UFSM

CIÊNCIA e NATURA

ISSN 2179-460X
OPEN ACCESS

Ci. e Nat., Santa Maria, v. 46, e74392, 2024 • https://doi.org/10.5902/2179460X74392
Submitted: 03/03/2023 • Approved: 17/08/2024 • Published: 22/11/2024

Chemistry

Potential antitumor effect of organochalcogenyl-benzoates in glioma cells

Potencial efeito antitumoral de organocalcogenil-benzoatos em células de glioma

Lauren Lúcia Zamin[I], **Elisa da Silva**[I], **Benhur Godoi**[I]

[I] Universidade Federal da Fronteira Sul, Cerro Largo, RS, Brazil

ABSTRACT

In the domain of brain malignancies, glioma, in particular the World Health Organization (WHO) Grade IV Glioblastoma, persists as having one of the worst prognoses in modern medicine. Despite the protocol leading to the convention of surgery with chemo-radiotherapy as the mainstay of treatment, mean survival rarely exceeds 18 months. In this work, we tested the antiglioma potential of three organochalcogenyl-benzoates containing an organoselenium group in their structure, obtained by chemical synthesis: 3-(phenylselanyl)prop-2-yn-1-yl nicotinate, 3-(phenylselanyl)benzoate)prop-2-yn-1-yl, and 3-((4-fluorophenyl)selanyl)prop-2-yn-1-yl benzoate. The tests were performed on a C6 rat glioblastoma cell line, which was treated with the compounds for different times (24, 48, and 72 hours) and concentrations (10-100 μM). After the treatments, MTT assays and cell counts were performed. All compounds showed cytotoxic effects, decreasing cell viability and the number of cells. For compound 3-((4-fluorophenyl)selanyl)prop-2-in-1-yl benzoate—which showed the most pronounced cytotoxic effects— analyses of cumulative population doubling, clonogenic ability, induction potential of senescence, and lipid peroxidation were performed. The compound was only able to induce a significant increase in lipid peroxidation, with no effect over the other parameters studied. The results presented here are unprecedented and promising, introducing new compounds with antitumor potential for glioma therapy.

Keywords: Cancer; Glioblastoma; Selenium; Organic compounds

RESUMO

No domínio das malignidades cerebrais, o glioma, em particular o Glioblastoma Grau IV da Organização Mundial da Saúde (OMS), persiste como tendo um dos piores prognósticos da medicina moderna. Apesar do protocolo convencional de cirurgia com quimiorradioterapia como base do tratamento, a

sobrevida média raramente ultrapassa 18 meses. Neste trabalho, testamos o potencial antiglioma de três organocalcogenil-benzoatos, contendo um grupo organoselênio em sua estrutura, obtidos por síntese química: nicotinato de 3-(fenilselenil)prop-2-in-1-ila, benzoato de 3-(fenilselanil)prop-2-in-1-ila e benzoato de 3-((4-fluorofenil)selenil)prop-2-in-1-ila. Os testes foram realizados em uma linhagem celular de glioblastoma de rato C6, que foi tratada com os compostos por diferentes tempos (24, 48 e 72 horas) e concentrações (10-100 μM). Após os tratamentos, foram realizados ensaios de MTT e contagem de células. Todos os compostos apresentaram efeitos citotóxicos, diminuindo a viabilidade celular e o número de células. Para o benzoato de 3-((4-fluorofenil)selenil)prop-2-in-1-ila, que apresentou os efeitos citotóxicos mais pronunciados, foram avaliados a duplicação cumulativa da população, capacidade clonogênica, potencial de indução de senescência e peroxidação lipídica. O composto só foi capaz de induzir aumento significativo na peroxidação lipídica, sem efeito sobre os outros parâmetros estudados. Os resultados aqui apresentados são inéditos e promissores, introduzindo novos compostos com potencial antitumoral para terapia de glioma.

Palavras-chave: Câncer; Glioblastoma; Selênio; Compostos orgânicos

1 INTRODUCTION

Cancer, or malignant neoplasm, is an invasive disease, especially when not diagnosed at the first stage, and is among the leading causes of death in the world (WHO, 2018). Malignant neoplasms are characterized as a tumor mass of cells that have lost control over cell growth and proliferation, overcoming immune defense mechanisms and cellular apoptosis (Rang et al., 2016). Among the neoplasms of the central nervous system (CNS), gliomas stand out as the most common primary tumor, representing about 40% to 60% of all tumors that affect the CNS (INCA, 2019).

Glioblastoma (GBM) is the most common and malignant glioma, with a high rate of morbidity and mortality, with a mean survival of only 12 to 15 months after diagnosis (Wen & Kesari, 2008). GBMs are mainly derived from glial cells, however, there is evidence to suggest that they can arise from several cell types, especially those with stem cell properties (Phillips et al., 2006).

Standard treatment for GBM usually includes maximal surgical resection, followed by radiation and concurrent use of Temozolomide, an oral chemotherapy agent (Ostrom et al., 2014). Extensive and complete surgical resection of GBMs is difficult because they are often located in eloquent areas of the brain, such as speech

control, motor functions and senses. Due to the high degree of invasiveness, radical tumor resection is not curative, as tumor cells remain infiltrated within the surrounding brain, leading to disease progression or recurrence later (Davis, 2016).

Despite maximal surgical resection and multimodal therapy, the percentage of patients who survive five years after the diagnosis of GBM is usually less than 5%, with about 70% of patients having disease progression within one year of diagnosis (Stupp et al., 2015). Thus, much still needs to be done to better understand the molecular characteristics of this disease, to increase the survival of these patients or even cure them (Yamanaka & Saya, 2009).

The search for new substances that can give rise to more effective drugs is constant, both in natural derivatives and in substances obtained by synthetic processes. In the group of synthetic compounds, esters derived from benzoates and organochalcogens deserve to be highlighted for their pharmacological and biological properties, and for their extensive applicability in the industrial area, being present in the structure of various drugs, for the most diverse pathologies (Kaushik et al., 2018; Klein, 2016; Kubanek, 2005; WHO, 1997).

