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Novel Heterocyclic Arylidene Derivatives as Anticancer Agents Against U87 Human Glioblastoma

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Novel heterocyclic arylidene derivatives as anticancer agents against U87 human glioblastoma cells

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ABSTRACT

The primary objectives of this interdisciplinary study were the synthesis of novel heterocyclic arylidenes and the investigation of their anticancer activity against U87 glioblastoma cell viability. Recently, novel hybrid derivatives have been considered as potential candidates for treating glioblastoma, demonstrating a synergistic anticancer effect in previous studies. 12 heterocyclic arylidenes with various functional groups, including halogens and boronic acid, were produced via a Knoevenagel condensation. These compounds and their starting reagents were then administered to U87 glioblastoma cancer cells at graded concentrations within a 12-well cell viability assay to determine each compound's lethal concentration 50 (LC_{50}). The LC_{50} of each compound was then compared to determine the effects of substituent type and position on anticancer activity. Although these arylidenes displayed some anticancer effects, their high LC_{50} suggest they have no significant effect on U87 glioblastoma cell viability and proliferation.

INTRODUCTION

Glioblastoma multiforme (abbreviated to GBM) is a malignant astrocytoma that derives from astrocytes, a type of glial cell that normally nourishes the nervous tissue and maintains the blood-brain barrier. GBM exhibits a high degree of invasiveness and an unprecedented proliferation rate, capable of infiltrating cranial nerve pathways and metastasizing throughout the brain's cerebrospinal fluid. Patients suffering from GBM present headaches, impaired balance and muscle coordination, temporary loss of consciousness, seizures, and visual problems. However, due to the lack of specific clinical symptoms, GBM can be easily misdiagnosed as an infection, inflammatory reaction, or an immunological disease; identifying GBM is further complicated if the patient already has a medical history of neurological disorders (Lee, 2016). Coupled with the limited regenerative capabilities of the central nervous system, patients that do undergo treatment do not experience a complete recovery, with most surviving only a median of one year and only 5% of patients surviving up to five (Haar, 2012; Eramo *et al*, 2006). Although treatment of glioblastoma has resulted in little success, the current standard procedure for treating glioblastoma involves surgical removal of the tumor followed by radiotherapy and chemotherapy with the anticancer, alkylating agent temozolomide (Davis, 2016; Fan *et al*, 2013).

Temozolomide acts as an alkylating agent that adds a methyl substituent group onto a guanine nucleotide, converting it into 6-O-methylguanine. Guanine is one of the four constituent bases in our DNA, encoding genetic information that is essential to cell survival and proliferation. The addition of a foreign chemical group onto guanine alters its structural and chemical characteristics, rendering the nucleotide unrecognizable to enzymes essential in DNA repair and replication ((Fan *et al*, 2013; Davis, 2016). Furthermore, increased concentration of O-6-methylguanine and the decreased concentration of normal guanine also lowers the encounter rate between these crucial enzymes and guanine. As a result, breakages in the DNA sequence accumulate, leading to missing segments of genetic information; these genetic inconsistencies encode dysfunctional or nonfunctional proteins that impair cancer cell survival (Fan *et al*, 2013).

One particular reason though that the standard treatment for glioblastoma is rather ineffective is due to glioblastoma's resistance to temozolomide (Haar, 2012; Davis, 2016). More specifically, the overexpression of a gene sequence known as MGMT is shown to confer a higher degree of resistance against this anticancer agent, rendering the only drug for treating glioblastoma less effective (Carbrini *et al*, 2015). The MGMT gene encodes for O^6-methylguanine DNA methyltransferase (abbreviated to MGMT), an enzyme that counteracts temozolomide's cytotoxic effects by initiating mismatch DNA repair (The Cancer Genome Atlas Research Network, 2008; Fan *et al*, 2013). MGMT facilitates DNA repair by converting 6-O-methylguanine, the alkylated nucleotide, into normal guanine. The activity essentially reverses temozolomide's DNA damaging effects, preventing cancer cell death and conferring glioblastoma with a resistance against it (Haar, 2012; Davis, 2016).

