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A novel organoselenium compound as antiproliferative agent against ovarian cancer cell lines

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Abstract: Ovarian cancer (OC) is considered a heterogeneous disease usually diagnosed at late stages as there are no symptoms and signs. Hallmarks and heterogeneity of ovarian cancer are limited and hard to establish according to the experiments *in vitro* models. Surgery, chemotherapy and other traditional treatments are accompanied by many toxic side effects. This study aimed to evaluate the anticancer activity of organoselenium compounds against sensitive cells A2780, cisplatin-resistant cells (A2780CP) and SKOV-3 cells. ((diselanediylbis (4,1-phenylene)) bis (azanediyl)) bis(3-methyl-1-oxobutane-1,2-diyl) diacetate, Compound 16, showed anticancer activity through changing the cell morphology and inhibit cell proliferation and this was confirmed by using MTT (3-(4,5-dimethylthiazoyl)-2,5-diphenyl-tetrazolium bromide).

keywords: Ovarian cancer cells, organoselenium, MTT assay, Proliferation assay.

1.Introduction

Ovarian cancer is considered one of the most common gynecologic malignancies in women worldwide [1]. Annually, 230,000 new cases and almost 140,000 death due to low survival rates [2, 3]. In 2019, there will be approximately 140,690 cancer cases diagnosed and 103,250 cancer deaths among the oldest old in the United States [4]. The most common cause of mortality and morbidity is ovarian cancer with nearly 1.4% incidence lifetime and 1.04% mortality lifetime annually [5].

The etiology of ovarian cancer is not fully clear so there are many factors implicated in this disease such as steroid hormones, ovulation, tumor suppressor genes, growth factors, germ cell depletion, tumor suppressor genes, cytokines, and environmental agents [6]. Identification of the risk factors of ovarian cancer is a basic goal for preventing death from this disease [7]. There are numerous risk factors of ovarian cancer such as Family history which is the main factor, related to the mutations in BRCA1 and 2. Besides the genetic factors, there are hormonal, Gynecologic, demographic and lifestyle factors [8, 9].

Cytoreductive surgery is the first line of ovarian cancer treatment in its early stages [10]. Combination chemotherapy between paclitaxel and platinum-based compounds is still the current regimen of choice for ovarian cancer treatment in late stages [11]. There are alternative ovarian cancer treatments such as hormonal therapy [12] and immunotherapy [13]. On the other side, patients treated with chemotherapy suffer from many side effects such as headache, fatigue, weakness, hair loss, nausea, vomiting, memory impairment and numbness [14]. Therefore, discovering a new chemotherapeutic drug is the main goal in the treatment of cancer.

Organoselenium compounds play an important role in the treatment of cancer through their many biological activities such as induction of apoptosis, reduction of oxidative stress, inhibition of DNA adduct formation and cell cycle arrest [15].

2.Material and methods

Cell line and drug

SKOV-3 cells are mutant p53 comparing to sensitive cells A2780 which express a wild type of p53 [16] sensitive and cisplatin-resistant cells were obtained from Jan Brábek, BIOCEV, Czech Republic. Nawah Scientific, Egypt , is the source of SKOV-3 cells. The cells were maintained in RPMI-1640 medium (BioWhittaker® Lonza, Cat.No.12-702F) supplemented by 10% fetal bovine serum albumin (Cat No: S-001B-BR, Life Science Group L UK) and (100 IU/mL) penicillin/ streptomycin (100 µg/mL) (Cat. No 17-602E Lonza,) in 37°C and 5% CO_2. Every two or three passages 1 µM of cisplatin (cis-diammineplatinum (II) dichloride) was added to A2780CP. The organoselenium compounds were synthesized and characterized by different analytical methods [17] and the chemical structure of compound **16** (Organoselenium pseudopeptides) shown in (**Figure 1**) . The compounds were dissolved in DMSO (Cat. No. 20385.02, Germany) and stored at –20°C.

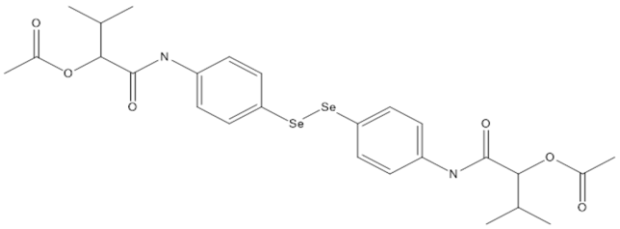

Figure 1: Chemical structure of ((diselanediylbis (4,1-phenylene)) bis (azanediyl)) bis(3-methyl-1-oxobutane-1,2-diyl) diacetate, compound **16**