Considering the pharmacological potential of these compounds, the objective of this work is to test the potential antitumor effect of benzoate derivatives containing an organochalcogen group—more specifically organoselenium—in their structure in a GBM cell line. The compounds tested here are new molecules, obtained through synthetic processes, from precursor organic substances.

2 MATERIAL AND METHODS

2.1 Cell culture

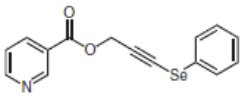
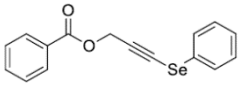
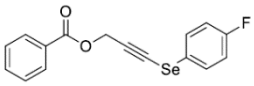
For this work, a C6 rat glioma cell line, kindly donated by Dr. Guido Lenz, professor of the Department of Biophysics at UFRGS and obtained from the American Type Culture Collection (ATCC) was used. The cells were maintained at 37°C/5% CO_2 in a

humidified atmosphere, in Dulbecco's Modified Eagle medium (DMEM) supplemented with 5% fetal bovine serum, $NaHCO_3$ 3.7 g/L, HEPES 2 g/L, Penicillin/Streptomycin 1% and Amphotericin 0.1%.

2.2 Compounds tested

This work tested the antiglioma effect of 3-organochalcogenyl-benzoates, named in the present work as compounds A, B and C. These compounds are new molecules, developed and produced in the Organic Chemistry Laboratory at UFFS, through coupling reactions catalyzed by copper iodide (CuI), between propargyl benzoates and diorganyl diselenides, under mild reaction conditions, as fully described in Gritzenco (2020). The compounds and their molecular characteristics are described in Chart 1.

Chart 1 – Organochalcogenyl Benzoates

	Compound A	**Compound B**	**Compound C**
Structural Formula			
Chemical Name	3-(phenylselanyl) prop-2-yn-1-yl nicotinate	3-(phenylselanyl) prop-2-yn-1-yl benzoate	3-((4-fluorophenyl) selanyl)prop-2-yn-1-yl benzoate
Molecular Formula	$C_{15}H_{11}NO_2Se$	$C_{16}H_{12}O_2Se$	$C_{16}H_{11}FO_2Se$
Molecular Weight	316,2133 g/mol	315,2253 g/mol	333,2157 g/mol

Source: Authors (2022)

2.3 MTT assay

Seeking to analyze whether the compounds were decreasing cell viability, the cells were plated in 96-well plates at a density of 5,000 cells/well, and treated with the compounds for 24, 48, and 72 hours, at the following doses: 10 μM, 50 μM, and 100 μM. After treatment, cell viability was measured using the MTT assay [3-(4,5-dimethyl-

2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide, Sigma], according to the protocol of Mosmann (1983). As a control, treatment with the vehicle dimethylsulfoxide (DMSO) at a maximum concentration of 0.5% was used.

2.4 Cell counting

To verify if the compounds were exerting the cytotoxic effect, the cells were treated with compounds A, B and C, at concentrations of 10 μM, 50 μM, and 100 μM for 24, 48, and 72 hours. After the time had elapsed, the cells were counted in a Neubauer chamber.

2.5 Cumulative population doubling (CPD)

To simulate the treatment cycle used in the clinic, which corresponds to 5 days of treatment and 23 days without, cells were plated in 12-well plates at a density of 10,000 cells/well and exposed to 50 μM and 100 μM treatments (compounds B and C) for 5 days. After this period, the cells were trypsinized, counted, and 1% of the volume was replated and left for another 23 days without treatment. Whenever cells reached 80% confluence, they were counted and replated. At the end of the 28 days, the population doubling (PD) rate was estimated with the following equation: PD= logN(t) log (N(to)) / log 2, where N(t) is the number of cells per well at the time of passage, and N(to) is the number of cells plated at the initial passage (Silva et al., 2016; Zamin et al., 2009). The sum of PDs, described as a cumulative PD (CPD) was then plotted against time of culture.

2.6 Clonogenic assay

To verify the ability of the treatments to decrease the formation of colonies, the cells were plated at a density of 10,000 cells/well in a 12-well plate and treated with Compound C at doses of 50 μM and 100 μM, for 5 days. Afterwards, the cells were trypsinized, counted, and plated at a density of 100 cells per well in a six-well plate. These cells were kept in culture medium without treatment for another 7 days; after

this time, they were stained with 0.5% crystal violet, and manually quantified to define the number of colonies formed (Franken et a*l*., 2006).

2.7 Senescence assay

To verify whether the treatments induce cell senescence, the cells were plated in 12-well plates, at a density of 10,000 cells/well and treated with Compound C at doses of 50 μM and 100 μM, for 5 days. After this period, the cells were maintained in DMEM medium without treatment for another 7 days. The cells were then washed in phosphate buffered saline (PBS), fixed in 4% formaldehyde for 15 minutes at room temperature, washed and incubated with a marker for β-galactosidase activity staining solution containing 1 mg/mL X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside, Sigma), 40 mM citric acid/sodium phosphate, pH 6.0, 5 mM potassium ferrocyanide, 5 mM potassium ferrocyanide, 150 mM NaCl, and 2 mM MgCl for 4-12 h at 37°C (Dimri et al., 1995; Zamin et al., 2009). The cells were photographed under a microscope and the results are presented as the ratio of β-galactosidase positive cells/total cells.

2.8 Thiobarbituric acid reactive substances (TBARS) assay

Malondialdehyde (MDA) is a breakdown product of the oxidative degradation of cell membrane lipids, and it is generally considered an indicator of lipid peroxidation. In this study, we evaluated lipid peroxidation induced by Compound C (100 μM) after 24 and 48 h incubation periods. Briefly, 2.5 x 10^5 cells per well were seeded in a six-well plate and treated as indicated. After the incubation time, cells were detached and counted in a hemocytometer, centrifuged, incubated with 500 μL of trichloroacetic acid (TCA) 20%, and sonicated for 5 × 30 s intervals at 40 V over ice. Then, 250 μL of TBA reagent was added to each sample and standard to be tested, and incubated at 95 °C for 45–60 min. Each sample and standard (100 μL) were loaded (in duplicate) into a clear 96-well plate and the absorbance at 532 nm was recorded using a microplate

reader. The content of MDA was calculated for each sample from a standard curve. The results were expressed as nmol MDA content per 1 x 10^6 cells (Martínez et al., 2020).