Recently, novel hybrid derivatives have been considered as potential candidates for treating glioblastoma, demonstrating an obstructive effect on the biochemical pathways necessary for cancer cell survival (Libby *et al*, 2018, de Moura Sperotto *et al*, 2019). The main premise of these studies is to chemically combine two or more compounds with known anticancer effects and observe their activity on harvested glioblastoma cells *in vitro*. The primary goal of these experiments is to provide an alternative set of anticancer agents that can

effectively counteract the aggressive proliferative nature of glioblastoma and other cancers (Cabrini *et al*, 2015). Therefore, the premise of my study investigates a primary question: are heterocyclic arylidenes, a type of novel hybrid derivative, effective anticancer agents against the proliferation of human glioblastoma brain cancer cells?

As part of a larger collaborative research effort, I synthesized 12 heterocyclic arylidenes from rhodanine-3-acetic acid and a set of benzaldehydes with various substituent groups in an enzyme-catalyzed controlled reflux. I then investigated the effects of these hybrid derivatives and their reagents in 12-well cell viability assays to determine each compound's lethal concentration 50 (LC_{50}) at which 50% of the cancer cell population is eliminated.

METHODOLOGY: CHEMISTRY

12 heterocyclic arylidenes from rhodanine-3-acetic acid and a set of benzaldehydes via a 3-hour, pancreatin-catalyzed Knoevenagel condensation (Fig. 1). This condensation reaction essentially marries rhodanine-3-acetic acid and a benzaldehyde with a carbon-carbon double bond; this double bond replaces the original aldehyde group on the benzaldehyde as shown on the figure.

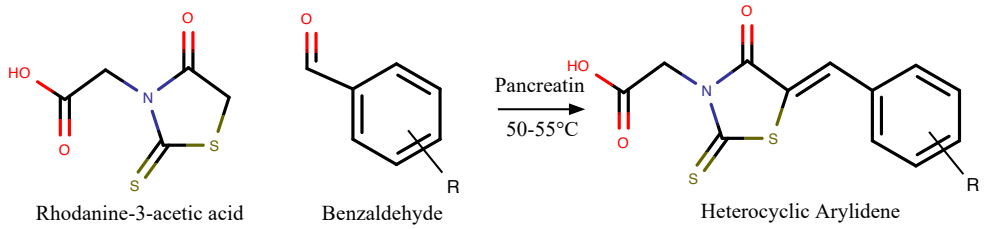


Fig. 1 Chemical equation of the Knoevenagel condensation of heterocyclic arylidenes

Before producing all 12 novel heterocyclic arylidenes, a series of experiments were conducted to determine the efficacy of different catalysts in the syntheses of these compounds. 8 enzymes were tested in a series of controlled refluxes, in which .176 g of 4-chlorobenzaldehyde and .239 g of rhodanine-3-acetic acid were refluxed in a water-dimethyl sulfoxide solution for 3 hours at 50-55°C. .2 g of the selected enzyme was also added to each controlled reflux reaction to determine their efficacy as a biocatalyst. Out of all 8 catalysts investigated, pancreatin had the highest and purest yield, producing 86% with no residual starting reagent.

After selecting the optimal catalyst, .239 g of rhodanine-3-acetic acid, .2 g of pancreatin, and .00125 mol of a selected benzaldehyde were refluxed using the same experimental set-up. These benzaldehydes varied depending on the type of substituent group and its placement on the compound's benzene ring. Benzaldehydes used during this study contained fluorine, chlorine, bromine, or boronic acids, with each substituent group placed on the 2nd, 3rd, or 4th position on the benzene ring. In total, 12 benzaldehydes with various substituent groups and positions were used. Although the benzaldehydes of interest were consistently .00125 moles, the amount of each compound varied depending on its molecular weight and physical state.

After adding each reagent into a 25-ml round-bottom flask and attaching a water-cooled condenser to the flask, the apparatus was placed on a magnetic hot plate set to 50-55°C and refluxed for 3 hours. Once the reflux reached completion, 30 mL of saturated NaCl solution were poured into the mixture to precipitate more product out of solution. The reaction mixture was then vacuum-filtered with 500 mL boiling deionized water to purify the solid product. After a 30 to 60-minute drying period, the product was transferred into a labelled vial and placed in a high vacuum system overnight to ensure complete desiccation.

