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ORIGINAL ARTICLE

Antioxidative and antiproliferative evaluation of 2-(phenylselenomethyl)tetrahydrofuran and 2-(phenylselenomethyl)tetrahydropyran

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Summary

Purpose: *To determine the antioxidant and antiproliferative influence of 2-(phenylselenomethyl)tetrahydrofuran (1a) and 2-(phenylselenomethyl)tetrahydropyran (2a) on colon cancer cell line HCT-116 and breast cancer cell line MDA-MB-231.*

Methods: *Cell viability was monitored in a dose-dependent manner using MTT assay. The concentration of superoxide anion radical ($O_2^{\bullet-}$) was determined spectrophotometrically. Spectrophotometric determination of nitrites (NO_2^-) was performed by using the Griess method. Determination of total glutathione (GSH) was also performed spectrophotometrically.*

Results: *HCT-116 cell line was more sensitive to the effects of the investigated substances than MDA-MB-231 cell line. Also, it was noticed that 1a produced greater effect compared to 2a. Moreover, both investigated compounds decreased to a certain degree the oxidative stress by decreasing the $O_2^{\bullet-}$ and thus the peroxynitrite concentration. At the same time, 1a and 2a acted more efficiently in promoting the endogenous antioxidative capacities (increased GSH concentration) providing better self-defence capabilities for cells.*

Conclusion: *Our findings showed that the investigated selenium compounds play an important role in reducing the levels of reactive oxygen species (ROS); therefore, we believe that, as antioxidants, they could prevent the processes arising as a consequence of oxidative stress, including cancer.*

Key words: *antioxidant, glutathione, nitrogen monoxide, proliferative effect, selenium compounds, superoxide anion radical*

Introduction

Selenium is an essential dietary component for humans. Although the anticancer role of selenium remains unclear, some mechanisms, such as antioxidant protection by selenoenzymes, tumor cell growth inhibition by selenium metabolites, cell cycle modifications, effects on DNA repair and apoptosis have all been described. Excessive consumption of dietary or pharmacological selenium supplements, mainly in the form of sodium selenite, has a potential to expose the body tissues to toxic levels of selenium with consequent negative effects on DNA integrity [1]. Oxidative stress causes production of ROS, which may cause carcinogenesis via genetic and epigenetic mechanisms. Elevated levels of ROS have been noted in many tumors, strongly implicating oxidative DNA damage in the etiology of cancer. It is well known that selenium exerts antioxidative characteristics whether it is incorporated in a complex compound or not [2]. Selenium has several anticancer properties related with protection against oxidative stress and is essential for the activity of some antioxidant enzymes with ability to scavenge free radicals [3]. Some studies have shown that selenium increases DNA repair capacity in human cells damaged by hydrogen peroxide and UV light. Selenium supplementation reduced the oxidative DNA damage in adnexectomized patients with BRCA1 mutations [4]. It is known that in rodents selenium supplementation is effective

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in reducing cancer incidence when nontoxic doses are provided [5]. Selenium is effective in various animal models against a diverse group of cancer-causing agents, including irradiation. It was demonstrated that selenium intake in humans has positive effects on the development of prostate, colon, lung, and breast cancer. Some trials have suggested that selenium supplementation at doses of 200 μg/day is effective in reducing the incidence of the above mentioned cancers [6]. The mammalian genome encodes 25 selenoproteins, each containing selenium in the form of the amino acid selenocysteine [7]. Some of these selenoproteins have antioxidant activity, although not all have been studied enough. Involvement of selenium in these proteins is one of the possible ways by which selenium reduces carcinogenesis [8]. Cancers of the colon and rectum show high levels of cyclooxygenase-2 (COX-2) expression. Decreasing levels of COX-2 and prostaglandin E2 (PGE2) induced by selenium supplementation cause inhibition of proliferation in colon cancer cell lines [9].

On the other hand, some selenium compounds, such as phenylselenoethers, have not been investigated enough in terms of antioxidant activity. During the last years, cyclic ether units are important synthetic targets in organic and medical chemistry due to their widespread occurrence in many complex natural compounds, exhibiting important biological activities [10]. These units can be found isolated in monocyclic or polycyclic compounds, fused with other cyclic ethers or forming spiro systems [11]. The presence of molecules with oxygenated heterocycles in nature is receiving considerable attention considering their capacity for modification of the transport of the metallic cations Na^+, K^+, and Ca^{2+} through the lipid membranes [12]. Also, this activity is responsible for their antibiotic [12], neurotoxic [13], antiviral [14] and cytotoxic properties [15] and as growth regulators [16] or inhibitors of the level of cholesterol in blood [17].

