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Synthetic 2-aryl-3-((piperidin-1-yl)ethyl)thiazolidin-4-ones exhibit selective *in vitro* antitumoral activity and inhibit cancer cell growth in a preclinical model of glioblastoma multiforme

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ABSTRACT

Glioblastoma multiforme (GBM) is the worst form of primary brain tumor, which has a high rate of infiltration and resistance to radiation and chemotherapy, resulting in poor prognosis for patients. Recent studies show that thiazolidinones have a wide range of pharmacological properties including antimicrobial, anti-inflammatory, anti-oxidant and anti-tumor. Here, we investigate the effect antiglioma *in vitro* of a panel of sixteen synthetic 2-aryl-3-((piperidin-1-yl)ethyl)thiazolidin-4-ones where 13 of these decreased the viability of glioma cells 30–65% (100 μM) compared with controls. The most promising compounds such as **4d**, **4l**, **4m** and **4p** promoted glioma reduction of viability greater than 50%, were further tested at lower concentrations (12.5, 25, 50 and 100 μM). Also, the data showed that the compounds **4d**, **4l**, **4m** and **4p** induced cell death primarily through necrosis and late apoptosis mechanisms. Interestingly, none of these 2-aryl-3-((piperidin-1-yl)ethyl)thiazolidin-4-ones were cytotoxic for primary astrocytes, which were used as a non-transformed cell model, indicating selectivity. Our results also show that the treatment with sub-therapeutic doses of 2-aryl-3-((piperidin-1-yl)ethyl) thiazolidin-4-ones (**4d**, **4l** and **4p**) reduced *in vivo* glioma growth as well as malignant characteristics of implanted tumors such as intratumoral hemorrhage and peripheral pseudopalisading. Importantly, 2-aryl-3-((piperidin-1-yl)ethyl)thiazolidin-4-ones treatment did not induce mortality or peripheral damage to animals. Finally, 2-aryl-3-((piperidin-1-yl)ethyl)thiazolidin-4-ones also changed the nitric oxide metabolism which may be associated with reduced growth and malignity characteristics of gliomas. These data indicates for the first time the therapeutic potential of synthetic 2-aryl-3-((piperidin-1-yl) ethyl)thiazolidin-4-ones to GBM treatment.

1. Introduction

Glioblastoma multiforme (GBM) is the most common and lethal primary brain tumor, exhibiting morphological and gene expression similarities with glial cells such as astrocytes, oligodendrocytes and its precursors [1]. Additionally, the presence of an inflammatory microenvironment composed of non-malignant cells, such as

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immune cells and fibroblasts, is associated with increased malignancy through modulation of glioma cell proliferation and angiogenesis, which results in a poor prognosis for patients and a challenge for oncology [2,3].

The most common treatment for GBM is the surgical resection, whenever possible [4], followed by radio and chemotherapy with temozolomide (TMZ) that is maintained as an adjuvant therapy for at least six months [5]. However, these tumors are resistant to therapeutic strategies and their high rate of proliferation and infiltrative growth patterns precludes curative neurosurgery, resulting in a low rate survival of 12 months for patients [6]. In addition, the blood-brain barrier (BBB) limits the drug delivery to central nervous system (CNS), favoring the development of chemoresistance [7]. As an alternative for the treatment, lipophilic compounds stand out because they have the ability to overcome BBB, reaching the target site of disease [7].

Recent studies have been reported the potential of biologically active five-membered thiazolidinones. Such heterocyclic class has occupied a prominent position in the medicinal chemistry field [8,9]. There are reports showing the wide range of pharmacological properties of thiazolidinones, including antimicrobial [10], anti-inflammatory [11], antioxidant [12] and anti-tumor activities [13]. These molecules are also considered peroxisome proliferator-activated (PPAR) receptors agonists showing hypoglycemic, anti-neoplastic and anti-inflammatory activities [14]. The mechanisms by which synthetic tiazolidinones contribute to anti-tumor activity remain controversial. However, data from literature suggest the involvement of these compounds in the control of cell proliferation by inducing apoptosis [15,16] or by acting as inhibitors of cyclo-oxigenases, which seems to be important in the cancer-related inflammation by producing inflammatory mediators related to angiogenesis [17].

Considering the important biological properties of thiazolidinones and that the actions described can be important for the treatment of GBM, this study aimed to investigate *in vitro* and *in vivo* antiproliferative activity and therapeutic potential of synthetic 2-aryl-3-((piperidin-1-yl)ethyl)thiazolidin-4-ones for the treatment of gliomas.

2. Materials and methods

2.1. Reagents

Dulbecco's modified Eagle's medium (DMEM), Fungizone, penicillin/streptomycin, 0.25% trypsin/EDTA solution and fetal bovine serum (FBS) were obtained from Gibco (Gibco BRL, Carlsbad, CA, USA). All other chemicals and solvents used were of analytical or pharmaceutical grade.