2.9 Statistical analysis

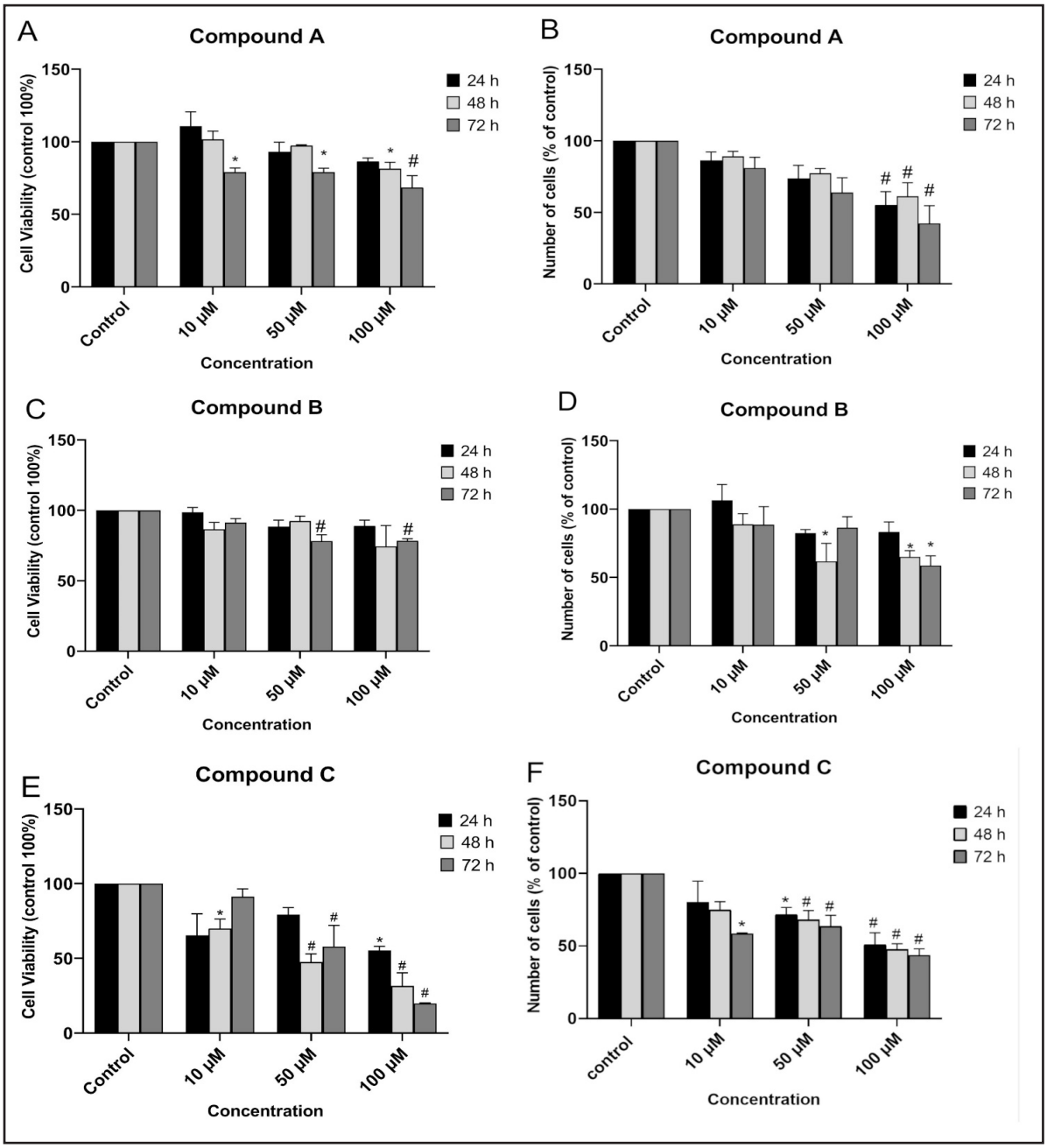
The sample size was at least three independent experiments for each protocol in triplicate. After obtaining the data, they were analyzed in a statistical program (Bioestat), and One-way ANOVA followed by Tukey's test were performed. Results were considered statistically different when they presented a $p<0.05$. The graphs were made in GraphPad Prism and are presented with the mean and standard error.

3 RESULTS

To verify whether compounds A, B and C induced cytotoxicity, cell viability was evaluated in the C6 cell line, using the MTT assay, after treatment for 24, 48, and 72 hours (Figure 1A, C and E). All tested compounds induced a significant reduction in cell viability, mainly at 100 µM after 72 h of treatment. At a concentration of 10 µM, only Compound A showed a significant reduction in cell viability after 72 hours of treatment. At a dose of 50 µM, all compounds reduced cell viability within 72 hours of exposure and Compound C also reduced cell viability within 48 hours of exposure.

To corroborate the results obtained with the cell viability test, a cell count test was performed after treatment with compounds A, B and C (Figure 1B, 1D, and 1F, respectively), after 24, 48, and 72 hours of treatment. The results obtained confirmed the cytotoxicity of the compounds with a dose of 100 µM at all times of treatment, which demonstrated the greatest effect. The concentration of 50 µM reduced the number of cells in 48 hours of treatment with Compound B and in 24, 48, and 72 hours with Compound C. The dose of 10 µM was not effective in reducing the number of cells in compounds A and B, but it did reduce the number of cells in Compound C after 72 hours of treatment.

