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Anticancer Activity of Some Boronic Acid Arylidene Heterocycles

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ABSTRACT

This research was done in order to investigate the anticancer activity of boronic acid arylidene heterocycles. Boronic acid, heterocycles, and aryl compounds have all independently exhibited various anti-microbial properties and have shown potential to be used in pharmaceuticals for the treatment of viruses and cancers. Using a one-step protocol developed by Dr. Murray, several different arylheterocyclic compounds were combined with an arylboronic acid to synthesize novel compounds. These were then tested for their anti-cancer activity on glioblastoma brain cancer cells. Although varied in potency, all had some amount of anti-cellular effect on the cancer cells. This is only the start of analyzing these compounds and further research should be done in order to realize their full potential.

INTRODUCTION

The purpose of this research project is to investigate the anticancer activity of boronic acid arylidene heterocycles. While similar studies have been conducted with these types of compounds, the specific compounds in this study have not previously been synthesized and tested. Dr. Murray has developed a simple one-step protocol for their chemical synthesis that facilitates the exploring of their biological activity.

Choosing the compounds for this research was based in part on the independent bioactivity of boronic acid and heterocycles. In recent years, the field of pharmacology has shown a growing interest in these compounds for medicinal purposes. Boronic acid has demonstrated anticancer [1], antibacterial [2], and antiviral [3] properties in past research, and one of the reasons behind the growing interest in boronic acid for therapeutic applications is that boron has been found in natural products of certain bacteria and is also involved in plant wellbeing, such as the building of cell walls [4]. The heterocyclic compounds chosen for the

anti-microbial properties of their family compounds were rhodanine [5,6], 2,4-thiazolidinedione [6,7], hydantoin and pseudothyiohydantoin [8,9], and 3-methyl-1-phenyl-2-pyrazoline-5-one [10].

While some of the previous research has put effort into investigating the mechanisms by which the compounds work, the purpose of this study is simply to look for the presence, if any, of anti-cancer properties of these novel compounds. The testing of these compounds were done on brain cancer cells. To date, brain cancer has been difficult to diagnose patients early on, and risk factors are not well understood. This combined with the complications of treating the disease due to the blood-brain barrier leads to a poor prognosis [11]. Thus, brain cancer cells were chosen as a good option for potentially expanding the body of knowledge concerning its treatment.

METHODS

Synthesizing Compounds:

Five different compounds were combined with the ortho, para, and meta substituted isomers of formylphenylboronic acid (thus three different reactions were performed for each compound). The compounds were rhodanine, 2,4-thiazolidinedione, pseudothiohydantoin, hydantoin, and 3-methyl-1-phenyl-2-pyrazoline-5-one. Ethanol was used as a biodegradable solvent, and calcium oxide was used as a nontoxic catalyst. The general protocol for the synthesis of all of the compounds was a nontoxic one-step procedure and is as follows. In a 25 mL round bottom flask, 20 mL of ethanol, 5 mmol of formylpheylboronic acid, 5 mmol of the variable compound, and 15 mmol of calcium oxide were added in that order. A stir bar was also added to the flask, which was then placed on a magnetic hot plate. A water-cooled condenser

was attached to the flask, and the mixture was heated under reflux for 4 hours. Afterwards the mixture was poured into an ice bath with 30 mL of saturated ammonium chloride as a neutralizing agent. After stirring for at least 10 minutes, vacuum filtration was performed in order to isolate the solid product. No further purification was performed.

The compounds were analyzed with NMR to determine if the reaction had successfully made the desired product. If the NMR indicated that traces of the reactants were still in the product, the compound was not used in testing with the cancer cells.

Cell line and cell culture:

The cells tested are glioblastoma brain cancer cells (U87MG cell line) and were ordered from American Type Culture Collection (ATCC). Minimal Essential Media (MEM) was obtained from Invitrogen and contained 10% fetal bovine serum and 1% penicillin and streptomycin. The media was changed 3 times per week and the cell plates were kept in an incubator at 37˚C and 5% carbon dioxide level.

Testing compounds:

The LD_{50} could not be obtained because of visibility limitations. The reason for this is that since a hemocytometer was used to count the cells, once the compound concentration exceeded 0.25M, it became too cloudy to accurately count the cells. Thus, in order to test the potency of the compounds, several fixed concentrations of the compounds were administered to the glioblastoma cancer cells. The cells were grown on plates with MEM, and then allocated onto new plates for the trials with roughly 500,000 cells on each plate. This was done by suspending the cells with trypsin, determining cell density with a hemocytometer, and then

distributing the appropriate amount of trypsin onto new plates accordingly. After setting up the plates and giving sufficient time for the cells to attach onto the plate (minimum 6 hours but was usually around 24 hours). The compounds were dissolved in DMSO and added to the plates in concentrations of 0.05M, 0.1M and 0.25M, with 1ml of DMSO and compound for every 3ml of MEM. DMSO was used because ethanol would have been the only other solvent that could have dissolved the compounds but would have killed the cells. Also, the properties of DMSO allow it to enter the cells, along with any substances it has dissolved. For each trial run, one plate with only cells and the media, as well as one plate with only pure DMSO added was also set up as controls. After the cells had been exposed to the compound for 24 hours they were again detached from the plate with trypsin and counted with a hemocytometer. 50µl of trypsin containing the cells in 50µl of trypan blue dye, in order to differentiate between the cells that were dead and alive, was counted using the hemocytometer. Three trials were performed for each compound. The data has been expressed in % death of the cells, and T-tests (one-tailed, unequal variance) were performed between the different compounds and the DMSO control results to determine if there was a significant difference in cell death.

