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Abstract

Glioblastoma multiforme, is a type of brain cancer that develops from glial cells, which surround neurons and provide support and insulation. Previous investigation has shown that some heterocyclic compounds are key in improving the properties of anticancer drugs by enhancing lipophilicity, polarity, and other varying physiochemical features. Synthetic heterocyclic compounds used as anticancer drugs attempt to imitate naturally-occurring ligands and substrates so as to disturb the natural balance in cells. Testing was done to determine the anticancer abilities of hybrid compounds, heterocyclic arylidenes, containing various functional groups, including boronic acids, through a three-day testing process. These compounds were previous synthesized by Jemma McLeish. This was done in order to determine whether the compounds have no effect on glioblastoma viability, increased viability of glioblastoma, or decreased viability of glioblastoma. Results show that only one compound, rhodanine + p-tolualdehyde, was successful, while the other three compounds, rhodanine + o-tolualdehyde, rhodanine + 2-fluoro-4-formylphenylboronic acid, and rhodanine + 3-fluoro-4-formylphenylboronic acid, caused growth acceleration.

Introduction

In the country, nearly 700,000 Americans are affected with brain cancer, either malignant, which are cancerous, or non-malignant (noncancerous). Those who are affected with malignant brain cancer have an average survival rate of 35%. Glioblastoma is an aggressive type of cancer that can attack the brain. It represents about 15% of all primary brain tumors (American Brain Tumor Association, 2019) with men being more likely to get them than women. It forms from star-shaped cells in the brain called astrocytes. In adults, it begins growth in the cerebrum, which is the largest part of the brain, but can be found anywhere in the brain. Eventually, the tumor is able to make their own blood supply, which helps it to grow, but also makes it easier to invade normal brain tissue. The tumor that forms causes brain swelling which eventually leads to increased pressure in the brain, which then can cause nausea, vomiting, and severe headaches. The patient can also exhibit neurological symptoms that can affect balance, neurocognitive function, or memory. Those affected with glioblastoma have a short life expectancy after diagnosis. According to the Cancer Research Institute, a quarter of newly diagnosed patients with glioblastoma survive for 24 months, and less than 10% of patients survive more than 5 years (2020). Glioblastoma is difficult to treat mainly due to the blood brain barrier, which prevents drugs from passing through and attacking the cancer cells directly. Current treatment includes temozolomide, but is not very effective since the cancer gains resistance to this drug very quickly.

Our study aimed to discover the anticancer abilities of hybrid compounds that have been synthesized by Jemma McLeish in 2011 (McLeish, 2017). The compounds used for testing are heterocyclic arylidenes, which consist of two parts—a heterocycle and an arylidene (Fig. 1).

The specific heterocycle present in the compounds I tested was rhodanine. Previous studies have shown that rhodanine and its related compounds exhibit anticancer (Jung et al., 2010; Ramesh et al., 2014), antidiabetic (Rakowitz, Maccari, Ottana, & Vigorita, 2006), antimicrobial (AbdelKhalek, Ashby, Patel, Talele, & Seleem, 2016), antiviral (Ramkumar et al., 2010), and antifungal activities (Rana, Desai & Jauhari, 2014). Compounds that contain the boronic acid functional group have shown anticancer (Achanta, Modzelewska, Feng, Khan, & Huang, 2006; Kumar, Hager, Pettit, Gurulingappa, Davidson, & Khan, 2003), antimicrobial (Eidam et al., 2010), and antiviral (Khanal et al., 2013) activities. By combining these two moieties, it is presupposed that it will exhibit at least one of the outcomes when tested on the U87MG glioma cell line. Additionally, compounds with methoxy and methyl substituents have been able to show antitumor and antiviral acitivities (Fathalla et al., 2009; Simic, Kolarevic, Brceski, Jeremic, & Vukovic-Gacic, 2016; Syam, Adelwahab, Al-Mamary, & Mohan, 2012).

Our study investigated the anticancer abilities of the following four compounds: rhodanine + p-tolualdehyde, rhodanine + o-tolualdehyde, rhodanine + 2-fluoro-4-formylphenylboronic acid, and rhodanine + 3-fluoro-4-formylphenylboronic acid (Fig. 2). All the compounds contain the same heterocycle, which is rhodanine, while the arylidene varies in substituents and position. Therefore, the brain cancer cells may either cause (1) no change in glioblastoma viability, (2) increased glioblastoma viability, or (3) decreased glioblastoma viability.