The aim of this study was to examine the antioxidant and (anti)proliferative influence of various concentrations of two newly synthesized selenium compounds, 2-(phenylselenomethyl) tetrahydrofuran (1a) and 2-(phenylselenomethyl) tetrahydropyran (2a) on the colon cancer cell line HCT-116 and breast cancer cell line MDA-MB-23,1 as well as to discuss the possible role of these selenium compounds in cancer prevention and treatment. Selenium supplementation could have an impact on reducing chemotherapy side effects as a consequence of the oxidative stress.

Methods

Chemicals

Dublecco's Modified Eagle Medium (DMEM) and PBS were obtained from GIBCO, Invitrogen, USA. 5,5'-dithio-bis(2-nitrobenzoic acid) and glutathione reductase were purchased from Sigma Chemicals Co., St Louis, MO, USA. Fetal bovine serum (FBS) and trypsin-EDTA were from PAA (The Cell Culture Company, Pasching, Austria). Dimethyl sulfoxide (DMSO), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), NADPH and nitro blue tetrazolium (NBT) were obtained from SERVA, Heidelberg, Germany. N-1-napthylethylenediamine dihydrochloride was purchased from Fluka chemie GMBH, Buchs, Switzerland. Sodium nitrite was obtained from Centrohem Stara Pazova, Serbia, Phosphoric acid from Alkaloid Skopje, FYROMacedonia, Sulfanilamide and sulfanilic acid from MP Hemija Belgrade, Serbia. PhSeCl and used alkenols (pent-4-en-1-ol and hex-5-en-1-ol) were purchased from Fluka, while PhSeBr was obtained from Acros organics, Geel, Belgium. All solvents and chemicals were of analytical grade.

Synthesis of 1a and 2a

Gas-liquid chromatography (GLC) analysis was performed with a Deni instrument, model 2000 (Haan, Germany) with capillary apolar columns. ^1H and ^{13}C NMR spectra were run in $CDCl_3$ on a Varian Gemini 200 MHz NMR spectrometer. IR spectra were obtained with Perkin-Elmer Model 137B and Nicolet 7000 FT spectrophotometers (Waltham, Massachusetts, USA). Microanalyses were performed in "Dornis and Colbe" laboratory (Höhenweg, Germany). Thin-layer chromatography (TLC) was carried out on 0.25 mm E. Merck precoated silica gel plates (60F-254) using UV light for visualization. For column chromatography, E. Merck silica gel (60, particle size 0.063-0.200 mm) was used.

Phenylselenoethers 1a and 2a were obtained from corresponding alcohols of pent-4-en-1-ol and hex-5-en-1-ol and from PhSeCl or PhSeBr in the presence of pyridine (Figure 1).

To a magnetically stirred solution of alkenol

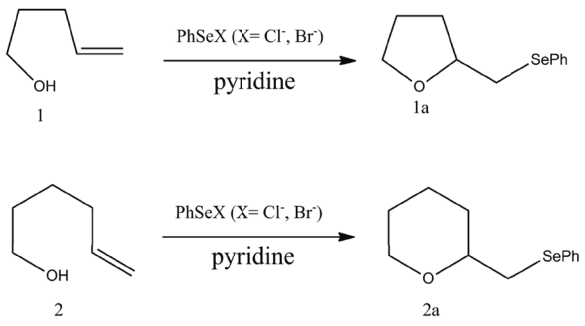

Figure 1. Phenylselenoetherification of pent-4-en-1-ol and hex-5-en-1-ol.