The initial screening and cell viability assay

To test the most active organoselenium compounds, ovarian cancer cells were screened against these compounds according to Skehan et al., procedures [18, 19]with minor modifications. Three ovarian cancer cell lines were seeded in a 96-well plate (2.5×10^4 cell/ml) (100 µl/well). After of incubation for 24h at 5% CO_2 and 37°C, cells were treated with 1 µM of each tested compound. Cisplatin and DMSO were used as a positive and negative control. MTT (3-(4,5-dimethylthiazoyl)-2,5-diphenyl-tetrazolium bromide (MTT) (5 mg/mL Phosphate Buffered Saline (PBS)) was added after 48h at 37°C and 5% CO_2 to solubilize formazan crystal. After 14h of incubation, Biotek plate reader (Gen5™).was used to measure the absorbance at $\lambda_{570-630}$ nm

Evaluation the IC50 value of the most cytotoxic compound depends on the results of initial screening. Ovarian cancer cell lines were treated with a serial dilution of compound **16** which was the most active compound against ovarian cancer cells. The cell viability was determined by using MTT after 48h of incubation. GraphPad prism 8 was used to calculate the IC50 value of compound **16**.

Proliferation assay

Ovarian cancer cell lines were seeded in a 96-well plate (100 µl/well). After 2h of seeding, the cells were treated with compound **16** which was the most active organoslenium compound. Cisplatin and DMSO were used as a positive and negative control. MTT was added after 4 days of incubation at 5% CO_2 and 37°C. After 14h of incubation with SDS to solubilize formazan crystal, Biotek plate reader (Gen5™).was used to measure the absorbance at $\lambda_{570-630}$ nm.

Selectivity index (SI)

With minor modifications to Bézivin et al., procedures [20]to evaluate the selectivity of compound **16** toward ovarian cancer cell lines [20-22], human skin fibroblast cells (HSF) were seeded at 5×10^4 (cell/mL) in a 96-well plate. After incubation for 24h at 37°C and 5% CO_2, serial dilution of compound **16 (50, 25, 12.5, 6.25, 3.125, and 1.56** µM**)** was added. The viability of HSF cells was evaluated by using MTT after 48h of incubation. The (SI) was calculated according to (Equation 1).

Equation 1: calculation of selectivity index where IC50 normal = the concentration of the tested compound that kills 50% of normal cells, IC50 cancer = the concentration of the tested compound that kills 50% of cancer cells

$$SI = \frac{IC50\ normal\ cells}{IC50\ cancer\ cell}$$

3.Results

1.1. Cytotoxicity and proliferative activity toward ovarian cancer cell lines

The anticancer activity of compound **16** against sensitive (A2780), cisplatin-resistant cells (A2780CP) and SKOV-3 cells was evaluated by using MTT (3-(4,5-dimethylthiazoyl)-2,5-diphenyl-tetrazolium

bromide). In this study, results showed that compound **16** was more cytotoxic against A2780CP rather than A2780 and SKOV-3 (**Table 1**) and **(Figure 2**). In addition to IC50 value calculations, human skin fibroblast cells were tested with compound **16** to investigate the selectivity index toward three ovarian cancer cell lines and (SI) was calculated according to (**Equation 1**). The morphology of the treated ovarian cancer cell lines showed that compound **16** was more cytotoxic to cancer cells rather than normal cells as increasing concentration of compound 60 led to the spherical shape of cells and floated **(Figure 3)**. Cell viability was determined by semi-logarithmic plotting between concentrations and cell viability (**Table 2**).

Table 1 selectivity index and IC50 value of the most active compound toward ovarian cancer cell lines, ± represents the standard deviation from three independent experiments

Cell line	IC50(µM)	SI
Cisplatin-resistant cells (A2780CP)	1.7±0.1	66.3
Sensitive cells (A2780)	2.6±0.4	43.3
SKOV-3	15±0.8	7.5
HFS	>50 µM	

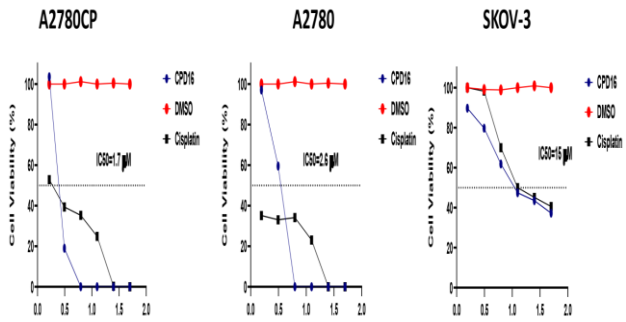

Figure 2: Ovarian cancer cells were treated with the IC50 value of compound **16**, incubated for 48h at 5% CO_2 and 37°C. After that, the cell viability was determined by using MTT and GraphPad prism 8 was used to calculate IC50 value.