Figure 1 – Cell viability and number of cells in C6 cell line

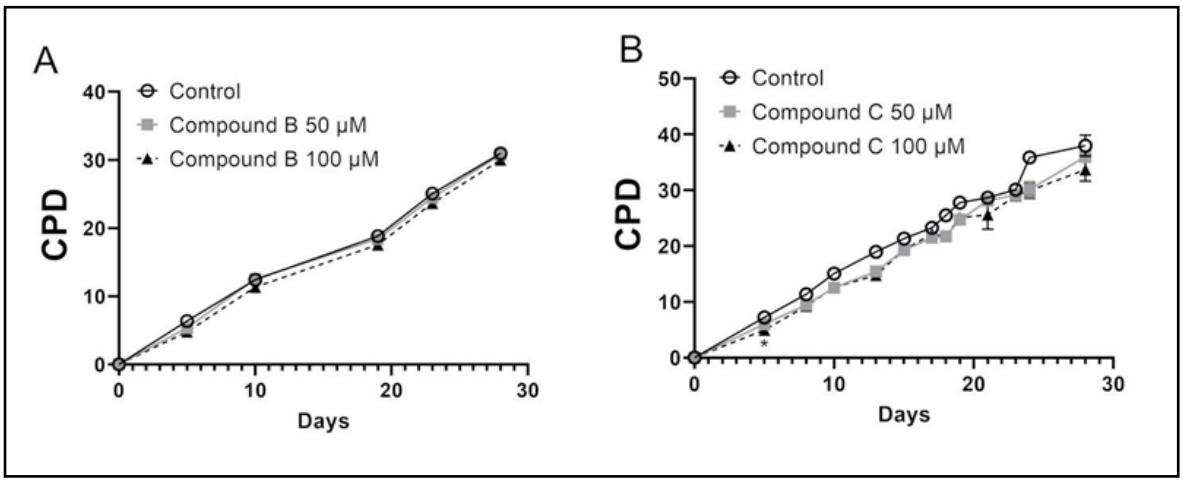


The cells were treated with compounds A, B and C at the indicated doses: 10 µM, 50 µM, and 100 µM, for 24, 48, and 72 hours each, followed by the MTT assay (A, C and E) and cell counting (B, D and F). Bars represent mean ± SEM. * $p<0.05$ and # $p<0.01$, in relation to the control
Source: Authors (2022)

To mimic the protocol used in the treatment of gliomas, a 5-day treatment cycle was performed with compounds B and C, at concentrations 50 and 100 µM, since they had the greatest cytotoxic effect (Figure 1). After this period, the cells were cultured for 23 days without treatment and the CPD was evaluated (Figure 2). There was no

significant difference in the rate of cell doubling over the experiment with compound B (Figure 2A). However, Compound C showed a CPD reduction in the first 5 days of treatment, and a modest reduction with no statistical significance at the end of the experiment (Figure 2B).

Figure 2 – Cumulative population doubling (CPD) of C6 cell line treated with Compound B (2A) and C (2B), at doses of 50 μM and 100 μM for 5 days, and cultured for another 23 days. Cells were counted in a Neubauer chamber on the indicated days. Results represent the mean ± SEM. ANOVA followed by Tukey. *p<0.05



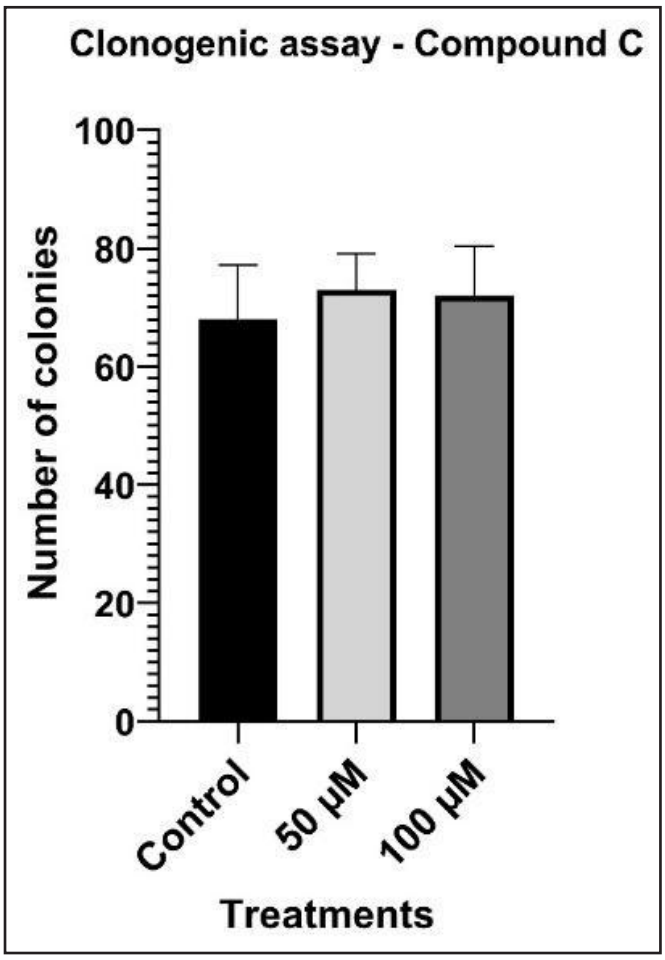
Source: Authors (2022)

To confirm this result, a clonogenic assay— the ability of cells to form colonies— was performed with Compound C, which showed a higher effect in the CPD test. As shown in Figure 3, there was no statistical significance of the treatments in relation to the control group.

Trying to find a possible mechanism by which Compound C would be decreasing the viability and the number of cells, the induction of senescence by the compound was tested. As indicated by results presented in Figure 4, the compound was not able to induce senescence. The blue dots in the figure indicate β-galactosidase positive cells, pointing to cellular senescence. As can be seen in the photographs, there is no significant difference in β-galactosidase activity between the treatments. Senescent cells have a characteristic morphology, which includes a much larger size and a flattened

