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Cytotoxicity of 1-(3, 5- bis(trifluoromethyl)phenyl)-3-(4-selenocyanatophenyl) urea against Triple Negative Breast Cancer Cells

***Miram Altouhamy[1], Fardous F. El-Senduny[2] , Saad Shaaban[3], Omali El-Khawaga[4]**

[1-] *M.Sc. student, biochemistry division, chemistry department, faculty of science, Mansoura University*

[2-] *Lecturer of Biochemistry, Faculty of Science, Chemistry department, Mansoura University*

[3-] *Associate Professor of Organic Chemistry, Chemistry Department, Faculty of Science, Mansoura University*

[4-] *Professor of Biochemistry, Faculty of Science, Chemistry department, Mansoura University*

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Abstract: Breast cancer is a major cause of death associated with cancer in women each year. Triple-negative breast cancer (TNBC) is considered as a subtype of breast cancer representing about 15% of breast cancer. It is not yet certain whether the poor prognosis of TNBC is due to the aggressive behavior or because of the lack of the targeted therapy. These malignant tumors pose a major health problem and targeting these tumors with traditional chemotherapy cannot be the only treatment as it has serious side effects. Accordingly, the present study aimed to evaluate the *in vitro* anticancer activity of organoselenium compounds against MDA-MB-231 and 4T1 TNBC cell lines. Compared with commercial anticancer drug cisplatin, 1-(3,5-bis(trifluoromethyl)phenyl)-3-(4-selenocyanatophenyl) urea showed anticancer action. Additionally, the inhibition activities were dose and time dependent as measured by MTT assay. These results suggest that the toxicity induced by 1-(3,5-bis(trifluoromethyl)phenyl)-3-(4-selenocyanatophenyl) urea might be efficient in the management of TNBC by utilizing several therapeutic approaches lowering the side effects related to existing TNBC treatments

keywords: Triple negative breast cancer, organoselenium, cytotoxicity, MTT assay, Proliferation assay

1.Introduction

Breast cancer is a leading cause of deaths associated with cancer world-wide and the most common cancer among women [1, 2]. Triple negative breast cancers (TNBCs), which signify 10-20% of cancer breast cases, are known to be extremely aggressive [3]. TNBCs are characterized with negative histotype for human epidermal growth factor 2 (HER2), progesterone receptor (PR) and estrogen receptor (ESR1) [4-7].

Chemotherapy, radiation and surgery are used in the treatment of many cancer types [8]. Chemotherapy is known to have many adverse side effects as it attacks all rapidly dividing cells (normal or tumor cells). These treatments reduce patients' quality of life and might be discontinued [9, 10].

Organoselenium compounds gained researchers interest recently as they have several *in vitro* biological activities related to psychological, neurodegenerative, endocrine, autoimmune and cardiovascular conditions [11, 12]. Different studies have shown the efficiency of organoselenium compounds with different structures as anticancer drugs. These results suggest an important role of the selenium atom within these compounds structures [13, 14]. The ability of organoselenium compounds as redox-modulating compounds has been described in therapy-refractory lymphomas management [15, 16]. Also, it was found that selenium, as an atom or incorporated into compounds structures, has synergistic interactions on leukemia, lung, breast, and colorectal cancer cell lines with chemotherapeutic agents including paclitaxel,

imatinib, irinotecan, and cisplatin, respectively [17, 18]. Therefore, there is still need for finding a new chemotherapy with fewer side effects, more selective and effective

2. Materials and methods

1. Cell line and reagents

All the chemicals utilized in the present research were purchased from Sigma chemical CO. (St. Louis, MO, USA) with analytical grade. The organoselenium compound 1-(3,5-bis(trifluoromethyl)phenyl)-3-(4-selenocyanatophenyl) urea (4c) (Figure 1) was synthesized and characterized by different analytical methods (under publication). The compound was dissolved in DMSO.

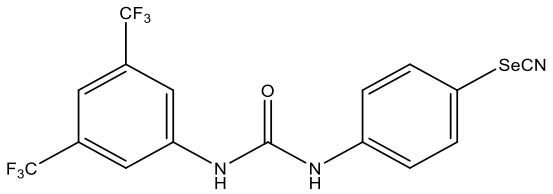

Fig 1. Chemical structure of 1-(3,5-bis(trifluoromethyl)phenyl)-3-(4-selenocyanatophenyl) urea (4c).

2. Cell culture and drug treatments

MDA-MB-231 and 4T1 were cultured in Dulbecco's Modified Eagle's medium (DMEM) culture medium containing 100 IU/mL penicillin, 10% FBS and 100 IU/mL streptomycin, and were grown at 37 °C in 5% carbon dioxide atmosphere. Dimethyl sulfoxide (DMSO) was used as a solvent for the organoselenium compounds. The stock solution was diluted to the needed concentrations. DMSO was used as control.

