragmentation. The new Schiff base compound showed well defined anti-cancer activity when treated with breast cancer cells (MCF-7). The compound blocked the cancerous cell's proliferation without apoptosis. As a consequence of the findings, it was recommended that this compound be used to treat breast cancer.

Key words: Thiadiazole Compound, Breast Cancer, DNA Laddering Assay

1. Introduction

Malignant neoplasms according to data provided by the World Health Organization are one of the leading causes of death after cardiovascular (Farooqiet al, 2018; Gomhaet al, 2018). Breast cancer is the most common cancer in women, and epidemiological studies have shown that it accounts for
approximately one-third of all female cancers. After lung cancer, breast cancer is the second leading cause of death and one of the leading causes of death in women between the ages of 40 and 55 (Abdelhamid et al., 2018; Farooq et al., 2018; Gomha et al., 2018). For decades, the most common type of anticancer pharmacotherapy has been chemotherapy (Perveen and Al-Taweel, 2018). Due to the high prevalence of the cancer in recent years, chemotherapy has made great progress and effectiveness of many drugs has been investigated. Given the limited therapeutic index medications and their side effects, prescribed for research, evaluation and optimization of drugs are very important (Chowrasia et al., 2017; Ferlay, 2007; Jemal, 2006). To evaluate the efficacy of new compounds for the treatment of tumors cell lines used in vitro condition. MCF-7 is a cell line of breast cancer which previously isolated from Caucasian woman of 69-year-old in 1970 (Lee et al., 2015). It is a widely used cell line for breast cancer that has been propagated by different groups for several years (Baguley and Leung, 2011; Shirazi, 2011).

Thiadiazole is a heterocyclic, five-membered compound containing two particle of nitrogen and one iota of sulfur. Four isoforms of this compounds are found in nature: 1,2,3-thiadiazole, 1,2,4-thiadizaoe, 1,2,5-thiadiazole, and 1,3,4-thiadiazole. Thiadiazole is the pyrimidine and oxadiazolebioisostere, Today, many pharmaceutical compounds are made from 1,3,4-thiadiazole ring compounds in their skeletons, the pharmacological effects of these compounds include a wide range of antiviral, antibacterial, antifungal, antiparasitic, anti-inflammatory and anticancer (Li et al., 2013). Thiadiazoles are prepared for crossing point the cell membrane considering their mesoionic nature. Their incredible liposolubility is a direct result of the presence of the sulfur atom (Haider et al., 2015). One of the compounds derived from thiadiazole is Imatinib, which is a tyrosine-kinase inhibitor. The compound is used to treat chronic myelogenous leukemia, gastrointestinal stromal tumors and a number of other malignancies. Also, other compounds containing thiadiazole rings such as doxorubicin are used to treat some cancers of the bladder, breast, stomach, lung, ovary, thyroid, soft tissue sarcoma, and multiple myeloma and leukemia. (Kheder et al., 2013; Saeed et al. 2019a, b; Saeed et al., 2020). The target from this work is to investigate anticancer impacts of a novel chemical compound 4,4'-[1,4-phenylenebis(1,3,4-thiadiazole-5,2-diyl)] bis (azaneylylidene) bis(methaneylylidene) diphenol, (Saeed et al., 2019a) against the MCF-7 cell line and its therapeutic effect in inhibiting the growth of breast tumors.

2. Materials and Methods

2.1. Cell line viability
The new Schiff base mentioned below (Figure 1) was previously prepared by Saeed et al., (2019). This new compound 4,4′-[1,4-phenylenebis(1,3,4-thiadiazole-5,2-diyl)] bis (azaneylylidene) bis (methaneylylidene) diphenol which was prepared previously from the reaction of the 5,5’[(1,4-Phenelene) bis (1,3,4-thiazol-2-amine)] and the para-hydroxybenzaldehyde using microwave method.

![Structure of the new Schiff base](image)

4,4′-((1E,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), involving human bosom malignancy (MCF-7).

In Dulbecco's Modified Eagle medium (DMEM) with 10 percent fetal bovine serum, at 37°C, 5 percent CO₂ and 95 percent humidity, cell lines had been cultured. At 1 * 10⁴ cells/well, cells were seeded onto 96-well culture plates and allowed to adhere. After 24 hours, cells were treated with different concentrations of the chemical compound. From stock solution (10.00 μg/ml), different concentrations of a novel compound base were used and re-suspended in DMSO. Triplicate concentrations of 250 and 300 mg/ ml were used to treat the cells. After 24, 48 and 72 hours of treatment, 20 ml of the solvent solution (DMSO) was applied to each well and incubated at 37°C. The compound-untreated cells were regarded as control cells (Al-Timimi, 2019; Al-Anssari et al., 2019). 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 for control after 48 hours'. In a micro titer plate reader, the absorbance for each well was measured at 540 nm and the percent cell viability (CV) was determined manually using the formula (Monks et al., 1991):

\[ CV = \frac{\text{Average abs of drug wells}}{\text{Average abs of control wells}} \times 100. \]
2.2. Acridine orange/propidium iodide (AO/PI) assay