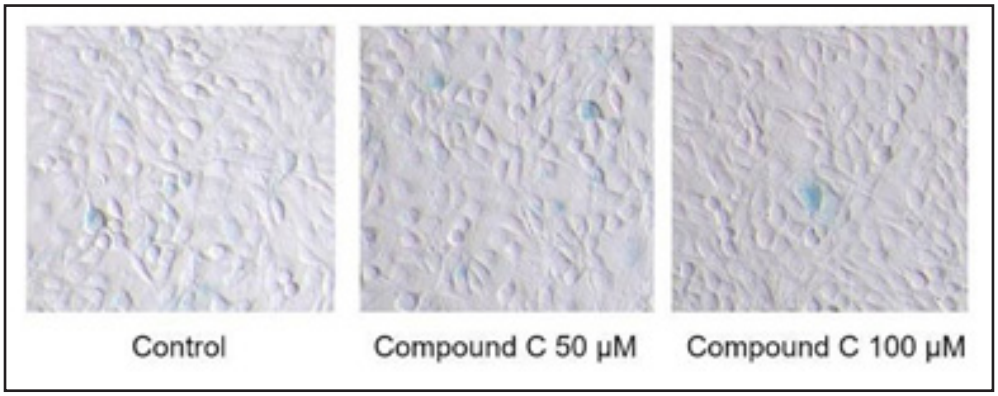
shape in the culture (Hayflick, 1965). The treatments were not able to provoke these morphological changes, corroborating non-occurrence of the senescence process.

Figure 3 – Colony formations in the C6 cell line treated for 5 days with Compound C, at doses of 50 μM and 100 μM, and cultured for another 7 days, followed by staining with crystal violet, and manually counted. Results represent the mean ± SEM. ANOVA follow by Tukey



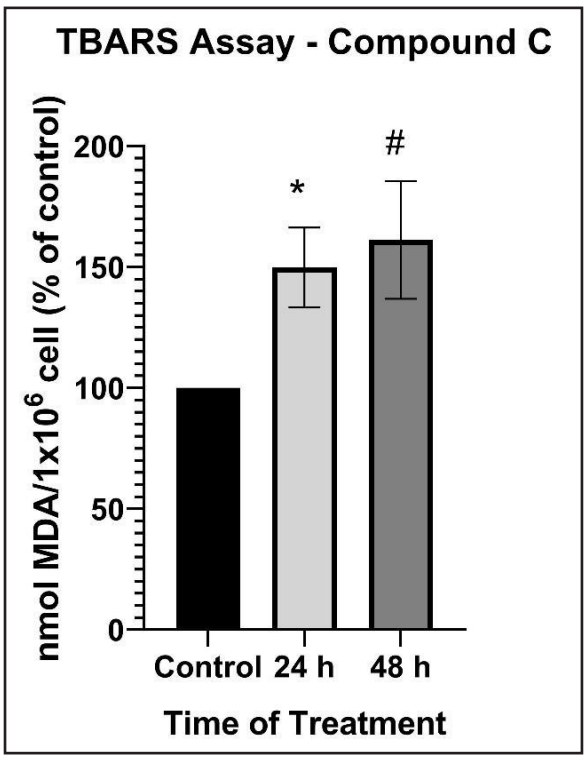
Source: Authors (2022)

Figure 4 – β-galactosidase activity observed in C6 cell line treated for 5 days with Compound C, at doses of 50 μM and 100 μM, and cultured for another 7 days. The image above represents a cell stained with β-Gal. The bar graph represents the ratio of β-gal-positive cells to total cells of at least three fields of three independent experiments. Results represent the mean ± SEM



Source: Authors (2022)

Figure 5 – TBARS assay. The C6 cell line was treated with Compound C, at 100 μM for 24 and 48 hours, and the lipid peroxidation was measured as an increase in MDA production. Results represent the mean ± SEM. ANOVA followed by Tukey. * $p<0.05$ and # $p<0.01$, in relation to the control



Source: Authors (2022)

Organochalcogen compounds are known for their oxidative stress modulating properties. Thus, the ability of Compound C (100 μM) to induce lipid peroxidation was tested after 24 and 48 hours of treatment. The results indicate an increase in lipid peroxidation after treatments (Figure 5).

4 DISCUSSION

The results presented in this study demonstrate cytotoxic effects of synthetic Organochalcogenyl benzoates on glioma cells. As they are synthetic and new chemical structures, there are few studies about the activity and pharmacological potential of these compounds. The lipophilic characteristic of the molecule, given by the aromatic rings present in the structures, is a promising indication of greater facility of penetration of the substance in the blood brain barrier (BBB), an essential characteristic for any drug that proposes CNS effects (Rang et al., 2016). This molecular characteristic, associated with the presence of selenium atoms (Se) in all structures, is what gives the direction of this work to the investigation of antigliomagenic activity.

In general, all compounds showed an effect of reducing cell number and viability. Compound C demonstrated the greatest cytotoxic effect, followed by compounds B and A. Difference between the results observed for the 3 compounds relies on the chemical structure, as shown in Chart 1. We observed that Compound B presents a more basic molecule, while the complexity of the others increases, with the addition of a fluorine to the fourth carbon of the second aromatic ring in Compound C, and the replacement of the third carbon of the first aromatic ring by a nitrogen, in Compound A.

Among the substances used in this study, the cytotoxicity of Compound B was tested in A172 human GBM cells by Gritzenco et al. (2021), after the development of the molecule. Results found in Gritzenco's work were similar to those obtained in this research, with cell number reduction after 24 hours of treatment with 50 and 100 μM, thus strengthening the antiglioma potential of this compound.

As the greatest cytotoxic effect was observed after treatment with compounds C and B, they were chosen for the CPD test to expand knowledge about its effects. In this trial, we observed a significant reduction in cell counts as the concentration of treatments increased (only in Compound C), in relation to the control. However, as the days without treatment pass, the cells recover, and at the end of the trial the count is uniform, with no significant difference between the control and the treatments. This result is related to that observed in the clinical treatment of GBM, where there is recovery of cell proliferation in the interval between treatments (Agani, 2018; Weller et al., 2014). Finding ways to overcome this recurrence continues to be a constant search in cancer research in general.

The clonogenic assay was used as a confirmation for PD. As can be seen, there was no difference in colony formation between the control and the treatment with Compound C, despite their impact on the initial cell count. This is another indication that the cells can recover after treatment, or that some of them are refractory to the cytotoxic effects of the compounds.