3. Assessment of cell inhibition rate

MDA-MB-231 and 4T1 cells, inhibition rates were assessed through MTT assay according to the method of Morgan *et al.* [19]. Cells were allowed to attach for 24 hours before treatment with the indicated concentrations of organoselenium compound at indicated times. Stock solution (10 mM) of the tested compounds was diluted to proper concentrations before treatment. The tested cells were subjected either to different concentrations of tested organoselenium, cisplatin as positive control, or DMSO alone as negative control, for indicated treatment time. Then MTT (5 mg/mL PBS) was added to the cells after 48 hours of treatment. Then, 100 µL of acidified sodium dodecyl sulfate solution was added to solubilize formazan crystals. The 96-well plate was incubated for another 14 hours at 37°C and 5% CO_2 then the absorbance was measured at wavelength 570-630 nm by Biotek plate reader (Gen5™). Half maximal inhibitory concentration (IC_{50}) was determined.

4. Cell proliferation

The inhibitory activities of organoselenium on MDA-MB-231 and 4T1 cells growth were determined using viable cells analysis (MTT assay) [19]. Cells were diluted and counted to the suitable population and density. Then cells were seeded in 96-well plates (Flat Bottom) at density of 5×10^4, 3×10^4 cells/ml, respectively and allowed to attach overnight at 37 °C. Cells were placed in full medium with serial dilutions of compound 4c (50, 25, 12.5, 6.25, 3.125 and 1.56 µM). After exposure of cells to the tested compound for 4 days, 10 µL of stock MTT solution (5 mg/mL) were added to all wells. Cells were further incubated for 14 hours at 37°C. The absorbance was determined at wavelengths 570-630 nm using Biotek plate reader (Gen 5^{th}).

5. Statistical analysis

GraphPad Prism 8 (GraphPad, San Diego, CA, USA) was used for the statistical analysis calculations. The significance of the inhibition results was calculated used one-way analysis of variance (ANOVA). Results with *P* value less than 0.05 were considered statistically significant. All results are represented as mean ± standard deviation (SD).

3. Results and Discussion

1. Cytotoxicity effect of 4c compound on 4T1 cells and MDA-MB-231

The evaluation of 4c compound cytotoxicity at different concentrations beginning from 50 to 1.56 µM, through MTT assay, revealed that the concentration that caused 50% inhibition in cell growth (IC50) was found to be 8 µM using a semi logarithmic plotting of cell viability *vs* that of the concentrations (Figure 2, Table 1). Compound 4c had a dose-dependent inhibition to the growth of MDA-MB-231 cells. Moreover, the cytotoxicity of compound 4c at different concentrations beginning from 50 to 1.56 µM was studied on 4T1 cell line through

MTT assay and revealed that the concentration that caused 50% inhibition in cell growth (IC50) was found to be 4.5 µM (Figure 2).

Table 1. Effect of the 4c compound on cell viability percentage on MDA-MB-231and 4T1 cells

Concentration (µM)	Cell viability (%)	
	MDA-MB-231	4T1
50	3.0	0.0
25	32.6	3.90
12.5	36.0	14.6
6.25	38.0	50.7
3.125	95.8	80.8
1.56	100.0	100.0

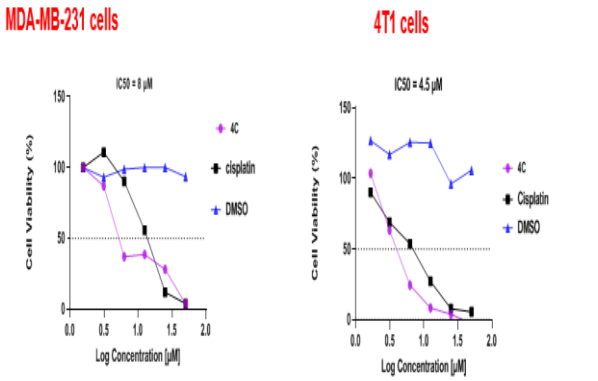

Fig 2. The effect of treatment with 4c compound on the viability of MDA-MB-231 and 4T1 cells by the MTT assay.

2. Morphological changes and cell proliferation inhibition of MDA-MB-231 by compound 4c.

The treatment of cells with 4c (Figure 1) at different concentrations, exhibited a major reduction in proliferative activity of MDA-MB-231 and 4T1 cells. The viability of TNBC cells treated with 4c were measured using MTT assay. It was noticed that, compared with cisplatin and DMSO, 4c usage at 9.8 µM for indicated time showed significant inhibitory activity ($P < 0.05$) against MDA-MB-231 cells. In comparison with DMSO, as well as the same results was obtained in the case of 4T1 cells at IC_{50} concentration **(**4.7 µM) (Figure 3). These results indicated that 4c has significant cytotoxic effect on proliferation of breast cancer cells.