After thoroughly drying to remove any residual water, final product was weighed to calculate its percent yield. A small sample of that product and their starting reagents were taken to conduct infrared (IR) and nuclear magnetic resonance (NMR) analyses. These IR and NMR readings of the final product to those of its reagents to determine the presence and purity of the product of interest.

METHODOLOGY: BIOLOGY

All 12 heterocyclic arylidenes and a majority of their starting reagents were tested on U87 human glioblastoma cancer cells using a 12-well cell viability assay, in which each assay tested one hybrid compound. This procedure covered three days and was conducted in triplicates to measure, compare, and verify data. Along with testing these heterocyclic arylidenes, their reagents were also tested. Over the course of several months, I had made 54 plates.

On the first day, glioblastoma cells were stained to determine the volume of cell culture needed to grow 10,000 cells in each well. 90 μL trypan blue dye were added into a 1.5 μL microcentrifuge tube, setting it aside for a later step. 2 mL of trypsin were then added to a glioblastoma cell plate in order to detach the cancer cells adhering to the plate's surface. Afterwards, 10 μL of the trypsinized cell suspension were pipetted into the 90 μL trypan blue dye and mixed thoroughly. The purpose of staining the glioblastoma cancer cells with trypan blue is to record the number of live cells after transferring 10 μL of the stained cell culture onto a hemocytometer grid. The number of cancer cells present was then calculated to determine the volume needed to culture 10,000 cells in each well on the 12-well plate. After adding the calculated volume with 13 mL media, 1,000 μL of the cell suspension mixture were pipetted into each well and incubated overnight at 37°C with 5% CO_2 to allow the glioblastoma cells to adhere to the wells.

On the second day, I made my dilutions for each well in my 12-well cell viability assay. 20 mg of my compound of interest was weighted and transferred it into a 1.5-mL microcentrifuge tube. Afterwards, 1 mL of dimethyl sulfoxide was added in order to dissolve the compound and make a primary stock solution. After organizing a set of 11 2-mL microcentrifuge tubes on a grid, 1,000 μL of media were pipetted into all 11 microcentrifuge tubes; 800 μL of media were added to the first tube. 200 μL of my primary stock solution were pipetted and diluted into 1,800 μL of media solution to produce a 2 mg/mL concentration. The mixture was then mixed and 1,000 μL of the first dilution was pipetted and added it to the next microcentrifuge tube in order to make a dilution that was half the concentration of my first. All subsequent concentrations on the plate were half of the previous concentration until all 11 microcentrifuge tubes are filled. On the 11^{th} tube, an excess 1,000 μL of the dilution was discarded so that each 2-mL microcentrifuge tube contained 1,000 μL of diluted compound. Along with these dilutions, there was a control that only contained 1,000 μL media. 1,000 μL of each dilution was administered to their corresponding wells on the 12-well plate and added 1,000 μL of media to the reserved control well. Once all the dilutions have been added to the 12-well plate, the glioblastoma cancer cells were again incubated in an enclosed chamber at 37°C with 5% CO_2 for another 24 hours.

On the third day, the media was removed and 1 mL methanol was added into each emptied well. These plates were allowed to rest for 5 minutes to the fix the cells. After emptying the wells of methanol, the glioblastoma cancer cells were treated with crystal violet stain for an additional 5 minutes to effectively stain the cells for easier visualization. The purpose of staining the glioblastoma cancer cells with crystal violet was to facilitate the cell viability count, allowing easy identification of the remaining cancer cells and to determine the percent of the cell population that died after administering the compound. After the 5-minute

setting period, the crystal violet stain was removed, and each well was washed with DI water to remove any residual stain. These plates were allowed to dry over the next couple of days.