Seeking to verify a potential mechanism by which Compound C—the compound which shows the higher cytotoxicity—would be affecting the viability and the number of cells, the effect of this treatment on cellular senescence and lipid peroxidation induction was investigated. Senescence describes metabolically active cells, without proliferative capacity and in a stable state of cell cycle arrest, in response to some type of stress (Ou et al., 2021). Senescence also works as a potent barrier to prevent tumorigenesis and as a tumor suppressor mechanism, since bypassing the senescence process is essential for the malignant transformation of the cell (Calcinotto et al., 2019; Dimri et al., 1995). Thus, drugs that induce senescence have an increase in their antitumor potential (Ou et al., 2021).

In this research no significant effect was found in the induction of this process by the treatments with Compound C. The absence of positive results may be related both to the fact that the compound is not capable of inducing cellular senescence,

and to the fact that the concentration and times tested may have been insufficient to induce the necessary effect.

The oxidative potential was verified using the TBARS test, which measures substances reactive to thiobarbituric acid resulting from lipid peroxidation (Martínez et al., 2020). A considerable increase in the peroxidation lipid index was observed with the treatment, indicating a possible mechanism of action of Compound C in glioma cells. However, it is necessary to analyze other parameters and conduct further assays to elucidate this potential mechanism of action. Additionally, it would be necessary to test normal cells to evaluate the effects of the compound on non-cancerous cells. Tumor cells proliferate preferentially under conditions of oxidative stress. Therefore, an ideal therapeutic approach would be to specifically target and destroy cancer cells through oxidation of redox-sensitive proteins, without affecting cells with a normal redox balance (Giles et al., 2003).

Giles et al. (2003), demonstrated the action of organochalcogenyl catalysts as potential anticancer agents, whose activity is controlled by the redox environment, and which are capable of mediating cell death only in the presence of oxidative stress. Ebselen organoselenide was tested in PC-12 cells, in an environment under strong oxidative stress, bringing encouraging results with effectiveness at low doses, and absence of toxic effects in cells with normal redox balance (Giles et al., 2003; Giles et al., 2021).

Benzoate derivatives and organochalcogens have an innate pharmacological potential due to their structure and chemical composition, and some synthetic substances are used as medicines, such as benzyl benzoate, bromophyllicide, and lysine dendrimer (Kubanek et al., 2005; Hong et al., 2016; Rosa et al., 2018). In addition, several studies suggest that Se has a modulating action in various conditions at the level of the nervous system, and not only the ability to prevent neoplasms, but also to act directly on the various tumorigenic processes and inflammatory activity (Klein, 2016; Li et al., 2015; Yakubov et al., 2014; Yakubov et al., 2021).

The most prominent effect shown in this work by the treatment with Compound C may result from the addition of the fluorine atom to the molecular structure. Fluoride is a halogen widely distributed in nature which has been used for many decades in dentistry for its preventive effect against caries. However, in recent years, its derivatives have gained great importance in the field of medicinal chemistry, being known as organofluor chemistry. The biological activity inherent to fluorine is linked to its high electronegativity and its reduced atomic radius, which allows it to be easily used as a substitute for the C-H bond. Fluorinated compounds can alter drug absorption and delivery processes, and improve the metabolic stability of compounds. Furthermore, its ability to modulate the physicochemical properties of molecules, such as lipophilicity and basicity, can be used to improve the permeation of the molecule through biomembranes and contribute to an increase in the bioavailability of compounds, in addition to improving the binding affinity with target receptors (Ferreira, 2013; Magalhães, 2018; Thomas, 2006).

Bartusik-Aebisher et al. (2021) demonstrated these capabilities of fluorine in a study performed with trastuzumab, where they developed a drug delivery system—Trastuzumab-dendrimer-fluor—through the synthesis and characterization of a series of fluorinated dendrimers. Trastuzumab is a monoclonal antibody, fundamental for the treatment of breast cancer with overexpression of Her-2. Research has shown that the drug delivery system was much more efficient than trastuzumab alone, with the presence of molecules with smaller masses and increased lipophilicity being the main reasons for this efficiency.

Fluorinated molecules can be found in several drug classes, such as anesthetics, anti-inflammatories, antidepressants, cholesterol absorption inhibitors, antibacterials, and antitumors. An example of a fluorinated drug, which is widely used in cancer medicine, is 5-fluorouracil (5-FU) with the molecular formula $C_4H_3FN_2O_2$. 5-FU is a fluoropyrimidine, which acts by inhibiting the synthetic enzyme nucleotide thymidylate synthase, and incorrectly incorporating fluoronucleotides into RNA and DNA in cancer

cells. It is widely used in the treatment of various types of cancer, such as breast, head, and neck, having its greatest impact on colorectal cancers, and is sometimes combined with other chemotherapeutic agents, such as leucovorin and methotrexate (Ferreira, 2013; Diasio & Harris, 1989; Longley, Harkin & Johnston, 2003).

5 CONCLUSIONS

The compounds tested in the present research are new synthetic structures, providing a pioneering spirit for their antiglioma potential. Despite the lack of pharmacological data due to the innovative nature of the compounds tested, the data presented here shows promising evidence for Compound C on glioma treatment since the compound induced cell number and viability decrease, in addition to oxidative stress.

ACKNOWLEDGEMENTS

We thank UFFS for providing the physical structure and personnel necessary to carry out this research, especially the technical staff responsible for the operation of the institution's laboratories. Special thanks are due to Maira Regina Giehl for producing the compounds used in this study.

The authors thank FAPERGS (Fundação de Apoio à Pesquisa do Rio Grande do Sul), CAPES (Coordination for the Improvement of Higher Education Personnel) and UFFS (Federal University of South Border) for financial support.

REFERENCES

Agani, C. A. J. O. (2018). *Estabelecimento de linhagem de glioblastoma humano resistente ao quimioterápico temozolomide*. [Trabalho de conclusão de curso, Universidade Federal do Rio Grande do Sul]. http://hdl.handle.net/10183/206854.

Bartusik-Aebisher, D., Chrzanowski, G., Bober, Z., & Aebisher, D. (2021). An analytical study of Trastuzumab-dendrimer-fluorine drug delivery system in breast cancer therapy *in vitro*. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, *133***,** 11105, 2021. https://doi.org/10.1016/j.biopha.2020.111053.