(0.086 g of pent-4-en-1 and 0.100 g of hex-5-en-1-ol, 1 mmol) and pyridine (0.079 g, 1 mmol) in dry dichloromethane (5 cm^3), solid PhSeCl (0.212 g, 1.1 mmol) or PhSeBr (0.260 g, 1.1 mmol) was added at room temperature. The reaction went to completion in a few minutes. The pale-yellow solution was washed with 1 M HCl (only in case of basic additives), and was then saturated with $NaHCO_3$ aqueous solution and water. The organic layer was dried over Na_2SO_4, concentrated and chromatographed. The product was obtained after the eluation of the traces of diphenyl diselenide from a silica gel-dichloromethane column in a 100% yields. The product was characterized and identified on the basis of its spectral data [18].

Preparation of drug solutions

Stock solutions of the 1a and 2a were made in dimethyl sulfoxide (DMSO) at the concentration of 100 mM, and diluted by a nutrient medium to various working concentrations. The concentration of DMSO in the most concentrated working solutions was 0.5% (v/v).

Cell preparation and culturing

The colon cancer adenocarcinoma cell line HCT-116 and breast cancer cell line MDA-MB-231 were obtained from the American Tissue Culture Collection (Manassas, VA, USA). These cells were propagated and maintained in DMEM and supplemented with 10% fetal bovine serum (PAA), and 100 IU/ml penicillin and 100 µg/ml streptomycin. The cells were grown in 75 cm^2 culture bottles and supplied with 15 ml DMEM until a confluence of 70-80%. After a few passages the cells were seeded in 96-well plate and cultured in a humidified atmosphere with 5% CO_2 at 37 °C.

Cell viability determination by MTT

The first screening test we performed was cell viability assay. HCT-116 and MDA-MB-231 cells were seeded in a 96-well plate (10^4 cells per well). After 24 h of incubation, the medium was replaced with 100 µl of each concentration (0.1, 1, 10, 50, 100 and 500 µM) of 1a and 2a for 24 and 72 h. Untreated cells served as a control. After 24 and 72 h of treatment, the cell viability was determined by MTT assay [19]. The proliferation test is based on the color reaction of mitochondrial dehydrogenase from living cells with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). At the end of the treatment period, MTT (final concentration 5 mg/ml PBS) was added to each well, which was then incubated at 37 °C in 5% CO_2 for 3 h. The colored crystals of the produced formazan were dissolved in 150 µl DMSO and the absorbance was measured at 570 nm on Microplate Reader (ELISA 2100C, Hamburg, Germany). Cell proliferation was calculated as the ratio of absorbance of the treated group divided by the absorbance of the control group, multiplied by 100 to give a proliferation percentage. The absorbance of the control group of cells served as viability of 100%.

Determination of superoxide anion radical (NBT assay)

The concentration of superoxide anion radical ($O_2^{\bullet-}$) in the sample was determined by spectrophotometric method [20], and is based on the reduction of nitroblue tetrazolium (NBT) to nitroblue-formazan in the presence of $O_2^{\bullet-}$. After treatement and after proper incubation with the investigated compounds (the same as in MTT test), assay was performed by adding of 10 µl of 5 mg/ml NBT to each well and then the cells were incubated for 3 h at 37 °C in 5% CO_2. To quantify the formazan product, formazan was solubilized in 10 µl DMSO and the resulting color reaction was measured spectrophotometrically on microplate reader at 550 nm (ELISA 2100C). The amount of reduced NBT was determined by the change in absorbance at 550 nm, based on molar extinction coefficient for monoformazan that is 15000 M^{-1} cm^{-1} and the results were expressed as nmol/ml.

Nitric oxide (NO) measurement (Griess assay)

The spectrophotometric determination of nitrites – NO_2^- (indicator of the NO^- level) was performed by using the Griess method [21]. Experiments were performed at room temperature or at 37 °C in a warm room, as noted. Typically, a nitrite standard solution (100 mM) was serially diluted from 100–1.6 µM in triplicate in a 96-well, flat-bottomed, microtiter plate. All samples were seeded also in triplicates in 96-well microtiter plate. Equal volumes of 0.1% (1 mg/ml) N-(1-naphthyl)ethylenediamine and 1% (10 mg/ml) sulfanilic acid (solution in 5% phosphoric acid) to form the Griess reagent, were mixed together immediately prior to application to the plate. Briefly, the Griess reaction is a diazotization reaction in which the NO-derived nitrosating agent (e.g., N_2O_3), generated from the acid-catalyzed formation of nitrous acid from nitrite (or the interaction of NO with oxygen), reacts with sulfanilic acid to produce a diazonium ion that is then coupled to N-(1-napthyl)ethylenediamine to form a chromophoric azo product that absorbs strongly at 550 nm. The absorbance at 550 nm was measured by using a Micro Plate Reader (ELISA 2100C) following incubation (usually 5–10 min). The results were expressed in nmol NO_2^-/ml from a standard curve established in each test, constituted of known molar concentrations of nitrites.