In addition, the morphological changes were observed in 4T1 and MDA-MB-231 cells treated 4c, as showed in (Figure 4) and (Figure 5), compared with cisplatin and DMSO, after treatment for 48h with 1.56 to 12.5 µM of 4c did not trigger noteworthy changes in morphology and cell number. However, the treatment at 25 - 50 µM resulted in remarkable morphological changes and major inhibition. Lengthy treatments triggered a decline in cell count compared to negative control cells treated with DMSO.

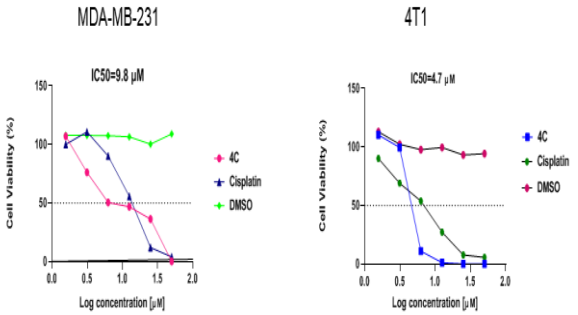

Fig 3. Effect of treatment with 4c compound on the proliferation activity on MDA-MB-231 and 4T1 cells.

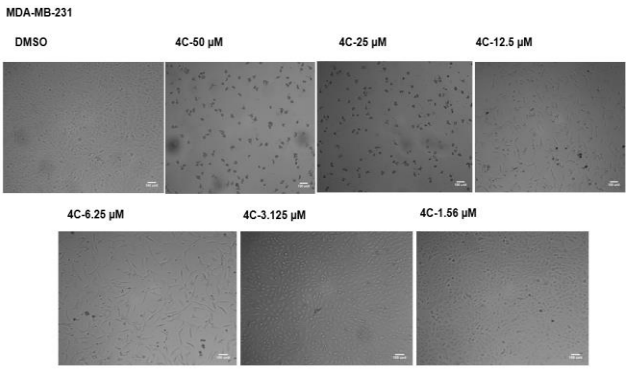

Fig 4. Effect of treatment with 4c compound on the morphology of MDA-MB-232 cells (an inverted microscope).

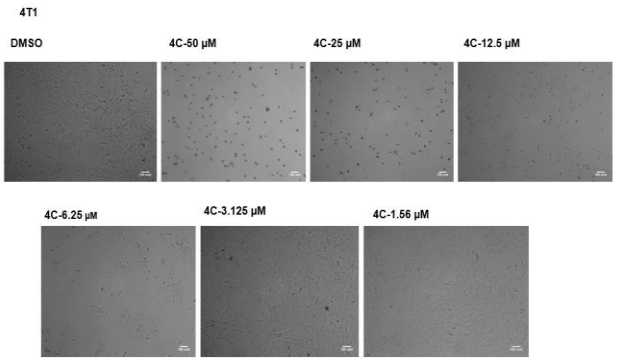

Fig 5. Effect of treatment with 4c compound on the morphology of 4T1 cells (an inverted microscope).

Discussion

Many cancer treatments are known to have many severe side effects, despite the availability of different therapeutic methods for cancer. Therefore, the most important consideration in developing or discovering new therapeutic agent for cancer is that the drug should have lower side effects, compared to known chemotherapeutic agents, with low toxicity to normal cells. TNBC is one of the cancers with high incidence of metastasis with low survival and prognosis comparing to other BC subtypes. Therefore, blocking metastasis might enhance patients' survival. There are no endocrine or targeted therapies for TNBC. The current agents (cytostatic and cytotoxic) targeting TNBC are designed to reduce tumor size and destroy malignant cells, however, they are not designed to block metastatic essential pathways.

Selenium is a vital trace element in the body with many biological functions including protecting against cancers, improving the immunity, and maintaining several physiological functions [11]. Therefore, investigation of the anti-cancer activity of new organoselenium compounds against TNBC cell lines was the aim of the study.

The treatment of TNBC cells by compound 4c showed differential activities against both tested cell lines (MDA-MB-231 and 4T1). These dissimilarities might be due to differences in cell type. Accordingly, 4T1 cells are aggressive type than MDA-MB231 cells. Many investigators showed that 4T1 cells have multiple mutated genes such as p53 and also resistant to 6-thioguanine that responsible for metastasis and aggressiveness behavior of the cells [20]. In addition, Kabir *et al.* indicated that substrate motif of ERK1/ERK2 kinase was found in MDA-MB231 cells, however, not in MCF-7 cells [21]. The present study might offer important evidence for the selection of therapeutic agent candidates for breast cancer management. According to our findings, the growth of MDA-MB-231 and 4T1 cells *in vitro* were significantly hindered by compound 4c. Further comprehensive studies on the antitumor mechanisms of organoselenium compound are required.

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