Methods

Day 1:

Glioblastoma cancer cells from the U87MG cell line were grown in cell culture plates using Minimal Essential Media (MEM) which was supplemented with 10% fetal bovine serum, penicillin, and streptomycin. The media was changed three times a week; the cell density of the plate was kept at less than 70-80% confluency. In order to set up the experimental plate, the media was removed and 2 mL of trypsin was added to the plate in order to wash the cells off. Afterwards, the cells were stained with trypan blue exclusionary dye and counted using a hemocytometer. Approximately 10,000 cells were then introduced into the wells of a 12-well cell plate. The cells were then left to incubate at 37℃ 5% CO_2.

Day 2:

A stock solution of the compound was made by weighing 20 mg of the compound and dissolving it into 1 mL dimethyl sulfoxide (DMSO). The stock solutions were used to create eleven separate concentrations of the compounds tested. The dilutions were prepared by adding 200 mL of the stock solution to 1800 mL of media to make a 2 mg/mL concentration. The next dilution was prepared by taking half of the previously made dilution and adding 1 mL of media (Fig. 3). The following dilution was made by taking half of the previous dilution and adding another 1 mL of media. The remaining dilutions were prepared by the same process. The cells were then incubated for 24 hours at 37ºC with 5% CO_2.

Day 3:

The cells were removed from the incubator. The media with the compounds was then removed from the wells. 1 mL of methanol was used to fix the cells so they will not degrade; it was left in the cell wells for approximately 5 minutes. 1 mL of crystal violet was then used to

stain the cells; it was also left in the cell wells for approximately 5 minutes. The crystal violet was removed; any excess crystal violet was removed using distilled water. Once the cells were stained, we were able to observe the cells through a digital microscope to allow cells to be counted. Nine areas within the well were counted and averaged. Finally, the total cell count was determined and the percentage of the control cells was calculated.

Once the total cell count was calculated, the data was analyzed through a linear regression in order to determine the LC_{50} of each of the compounds. LC_{50} is described as the median lethal dose, which is the concentration of the compound that is lethal to 50% of the population. The higher the LC_{50}, the less toxic the compound; a lower LC_{50} indicates a more toxic compound.

Results

We inspected four various compounds in order to determine its anticancer abilities. Only the first compound, rhodanine + p-tolualdehyde, was successful while the other three compounds, rhodanine + o-tolualdehyde, rhodanine + 2-fluoro-4-formylphenylboronic acid, and rhodanine + 3-fluoro-4-formylphenylboronic acid, were not successful since they caused growth acceleration. The percentages of the control for each compound has been calculated (Fig. 4).

The LC_{50} of the rhodanine + p-tolualdehyde is 0.28 mg/mL. The concentrations that show statistical significance are 1 mg/mL, 0.25 mg/mL, 0.063 mg/mL, 0.032 mg/mL, and 0.016 mg/mL. The LC_{50} of rhodanine + o-tolualdehyde is 0.25 mg/mL. The concentration that shows statistical significance is 0.5 mg/mL. The LC_{50} of rhodanine + 2-fluoro-4-formylphenylboronic acid is 0.75 mg/mL. The concentrations that show statistical significance are 2 mg/mL and 1 mg/mL. The LC_{50} of rhodanine + 3-fluoro-4-formylphenylboronic acid is 0.375 mg/mL. The concentrations that show statistical significance are 1 mg/mL and 0.063 mg/mL.

Discussion

Only rhodanine + p-tolualdehyde was successful while rhodanine + o-tolualdehyde, rhodanine + 2-fluoro-4-formylphenylboronic acid, and rhodanine + 3-fluoro-4-formylphenylboronic acid accelerate cell growth, increasing glioblastoma viability. The percentages of the control for rhodanine + o-tolualdehyde, rhodanine + 2-fluoro-4-formylphenylboronic acid, and rhodanine + 3-fluoro-4-formylphenylboronic acid have exceeded 100%; the number of cells present in the wells after treatment exceeded the control, showing that the compounds accelerated growth (cell viability increased). Between rhodanine + p-toluadehyde and rhodanine + o-tolualdehyde, it seems that the position of the methyl group on the arylidene seems to make a slight difference. With the LC_{50} of rhodanine + p-tolualdehyde being 0.28 mg/mL and rhodanine + o-tolualdehyde being 0.25, the latter compound would be slightly more effective since it requires a smaller concentration of the drug to cause half of the cell population to die. However, when the methyl group is replaced by the presence of the boronic acid, it seems to accelerate the growth greatly, making rhodanine + 2-fluoro-4-formylphenylboronic acid and rhodanine + 3-fluoro-4-formylphenylboronic acid dangerous to pursue further research. The statistical significances that were noted in each of the compounds were probably due to the acceleration of the growth rather than the compound itself. This acceleration, or increase in cell viability, has also been observed in Jemma McLeish's research when tested on the AU565 breast cancer cells (McLeish, 2017).