Determination of total glutathione

Similarly, as in cell viability assay and in the determination of NO_2^- and $O_2^{\bullet-}$, measuring of the content of total GSH was performed on HCT-116 and MDA-MB-231 cells. Experiments were performed at room temperature. Cells were seeded in triplicates on a 96-well plate ($5x10^4$ cells per well). The treatment was performed with 100 µl of the same concentrations of 1a and 2a for 24 and 72 h as in the above described assays.

This assay is based on the oxidation of the reduced form of GSH with reagent with active thiol group, i.e. 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) when a yellow product of 5'-thio-2-nitrobenzoic acid (TNB) is formed [29]. Color reaction was measured spectrophotometrically on microplate reader at 405 nm (ELISA 2100C) following incubation for 5 min. The formed glutathione disulphide (GSSG) was immediately reduced to GSH with glutathione reductase (GR). The results were expressed in nmol/ml from a standard curve established in each test, constituted of known molar GSH concentrations.

Statistics

The data were expressed as mean ± standard error (SE). Biological activity was the result of 3 individual experiments, performed in triplicate for each dose. Statistical significance was determined using the Student's t-test or the one-way ANOVA test for multiple comparisons. A p value < 0.05 was considered as significant. The magnitude of correlation between variables was done using SPSS (Chicago, IL) statistical software package (SPSS for Windows, version 17, 2008). The IC_{50} values were calculated from the dose curves by a computer program (CalcuSyn).

Table 1. Growth inhibitory effects - IC_{50} values (μM) of 1a, and 2a on HCT-116 and MDA-MB-231 cells lines after 24 and 72 h exposure

Tested compounds	*IC_{50}*			
	HCT-116		*MDA-MB-231*	
	24 h	*72 h*	*24 h*	*72 h*
1a	>500 μM			
2a				

Results

Cell viability assay (MTT assay)

The results showed that both selenium compounds exerted enhanced proliferation effect and had no cytotoxic effect, with IC50 values of >500 μM (Table 1).

HCT-116 and MDA-MB-231 cells treated with 1a and 2a exhibited higher proliferation after 24 h as well as after 72 h from treatment. From data obtained with this assay (Figures 2 and 3) a slightly greater increase in cell viability was noticed after 24 h in comparison to 72 h. This implies that our selenium compounds exerted acute influence on cell viability. Also, a greater influence of 1a on cell proliferation compared to 2a was noticed, and HCT-116 cells were more sensitive than MDA-MB-231 cells.

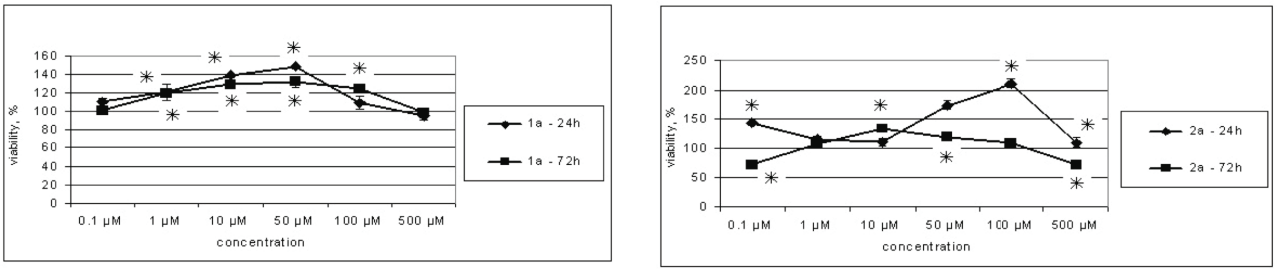

Figure 2. The dose response curve of the effect of 1a and 2a on HCT-116M growth after 24 and 72 h of exposure. The cells were treated with selenium compounds in a concentration range from 0.1-500 μM. The antiproliferative effect was measured by MTT assay after 24 and 72 h exposure. All values are mean ± standard error, n=3, *p < 0.05 as compared with control.