RESULTS

Out of the fifteen reactions performed, fourteen made a solid product. When analyzed with NMR, six were found to have successfully undergone the reaction completely. This included all three rhodanine reactions, two of the 2,4-thiazolidinedione reactions, and one reaction with pseudothiohydantoin. The structures of the products are shown in Figure 1.

Table 1 shows the numerical results of the trials and data analysis, while Figure 2 gives a visual representation of the data (the compounds were arbitrarily named to keep them ordered).

Table 1 also includes the results of the t-tests and, based on the p-value given, if the amount of cell death increased significantly when adding that compound at that concentration. It was found that based on the t-tests, all of the compounds were associated with an increase in cell death at some dosage. However, several of the compounds were not consistent in the sense that they had a significant p-value for one concentration but not at a higher concentration. Notably, compound 4-R3 showed a significant increase in cell death for all three concentrations.

DISCUSSION

Looking at the data, it is somewhat difficult to interpret the results. This is due to several reasons. First, although all of the compounds have at least one concentration at which a significant increase in cell death occurred, a greater concentration did not always correlate with greater amounts of cell death. Furthermore, as aforementioned, some of the compounds were inconsistent in their significance in that sometimes at one concentration a significant p-value would be obtained, but such would not be the case at a higher concentration for the same compound. It appears that one of the reasons for this is that sometimes at those higher concentrations, even if the cell death increased, standard deviation was large enough to result in an insignificant p-value. If this high standard deviation is due to random error, further research with more trials per compound and concentration should decrease the standard deviation and result in more consistent data.

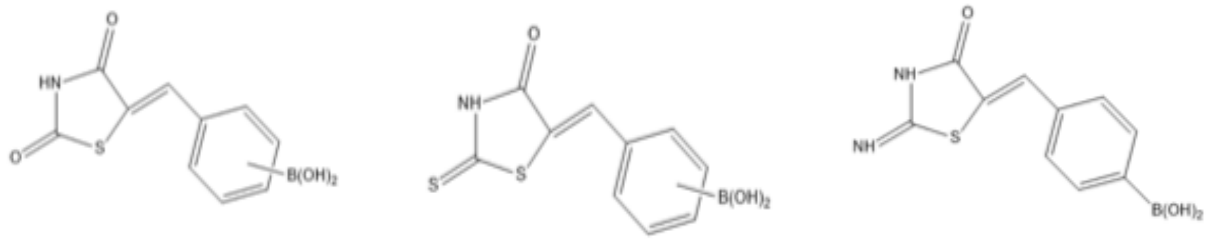
Another factor that affected the data collected was that adding DMSO to the cells seemed to make them more tightly adhered to the cell plate and more resistant to trypsin. Under normal conditions with only the cells and MEM, the cells detach fairly easily from the plate after adding trypsin and mixing with a pipette. However, whenever the plates contained DMSO, it became

much more difficult to get the cells to detach, to the point where a substantial amount of the cells that were alive were still attached to the plate and thus did not contribute to the number of alive cells when counting with a hemocytometer. As a result, even though the relative potency would not be affected because the control plate contained DMSO, the absolute percentages of cell death would be positively skewed. To fix this issue, gridded plates should be used in future trials so that cells can be counted even if they are still attached to the plate.

However, even though the effect that DMSO had on the cell attachment to the plate caused potential issues in accurate data collection, it may also be an indicator of the therapeutic effects of DMSO on cancer cells. The particular cancer cell line we used has an anchorage-independent trait [12], but the fact that the cells become more strongly attached to the plate means that they are most likely losing that characteristic. This would suggest that DMSO can somehow influence cancer cells to behave more like normal healthy cells, and further research should be done to investigate this more thoroughly.

Although the results of this study did not provide any decisive evidence on whether or not these compounds have anti-cancer properties, there is some indication that these compounds as well as DMSO in itself has some potential applications for cancer treatment. Furthermore insight was gained what should be further studied and how to improve the methodology in order to facilitate future research. As this was the first time these compounds were tested on cells, it is only the start of evaluating their cancer-fighting properties and medicinal applications. Thus, the next step of research will be to test the compounds on normal cells as well to verify that the effect the compounds have had on the cancer cells are anti-cancer properties and not merely cytotoxic. This is important to determine because if it is the latter, they will not have as much

value and potential in the pharmaceutical realm for cancer treatment. After that, the mechanism by which they carry out their anti-cancer activity should be studied.



1a. 1b. 1c.

Figure 1. a) The structure of 2-R1, 3-R1, and 4-R1 (boronic acid combined with rhodanine). b) The structure of 2-R2 and 3-R2 (boronic acid combined with 2,4-thiazolidinedione). c) The structure of 4-R2 (boronic acid combined with pseudothiohydantoin).