Calcinotto, A., Kohli, J., Zagato, E., Pellegrini, L., Demaria, M., & Alimonti, A. (2019). Cellular senescence: aging, cancer, and injury. *Physiological reviews*, *99*(2), 1047-1078, 2019. http://doi.org/10.1152/physrev.00020.2018.

Davis, M. E. (2016). Glioblastoma: overview of disease and treatment. *Clinical journal of oncology nursing*, *20*(5 Suppl), S2-S8. http://doi.org/10.1188/16.CJON.S1.2-8.

Diasio, R. B., & Harris, B. E. (1989). Clinical pharmacology of 5-fluorouracil. *Clinical Pharmacokinetics*, *16*(4), 215–237. http://doi.org/10.2165/00003088-198916040-00002.

Dimri, G. P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E. E., ... & Campisi, J. (1995). A biomarker that identifies senescent human cells in culture and in aging skin *in vivo*. *Proceedings of the National Academy of Sciences of the United States of America, 92*(20), 9363–9367. http://doi.org/10.1073/pnas.92.20.9363.

Ferreira, Á. L. G. (2013). *Novos Processos de Fluoração em Química Farmacêutica Industrial*. [Dissertação de Mestrado em Química Farmacêutica Industrial, Universidade de Coimbra]. https://hdl.handle.net/10316/37508

Franken, N. A. P., Rodermond, H. M., Stap, J., Haveman, J., & Van Bree, C. (2006). Clonogenic assay of cells *in vitro*. *Nature protocols*, *1*(5), 2315–2319. http://doi.org/10.1038/nprot.2006.339.

Giles, N. M., Giles, G. I., Gutowski, N. J., & Jacob, C. (2003). Redox catalysts as sensitizers towards oxidative stress. *FEBS lett*ers, *535*(1-3), 179-182. http://doi.org/10.1016/S0014-5793(02)03890-5.

Giles, N. M., Giles, G. I., Holley, J. E., Gutowski, N. J., & Jacob, C. (2003). Targeting oxidative stress-related diseases: organochalcogen catalysts as redox sensitizers. *Biochemical pharmacolpgy, 66*(10), 2021–2028. http://doi.org/10.1016/S0006-2952(03)00544-6.

Gritzenco, F. (2020). *Síntese de benzoatos 3-(organocalcogenil)prop-2-in-1-ílicos catalisada por cobre: aplicação sintética e potencial farmacológico.* [Dissertação de mestrado (Programa de Pós-graduação em Ambiente e Tecnologias Sustentáveis, Universidade Federal da Fronteira Sul]. https://rd.uffs.edu.br/handle/prefix/3972.

Gritzenco, F., Kazmierczak, J. C., Anjos, T., Sperança, A., Peixoto, M. L. B., Ledebuhr, K. N. B., Bruning, C.A., ... & Godoi, B. (2021). Base-Free synthesis and synthetic applications of novel 3-(Organochalcogenyl)prop-2-yn-1-yl esters: promising anticancer agents. *Synthesis, 53*(15), 2676-2688. http://doi.org/10.1055/a-1477-6470.

Hayflick, L. The limited *in vitro* lifetime of human diploid cell strains. (1965). *Experimental Cell Research, 37*, 614-636. http://doi.org/10.1016/0014-4827(65)90211-9.

Hong, J., Zeng, X. A., Brennan, C. S., Brennan, M., & Han, Z. (2016). Recent advances in techniques for starch esters and the applications: a review. *Foods, 5*(3), 50. http://doi.org/ 10.3390/foods5030050.

INCA - Instituto Nacional do Câncer. (2019). *Estimativa 2020: incidência de câncer no Brasil* / Instituto Nacional de Câncer José Alencar Gomes da Silva – Rio de Janeiro: INCA Available at: https://www.inca.gov.br/sites/ufu.sti.inca.local/files/media/document/estimativa-2020-incidencia-de-cancer-no-brasil.pdf. Access in: 03/07/2022.

Kaushik, C. P., Pahwa, A., Kumar, D., Kumar, A., Singh, D., Kumar, K., & Luxmi, R. (2018). Synthesis and antimicrobial evaluation of (1-(2-(Benzyloxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl) methyl benzoate analogues. *Journal of heterocyclic chemistry*, *55*(7), 1720-1728. http://doi.org/10.1002/jhet.3209.

Klein, D. (2016) *Química Orgânica*. *Compostos aromáticos.* 2. ed. Rio de Janeiro: Ltc, 18,154-195.

Kubanek, J., Prusak, A. C., Snell, T. W., Giese, R. A., Hardcastle, K. I., Fairchild, C. R. Aalbersberg, W., ... & Hay, M. (2005). Antineoplastic Diterpene−Benzoate Macrolides from the Fijian Red Alga *Callophycus serratus*. *Organic letters*, *7*(23), 5261-5264. http://doi.org/10.1021/ol052121f.

Li, T., Smet, M., Dehaen, W., & Xu, H. (2015). Selenium − Platinum coordination dendrimers with controlled anti-cancer activity. *ACS applied materials & interfaces, 8*(6), 3609-3614. http://doi.org/10.1021/acsami.5b07877.

Longley, D. B., Harkin, P., & Johnston, P. G. (2003). 5-Fluorouracil: mechanisms of action and clinical strategies. *Nature reviews cancer*, *3*, 330-338. http://doi.org/10.1038/nrc1074.

Magalhães, H. I. C. (2018). *Efeitos do Flúor na Saúde Humana*. [Dissertação de Mestrado em Ciências Farmacêuticas. Universidade Fernando Pessoa]. http://hdl.handle.net/10284/7327.

Martínez, M. A., Rodríguez, J. L., Lopez-Torres, B., Martínez, M., Martínez-Larrañaga, M. R., Maximiliano, J. H., ... & Ares, I. (2020). Use of human neuroblastoma SH-SY5Y cells to evaluate glyphosate-induced effects on oxidative stress, neuronal development and cell death signaling pathways. *Environment international*, *135*, 105414. http://doi.org/10.1016/j.envint.2019.105414.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods*, *65*(1-2), 55-63. http://doi.org/10.1016/0022-1759(83)90303-4.