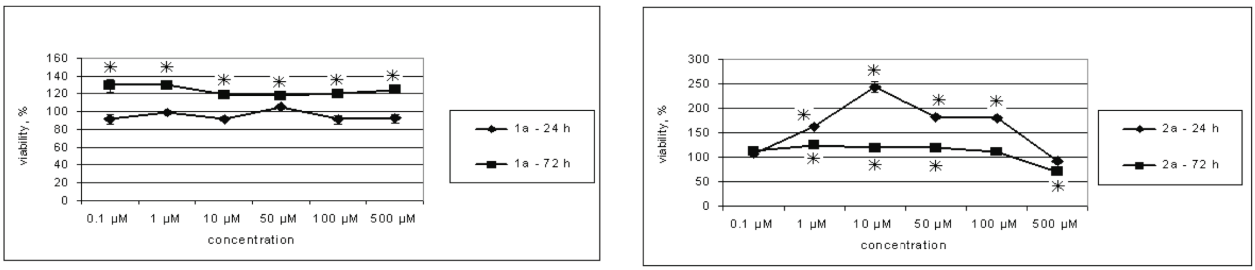

Figure 3. The dose response curve of the effect of 1a and 2a on MDA-MB-231 cell growth after 24 and 72 h of exposure. The cells were treated with selenium compounds in a concentration range from 0.1-500 μM. The antiproliferative effect was measured by MTT assay after 24 and 72 h exposure. All values are mean ± standard error, n=3, *p < 0.05 as compared with control.

Determination of superoxide anion radical (NBT assay)

Table 2 shows shows release of $O_2^{\bullet-}$ as nmol/ml after 24 h and 72 h of incubation with 1a and 2a. HCT-116 cells exhibited a slightly greater decrease of $O_2^{\bullet-}$ concentration in comparison to MDA-MB-231 cells after treatement with both selenium compounds, whereas decrease was more evident with 1a and after 72 h. These findings imply that the investigated selenocompounds 1a and 2a have antioxidative properties.

Nitric oxide (NO) measurement (Griess assay)

We compared the level of nitrites (NO_2^-) production, expressed as nmol/ml in HCT-116 and MDA-MB-231 cell line (Table 3) treated with various doses of 1a, and 2a in the same manner as in MTT and NBT assay. Both cell lines exhibited increased of NO_2^- concentration after treatement with both selenium compounds, namely 1a and 2a induced dose-dependent increase of NO_2^- concentration, whereas 1a showed slightly greater increased in NO_2^- concentration. Both selenium compounds induced greater increase of NO_2^- level after 72 h compared to 24 h. The data show that the investigated selenium compounds at the given conditions produced higher content of NO_2^- in MDA-MB-231 cells than in HCT-116 cells and that MDA-MB-231 cells were more sensitive on NO_2^- content changing than HCT-116 cells.

Determination of total glutathione (GSH)

Under our laboratory conditions we noticed a very similar behavior for 1a in both cell lines as well as for 2a in both cell lines. Namely, the content of GSH induced by 1a and 2a in HCT-116 and MDA-MB-231 cells was almost the same (Table 4). After 24 h from treatment the total GSH decreased, while it increased after 72 h. In the HCT-116 cell line GSH content was evidently higher after 72 h from treatment than after 24 h. This decrease after 24h was more evident in the HCT-116 cells and the increase of GSH after 72 h was more evident in the MDA-MB-231 cells. Quantitatively expressed, GSH concentration was higher in the HCT-116 cell line. This behavior observed with 1a and 2a implies an acute antioxidative action of GSH.