Pursuing further research with rhodanine + o-tolualdehyde, rhodanine + 2-fluoro-4-formylphenylboronic acid, and rhodanine + 3-fluoro-4-formylphenylboronic acid would not be useful since these three compounds have been shown to accelerate cell growth, increasing the cell viability. Rhodanine tends to be a promiscuous compound, meaning that it has multi-target

activity. Therefore, it would still be beneficial to continue further research on rhodanine + p-tolualdehyde in hopes of determining its mode of action that would shed some light on rhodanine's possible targets.

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Figures and Tables

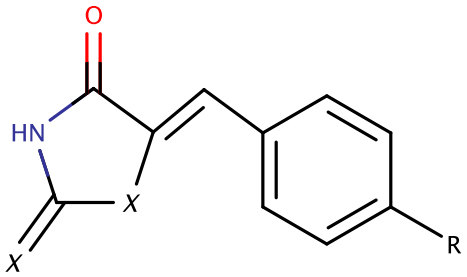

Figure 1. General structure of heterocyclic arylidene. (X = sulfur, R = boronic acid, methyl)

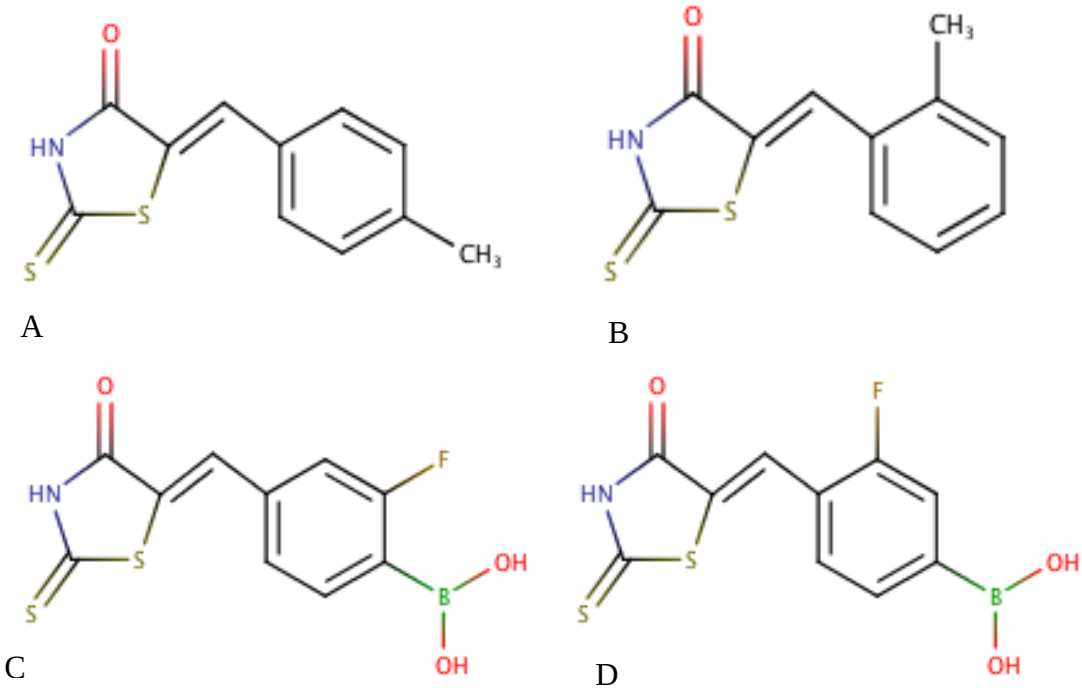

Figure 2. A. Rhodanine + p-tolualdehyde. B. Rhodanine + o-tolualdehyde. C. Rhodanine + 2-fluoro-4-formylphenylboronic acid. D. Rhodanine + 3-fluoro-4-formylphenylboronic acid

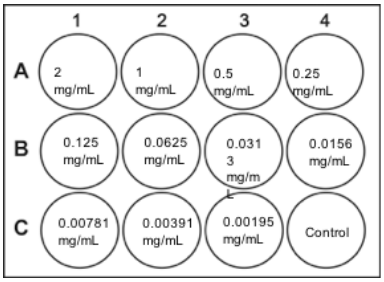

Figure 3. The 12-well cell plate setup including dilutions.

Figure 4. The effects of heterocyclic arylidenes on the U87MG glioma cell line compared to untreated U87MG glioma cells. Statistical significances ($p \leq 0.05$ for t-test) between untreated cells and treated cells are indicated by an asterisk. The LC_{50} for each compound are indicated by a red arrow.