Staining with acridine orange (AO)/propidium iodide (PI) color was used to evaluate cell apoptosis at different concentrations of the chemical compound (Fani et al, 2015, Sulaiman et al, 2018). The untreated cells of MCF-7 were viewed as a control. An aliquot of 1 µl of 0.5 mg/ml acridine orange/propidium iodide (AO/PI) reagent was added to each well contains different concentrations of novel compound base composition according to the previous step and incubated for 10 min at room temperature. 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 (Al-Timimi, 2019). Green and red cells represent living cells 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 added 0.5 mL of TES lysis buffer and vortex, next added 20 µL of RNase and mix well. Then incubated for 30-120 minutes at 37°C. Afterward added 20 µL of proteinase K and mix by flipping the tip of the tube and incubated at 50°C for at least 90 minutes. After mixed the samples of DNA with loading buffer, samples loaded 10-20 µL of DNA samples to each well of a standard 1% agarose gel containing 0.5 µg/mL ethidium bromide (Al-Timimi, 2019).

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 Saeed et al, (2019). Evaluation of cytotoxicity and viability of breast cancer cells exposed to novel 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 cell line and growth inhibition in MCF-7 cell line showed the effectiveness of the product. The percentage of cell viability in culture medium of the MCF-7 cell 72 hours after
exposure to different concentrations of new Schiff is shown in Table 1. After staining using ethidium bromide in agarose gel, evidence of DNA fragmentation was observed. The present study showed inhibitor activity of viability of MCF-7 cell after 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).

Table 5: Percentage of breast cancer cell line that remain viable after treated with novel compound

<table>
<thead>
<tr>
<th>Compounds concentrations</th>
<th>viable cells % 1 (mean)</th>
<th>viable cells % 2 (mean)</th>
<th>viable cells % 3 (mean)</th>
<th>viable cells % Control (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250(µM)</td>
<td>56.6</td>
<td>58.3</td>
<td>61.3</td>
<td>58.7</td>
</tr>
<tr>
<td>300(µM)</td>
<td>63.6</td>
<td>69.5</td>
<td>75.5</td>
<td>69.5</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 2. A) Under light microscope, Vero cell line as control (at 10x); B) cancerous cell (MCF-7) not treated with novel compound, pigmented with the AO/PI (at 10x); C) (MCF-7) treated with the novel compound, pigmented with the AO/PI (at 10x); D)(MCF-7)treated with the novel compound, pigmented with the AO/PI (at 100x).
3.2. DNA laddering assay

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

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

4. Discussion

One of the known malignant tumors is breast cancer in the female population of the world. Due to its histological features and despite attempts to detect breast cancer, it is almost considered a problem in treatment. Today, extensive medical research is focused on new anticancer drugs to reduce cancer problems. Due to the recurrence of the disease, high treatment costs and drug resistance and side effects in chemotherapy, the use of new drugs and new compounds is always considered by researchers (Senawong et al, 2014, Dean and Rhodes 2014)

Recently, several pharmacophores containing 1, 3, 4-thiadiazole ring have been reported with potential anticancer activity. 4-Thiadiazole that have amino group were have inhibition activities against many tumors. MTT assays have investigated new thiadiazoles with thiazolidin-4-one moieties and in vitro anti-proliferative activity in human breast adenocarcinoma cells (MCF-7) (Ibraheem et al, 2018). The
results of this study show the activity of new compound 4,4’-[1,4-phenylenebis(1,3,4-thiadiazole-5,2-diyl)] bis (azaneylylidene) bis (methaneylylidene) diphenol which was prepared previously from the reaction of the 5,5’[(1,4-Phenelene) bis (1,3,4-thiadiazol-2-amine)] and the para-hydroxybenzaldehyde using against the breast cancer MCF-7 cells, this due to the presence of amino-group in thiadiazole compounds. In the current study, no DNA fracture happens which is consistent with the results of other researchers. (Shapiro et al, 2001; Sharief and Gani, 2004; Al-Timimi, 2019). The shortfall of caspase-3 outcomes in apoptosis of MCF-7 cell lines without proof of DNA laddering (Al-Timimi, 2019). Song et al. (2011) reported anticancer evaluation of novel fluorinated pyrazolo[3,4-d] pyrimidine with a 1, 3, 4-thiadazole against HL-60 (human leukemia cancer cell) by MTT assay. Wang et al. (2019) showed that 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 by the CCK-8 assay. Rahman et al 2014, were reported the novel 1, 3, 4-thiadiazole analogues with expected anticancer activity against A549 (human lung carcinoma) cell line using sulforhodamine B assay.

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

References


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