Table 2: Effect of compound **16** on cell viability percentage on ovarian cancer cell lines

Concentration/Cell viability (%)	A2780CP	A2780	SKOV3
50 µM	0%	0%	21%
25 µM	0%	0%	23%
12.5 µM	0%	0%	24%
6.25 µM	0%	22%	30%
3.125 µM	0%	88%	45%
1.56 µM	24%	99.7%	81%

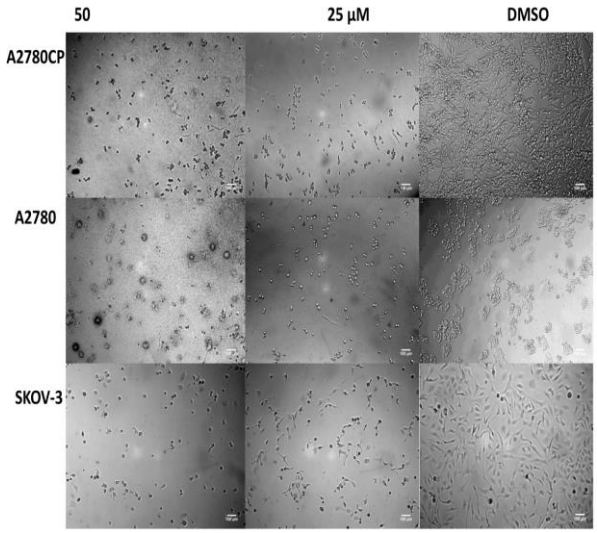

Figure 3: compound **16**changed the morphology of the cells comparing to negative control (DMSO), by increasing the concentration of the drug the cells were dead

Compound 16 inhibited cell proliferation and morphological changes in treated ovarian cancer cells.

To study the ability of cancer cells to proliferate, a proliferation assay test was made by using MTT. The results showed the inhibitory effect of compound **16** on the proliferation of ovarian cancer cells. Compound **16** significantly reduced the proliferation of sensitive cells A2780 rather than A2780CP and SKOV-3 as shown in (**Figure 4**). The IC50 value of the proliferation was calculated by GraphPad prism 8 (**Figure 5**).

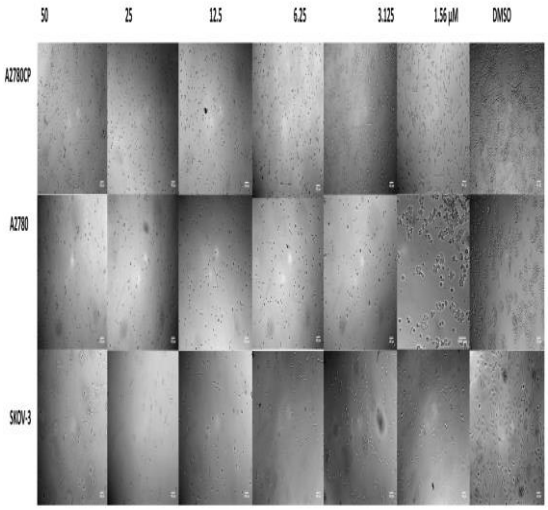

Figure 4: After 2h of seeding, ovarian cancer cells were treated with the IC50 value of compound **16**. After 4 days of incubation at 5% CO_2 and 37°C, the viability was determined by using MTT

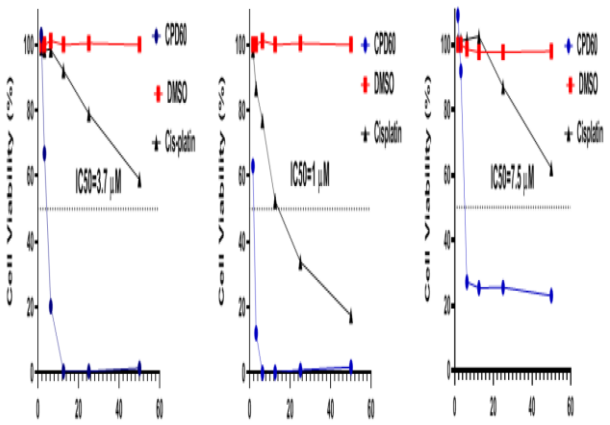

Figure 5: GraphPad prism 8 was used to calculate the IC50 value of the proliferation.

Discussion

The 5th leading cause of death is ovarian cancer with high mortality rate due to the lack of diagnosis, early symptoms, limited treatment and drug resistance [23]. Selenium is an essential trace element discovered by Berzelius which is characterized by many biological activities such as improving immunity, maintaining various biological functions and protecting against cancer [24, 25]. Therefore, the aim of this study was the evaluation the cytotoxicity of these organoselenium compounds on ovarian cancer cell lines

MTT assay is one of the most valuable cytotoxic assays to evaluate the intracellular effects on metabolic activity, viability and cell proliferation [26]. MTT is able to pass through the cell membrane and converted by cell plasma enzymes to the water-insoluble formazan crystals by living cells and this determines the mitochondrial activity [27, 28]. According to Suzuki et al., Selenium (Se) compounds are well known to induce the cell death and inhibit proliferation of the cells in human cancer cells such as MCF-7 and (HSC-3), human oral squamous cell carcinoma, [29].

Treatment of ovarian cancer cells with compound 60 showed differential activities against three tested cell lines. These differences may be due to cell-type differences. SKOV-3 cells are the most aggressive type rather than sensitive and cis-platin resistant cells. Studies showed that SKOV-3 cells have multiple mutated genes such as p53 and p21 activated kinase 4 which responsible for invasion, cell migration and proliferation [30].

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