Table 2. Effect of 1a and 2a on HCT-116 and MDA-MB-231 cell lines after 24 and 72 h of exposure on superoxide anion radical ($O_2^{\bullet-}$) production expressed as nmol/ml

Compound concentration (μM)	*After 24 h*			
	HCT-116		*MDA-MB-231*	
	1a	*2a*	*1a*	*2a*
0	29.60±0.30	29.60±0.30	40.71±2.20	40.71±2.20
0.1	31.10± 0.50	32.50±1,.35*	29.96±0.20*	32.66±2.77*
1	29.40± 0.01	32.20±0.70*	34.28±0.04*	34.29±1.59*
10	29.30± 0.40	30.70±0.05	40.16±0.08	33.09±0.96*
50	31.20±1.10	30.00±0.10	33.20±0.48*	34.69±1.61*
100	30.80 ±0.30	27.40±0.10	31.88±0.20*	34.45±1.31
500	27.90± 0.50	27.70±1.00	50.36±0.60*	31.63±1.38*
	After 72 h			
Compound concentration	*HCT-116*		*MDA-MB-231*	
	1a	*2a*	*1a*	*2a*
0	34.40 ± 0.20	34.40±0.20	28.75±0.61	28.75±0.61
0.1	23.9 ± 2.10*	23.90±1.75*	27.24±0.68*	30.45±0.39
1	26.10 ± 1.30*	25.60±0.90*	26.36±0.12*	32.83±1.70
10	23.8 ± 0.50*	24.60±0.10*	25.32±0.52*	30.78±2.02
50	23.6±0.60*	23.20±0.05*	27.60±0.24	30.74±1.32
100	20.4± 0.50*	25.50±2.10*	25.84±0.08*	31.55±0.98
500	20.3 ± 0.90*	22.0±0.40*	24.84±0.28*	32.35±0.76

All values are mean ± standard error. Biological activity is the result of 3 individual experiments, performed in triplicate for each dose, n=3. *p < 0.05 as compared with control

Table 3. Effect of 1a and 2a on HCT-116 and MDA-MB-231 cell lines after 24 and 72 h of exposure on the nitrite (NO_2^-) production expressed as nmol/ml

	After 24 h			
Compound concentration (μM)	*HCT-116*		*HCT-116*	
	1a	*1a*	*1a*	*1a*
0	17.33±0.57	17.33±0.57	15.65±0.69	15.65±0.69
0.1	18.39± 2.70	12.99±0.53	16.06±0.64	24.44±2.06
1	17.59± 1.25	16.26±0.56	19.76±1.18	28.14±3.72*
10	18.77± 0.53	16.16±0.89	23.85±1.88*	27.68±2.85*
50	18.08±0.34	13.82±1.14*	20.19±2.17	29.54±3.54*
100	18.50 ±1.12	14.02±0.16*	23.60±2.51*	25.33±2.31*
500	19.36± 1.80	20.02±0.76*	27.12±2.51*	24.73±2.23
	After 72 h			
Compound concentration	*HCT-116*		*HCT-116*	
	1a	*1a*	*1a*	*1a*
0 μM	17.52±0.76	17.52±0.76	22.89±0.41	22.89±0.41
0.1 μM	15.25±0.21	15.09±1.12	21.45±2.35	17.94±0.91
1 μM	17.25±2.17	14.67±0.43	15.96±0.90	17.98±0.49
10 μM	20.35±0.36	15.28±0.67	22.50±2.85	18.53±1.84
50 μM	14.65±0.89	15.23±1.29	30.15±2.58	28.78±2.53*
100 μM	16.28±0.89	16.52±1.50	29.90±3.33	31.36±2.41*
500 μM	19.48±0.66	34.5±1.65*	36.32±2.12*	38.27±0.80*

All values are mean±standard error. Biological activity is the result of 3 individual experiments, performed in triplicate for each dose, n=3. *p < 0.05 as compared with control

Table 4. Effect of 1a and 2a on HCT-116 and MDA-MB-231 cell lines after 24 and 72 h of exposure on the total glutathione production expressed as nmol/ml