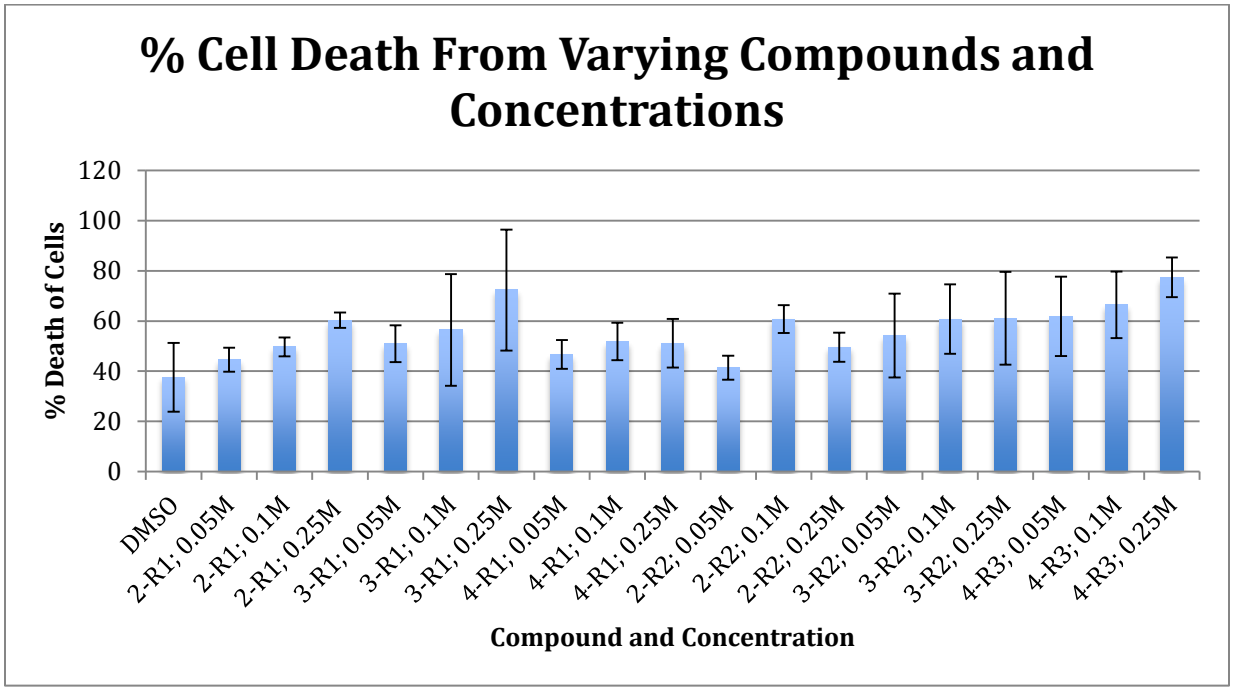


Figure 2. A chart showing the average percentage of cell death from different compounds at different concentrations. Error bars are shown of one positive and negative standard deviation. Compound 4-R3 at 0.25M had the highest value. Compound 3-R1 at 0.25M followed close behind, but did not have a significant p-value from the t-test performed because of the high standard deviation.

Compound & Concentration	Average % Cell Death	Std Deviation	p-value	Significant? (<0.05)
DMSO	37.57	13.7257301	N/A	N/A
2-R1; 0.05M	44.6	4.76235236	0.100246547	no
2-R1; 0.1M	49.63333333	3.775358703	0.015212137	**yes**
2-R1; 0.25M	60.33333333	3.113411848	0.000263529	**yes**
3-R1; 0.05M	50.96666667	7.294746968	0.031720453	**yes**
3-R1; 0.1M	56.43333333	22.22978482	0.137841199	no
3-R1; 0.25M	72.33333333	24.14711853	0.059509215	no
4-R1; 0.05M	46.66666667	5.773502692	0.065673074	no
4-R1; 0.1M	51.83333333	7.421814693	0.026911422	**yes**
4-R1; 0.25M	51.13333333	9.650043178	0.057800449	no
2-R2; 0.05M	41.4	4.757099957	0.236312287	no
2-R2; 0.1M	60.76666667	5.589573627	0.000977389	**yes**
2-R2; 0.25M	49.56666667	5.831237719	0.028767417	**yes**
3-R2; 0.05M	54.23333333	16.75599395	0.109220749	no
3-R2; 0.1M	60.76666667	13.86590543	0.038456074	**yes**
3-R2; 0.25M	61.06666667	18.47953823	0.072055038	no
4-R3; 0.05M	61.83333333	15.80263691	0.048251885	**yes**
4-R3; 0.1M	66.5	13.28645927	0.019253185	**yes**
4-R3; 0.25M	77.43333333	7.941242556	0.000344942	**yes**

Table 1. Numerical values of data collected and results of t-tests. As shown, all compounds showed a significant increase in cell death based on the t-tests, but were inconsistent in terms of increasing concentration and staying significant. Compound 4-R3 was the only one to show a significance for all three concentrations.

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