Ostrom, Q. T., Bauchet, L., Davis, F. G., Deltour, I., Fischer, J. L., Langer, C. E., Pekmezci, M., ... & Barnholtz-Sloan, J. S. (2014). The epidemiology of glioma in adults: A "state of the science" review. *Neuro-oncology*, 16(7), 896–913. http://doi.org/10.1093/neuonc/nou087.

Ou, H-L., Hoffmann, R., Gonzalez-Lopez, C., Doherty, G. J., Korkola, J. E., & Munoz-Espin, D. Cellular senescence in cancer: from mechanisms to detection. (2021). *Molecular oncology*, *15*(10), 2634–2671. http://doi.org/10.1002/1878-0261.12807.

Phillips, H. S., Kharbanda, S., Chen, R., Forrest, W. F., Soriano, R. H., Wu, T. D., Misra, A., ...& Aldape, K. (2006). Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell*, *9*, 157–173. http://doi.org/10.1016/j.ccr.2006.02.019.

Rang, H. P., Ritter, J. M., Flower, R. J., & Henderson, G. (2016). *Pharmacology*. 8. ed. Rio de Janeiro: Elsevier.

Rosa, S. G., Brüning, C. A., Pesarico, A. P., Souza, A. C. G., & Nogueira, C. W. (2018). Anti-inflammatory and antinociceptive effects of 2,2`-dipyridyl diselenide through reduction of inducible nitric oxide synthase, nuclear factor-kappa b and c-jun n-terminal kinase phosphorylation levels in the mouse spinal cord. *Trace Elements*, *48*, 38-45. http://doi.org/10.1016/j.jtemb.2018.02.021.

Silva, A. O., Dalsin, E., Onzi, G. R., Filippi-Chiela, E. C., & Lenz, G. (2016). The regrowth kinetic of the surviving population is independent of acute and chronic responses to temozolomide in glioblastoma cell lines. *Experimental cell research*, *348*(2), 177–183. http://doi.org/10.1016/j.yexcr.2016.09.014.

Stupp, R., Taillibert, S., Kanner, A. A., Kesari, S., Steinberg, D. M., Toms, S. A., Taylor, L. P., ... & Ram, Z. (2015). Maintenance therapy with tumor-treating fields plus temozolomide vs temozolomide alone for glioblastoma: a randomized clinical trial. *JAMA*, *314*(23), 2535–2543. http://doi.org/10.1001/jama.2015.16669.

Thomas, C. J. (2006). Fluorinated natural products with clinical significance. *Current topics in medicinal chemistry*, *6*(14), 1529-1543. http://doi.org/10.2174/156802606777951109.

Weller, M., Van Den Bent, M., Hopkins, K., Tonn, J. C., Stupp, R., Falini, A., Cohen-Jonathan-Moyal, E, ... & Wick, W. (2014). EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma. *The Lancet. Oncology, 15*(9), e395-e403. http://doi.org/10.1016/S1470-2045(14)70011-7.

Wen, P. Y., Kesari, S. (2008). Malignant gliomas in adults. *The New England journal of medicine*, *359*(5), 492-507. http://doi.org/10.1056/NEJMra0708126.

WORLD HEALTH ORGANIZATION – WHO (1997). *Model Prescribing Information: drugs used in skin diseases.* Geneva.

WORLD HEALTH ORGANIZATION – WHO (2018). *Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region*. Geneva.

Yakubov, E., Buchfelder, M., Eyüpoglu, I. Y., & Savaskan, N. E. (2014). Selenium action in neuro-oncology. *Biological Trace Element Research*, 161, 246–254. http://doi.org/10.1007/s12011-014-0111-8.

Yakubov, E., Eibl, T., Hammer, A., Holtmannspötter, M., Savaskan, N., & Steiner, H-H. (2021). Therapeutic potential of selenium in glioblastoma. *Frontiers in Neuroscience***,** 15, 1-12. http://doi.org/10.3389/fnins.2021.666679.

Yamanaka, R., Saya, H. (2009). Molecularly targeted therapies for glioma. *Annals of Neurology*, 66, 717-729. http://doi.org/10.1002/ana.21793.

Zamin, L. L., Filippi-Chiela, E. C., Dillenburg-Pilla, P., Horn, F., Salbego, C., & Lenz, G. (2009). Resveratrol and quercetin cooperate to induce senescence-like growth arrest in C6 rat glioma cells. *Cancer Science*, 100(9), 1655-1662. http://doi.org/10.1111/j.1349-7006.2009.01215.x

Authorship contributions

1 – Lauren Lúcia Zamin

Doutorado em Biologia Celular e Molecular pela Universidade Federal do Rio Grande do Sul
https://orcid.org/0000-0002-3452-1483 • lauren.zamin@uffs.edu.br
Contribution: Conceptualization, Methodology, Software, Formal Analysis, Research, Resources, Data Curation, Writing – original draft, Writing - review & editing, Visualization - Supervision - Project Administration

2 – Elisa da Silva

Mestrado em Ambiente e Tecnologias Sustentáveis pela Universidade Federal da Fronteira Sul
https://orcid.org/0000-0002-8455-7839 • elisadasilva16@hotmail.com
Contribution: Conceptualization, Methodology, Software, Formal Analysis, Research, Resources, Data Curation, Writing – original draft, Writing - review & editing

3 – Benhur Godoi

Doutorado em Química pela Universidade Federal de Santa Maria.
https://orcid.org/0000-0001-7437-9656 • benhur.godoi@uffs.edu.br
Contribution: Formal Analysis, Research, Resources, Data Curation, Writing - review & editing, Visualization - Supervision

How to quote this article

Zamin, L. L., Silva, E. da, & Godoi, B. (2024). Potential antitumor effect of organochalcogenyl-benzoates in glioma cellss. *Ciência e Natura*, 46, e74392. https://doi.org/10.5902/2179460X74392