2.2. Thiazolidinone synthesis

The 2-aryl-3-((piperidin-1-yl)ethyl)thiazolidin-4-ones **4a**–**p** were synthesized by one-pot reactions from 1-(2-aminoethyl) piperidine, arenealdehydes and mercaptoacetic acid in according to a previous paper published by us [18]. Analogues thiazolidinones containing arenealdehydes groups substituted by electron-withdrawing (F, Cl, NO_2) or electron-releasing groups (OH, OCH_3, CH_3) at 2- 3- and 4-positions were used to study the cytotoxic potential. The general structure of thiazolidinones **4a**–**p** is shown in Fig. 1.

2.3. General cell culture procedures

2.3.1. Glioma cultures

Rat C6 malignant glioma cell line was obtained from American Type Culture Collection (Rockville, MD, USA). Cells were grown and maintained in low-glucose DMEM containing 0.1% fungizone and 100 U/L penicillin/streptomycin and supplemented with 5% FBS. Cells were kept at 37 °C in a humidified atmosphere with 5% CO_2.

2.3.2. Primary astrocyte cultures

Astrocyte cultures were prepared as follow: cortex of newborn Wistar rats (1–2 days old) were removed and dissociated mechanically in a Ca^{+2} and Mg^{+2} free balanced salt solution (pH 7.4; 137 mM NaCl, 5.36 mM KCl, 0.27 mM Na_2HPO_4, 1.1 mM KH_2PO_4, and 6.1 mM glucose). After centrifugation at 1000 *g* for 5 min, the pellet was suspended in DMEM supplemented with 10% FBS. The cells (5×10^4) were seeded in poly-L-lysine-coated 96-well plates. Following 4 h of seeding, plates were gently shaken and washed with PBS and medium was changed to remove neuron and microglia contaminants. Cultures were allowed to grow to confluence by 20–25 days. Medium was replaced every 4 days [19,20]. The procedures were approved by the Ethics Committee of Federal University of Pelotas (Protocol number 9219).

2.3.3. In vitro cell culture treatment

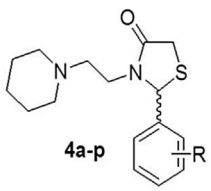
Sixteen synthetic 2-aryl-3-((piperidin-1-yl)ethyl)thiazolidin-4-ones (**4a** to **4p**) were firstly dissolved in sterile DMSO at the concentration of 100 mM (stock solution) and further diluted in DMEM with 5% (glioma) or 10% (astrocytes) FBS to obtain 12.5, 25, 50 and 100 μM. The C6 glioma cell line was seeded at 1×10^3 cells/well in DMEM/5% FBS in 96 multiwell plates in a final volume of 100 μL and the cells were allowed to growth for 24 h. Astrocyte cultures were prepared as described above. Cell cultures were exposed to synthetic 2-aryl-3-((piperidin-1-yl)ethyl)thiazolidin-4-ones (12.5–100 μM) for 48 or 72 h. Appropriate controls containing DMEM 5%/10% FBS or 0.01% DMSO were performed.

2.3.4. Cell viability assay

Dehydrogenases-dependent 3(4,5-dimethyl)-2,5diphenyl tetrazolium bromide (MTT) reduction was used to estimate viability of glioma and astrocyte cell cultures. This method is based on the ability of viable cells to reduce MTT and form a blue formazan product. MTT solution (sterile stock solution of 5 mg/mL) was added to the incubation medium in the wells at a final concentration of 0.5 mg/mL. The cells were left for 60 min at 37 °C in a humidified 5% CO_2 atmosphere. The medium was then removed and plates were shaken with DMSO for 30 min. The optical density of each well was measured at 492 nm and results were expressed as percentual of control [21].

2.3.5. Propidium iodide assay

Cell damage was assessed by fluorescent image analysis of propidium iodide (PI) uptake. At the end of the treatments, C6 glioma and astrocyte cell cultures were incubated with PI (7.5 μM) for 1 h. PI fluorescence was excited at 515–560 nm using an



R = 2-F (**a**) 2-Cl (**d**) 2-NO_2 (**g**) 3-OH (**j**) 3-OCH_3 (**m**) 2,6-Cl (**p**)
3-F (**b**) 3-Cl (**e**) 3-NO_2 (**h**) 4-OH (**k**) 4-OCH_3 (**n**)
4-F (**c**) 4-Cl (**f**) 4-NO_2 (**i**) 2-OCH_3 (**l**) 4-CH_3 (**o**)

Fig. 1. General structure of 2-aryl-3-((piperidin-1-yl)ethyl]thiazolidin-4-ones (**4a**–**p**).