	After 24 h			
Compound concentration (μM)	*HCT-116*		*MDA-MB-231*	
	1a	*2a*	*1a*	*2a*
0	73.95±2.41	73.95±2.41	43.29±0.96	43.29±0.96
0.1	76.21±0.03	78.63±0.48*	44.14±0.45	42.93±0.06
1	72.49±1.53	76.76±0.26	38.31±0.16*	41.18±0.42
10	73.51±1.28	72.74±0.19	40.39±0.25	35.74±0.45*
50	77.42±0.67	68.00±1.18*	40.32±0.51*	34.27±1.34*
100	75.42±0.57	67.36±0.54*	40.07±1.98*	35.99±0.32*
500	59.30±0.25*	52.58±1.62*	42.74±0.32	39.27±0.61*
	After 72 h			
Compound concentration	*HCT-116*		*MDA-MB-231*	
	1a	*2a*	*1a*	*2a*
0	124.30±2.01	124.30±2.01	41.83±0.97	41.83±0.97
0.1	109.62±0.83*	121.37±0.16	39.69±0.25	41.97±1.21
1	94.01±0.38*	99.84±1.37*	37.58±0.19*	36.34±0.61*
10	99.46±0.22*	108.57±0.28*	43.60±0.03	39.62±1.79
50	108.12±0.03*	111.88±0.09*	48.70±0.23*	47.74±1.63*
100	125.45±0.10	131.05±0.54*	63.73±2.52*	61.82±0.79*
500	130.39±1.66*	125.23±0.96	73.86±0.60*	62.97±2.84*

All values are mean±standard error. Biological activity is the result of 3 individual experiments, performed in triplicate for each dose, n=3. *p < 0.05 as compared with control

Discussion

So far, a large number of inorganic and organic metal compounds has been investigated. Most of them exhibit cytotoxic activity [23,24], while some of them exert stimulating effects on cell growth [25]. Antioxidants induce protection of cells against reactive oxygen and nitrogen species and exhibit enhanced cell growth [26]. Because the basic structure of 1a and 2a is tetrahydrofuran and tetrahydropyran respectively, whose derivatives are biologically active substrates [27], we focused on the biological activity of ethers of these chemicals, i.e. of 1a and 2a. Kinetics of 1a and 2a are already published [28], but there is no report on the biological activity of these selenocompounds. According to the chemistry of the investigated compounds, in this work we expected a positive impact on cell proliferation. We also expected to record an antioxidative behavior. We found that these compounds have an antioxidative potential which could be convenient for some future *in vivo* investigations, especially because they are not cytotoxic. Many of potent antioxidant drugs are unfortunately cytotoxic [29].

One feature of cancer cells is the dysfunction of protecting mechanisms against ROS [30]. As we noticed a decrease in $O_2^{\bullet-}$ level, we subsequently observed a great increase in NO_2^- content. This is in accordance with our assumptions because NO· has a half-life of only several seconds in an environment rich in superoxide anion radical [31], while in the surroundings with low level of superoxide anion radical NO· has much greater stability [32]. Superoxide anion radical has a high affinity for NO· forming peroxynitrite anion (ONOO–). Considering the conditions when 1a and 2a affect the $O_2^{\bullet-}$ production, NO· remains more stable and therefore more approachable for detection.

To our knowledge this is the first report of estimation of the antioxidative behavior of 1a and 2a. There are plenty of selenium compounds which express a tendency to act as antioxidants. The total GHS content is a very important parameter since, by following the GSH levels, one can assume the level of the oxidative stress in treated cells.

It is known that ROS can induce increase in GSH level [33]. Our selenium-based compounds, synergistically with GSH, lowered the concentration of superoxide anion radical. Twenty four hours after treatment it was noticed acute GSH consuption which lowered $O_2^{\bullet-}$ level to a certain extent. However, since after 72 h cells extensively proliferated (shown in MTT assay results) GSH was de novo synthetised and synergistically interacted with 1a and 2a suppressing the $O_2^{\bullet-}$ generation to a greater extent. This explained the greater decrease of $O_2^{\bullet-}$ level after 72 h compared to 24 h.

According to our findings it can be assumed that in our experimental conditions HCT-116 cell line is more sensitive than MDA-MB-231 cell line. Also, it was noticed that 1a produces greater antioxidative effect in comparison to 2a. Moreover, both investigated compounds decreased to a certain extent the oxidative stress by decreasing the $O_2^{\bullet-}$ and thus the peroxynitrite concentration. At the same time, 1a and 2a acted more efficiently in promoting the endogenous antioxidative capacities (increased GSH concentration), providing better self-defence capabilities for cells.

In future investigations we will focus on the cotreatment of 1a and 2a with cytostatic drugs in order to investigate whether this combination would retain positive cytotoxic impact and at the same time would lower negative side-effects, such as impact on normal bone marrow cells of the cytostatic drugs because of increase of the radical reactive species content.

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