To determine the LC_{50} of each compound, I counted my cell assays with a digital microscope. 9 points were designated on cell well to standardize counting among all wells; each point had a 25 mm^2 field of view. Within these points, the number of visible cells were counted, and the number of cell counts for a specific dilution from all three cell viability plates were averaged. From these values, the standard deviation and standard error mean was calculated. T-tests (with α of 0.05) were also conducted on each value to determine its statistical significance. These values were then applied to a regression line to determine the LC_{50}.

RESULTS: CHEMISTRY

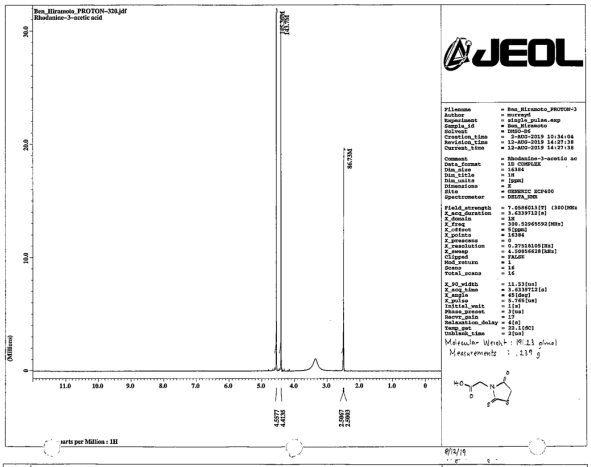
In terms of the chemical aspect of this study, pancreatin can be used as a biocatalyst in the syntheses of novel heterocyclic arylidenes containing rhodanine-3-acetic acid and select set of benzaldehydes. However, NMR analyses revealed that residual aldehydes still remained in 50% of the heterocyclic arylidenes, placing doubt on pancreatin as a promising catalyst.

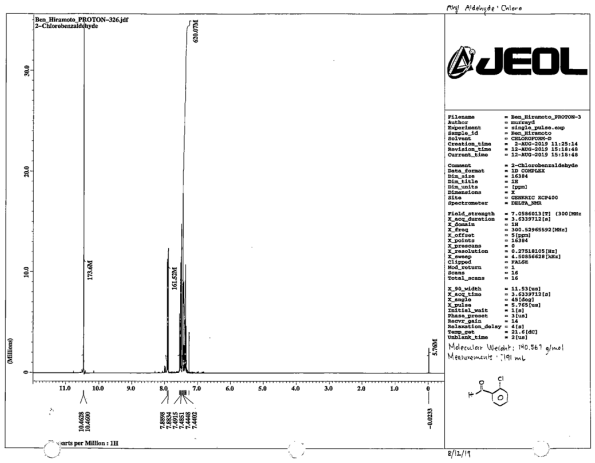
Name	Rhodanine derivative	Benzaldehyde	Percent Yield	Aldehyde in IR and NMR spectra
R3-2CBA	Rhodanine-3-acetic acid	2-chlorobenzaldehyde	86.6%	Present
R3-3CBA	Rhodanine-3-acetic acid	3-chlorobenzaldhyde	66.0%	Present
R3-4CBA	Rhodanine-3-acetic acid	4-chlorobenzaldehyde	85.1%	Absent
R3-2FBA	Rhodanine-3-acetic acid	2-fluorobenzaldehyde	70.9%	Absent
R3-3FBA	Rhodanine-3-acetic acid	3-fluorobenzaldehyde	55.9%	Present
R3-4FBA	Rhodanine-3-acetic acid	4-fluorobenzaldehyde	80.5%	Present
R3-2BBA	Rhodanine-3-acetic acid	2-bromobenzaldehyde	73.2%	Absent
R3-3BBA	Rhodanine-3-acetic acid	3-bromobenzaldehyde	96.4%	Present
R3-4BBA	Rhodanine-3-acetic acid	4-bromobenzaldehyde	85.5%	Present
R3-2FPBA	Rhodanine-3-acetic acid	2-formylphenylboronic acid	30.5%	Absent
R3-3FPBA	Rhodanine-3-acetic acid	3-formylphenylboronic acid	4.70%	Absent
R3-4FPBA	Rhodanine-3-acetic acid	4-formylphenylboronic acid	34.9%	Absent

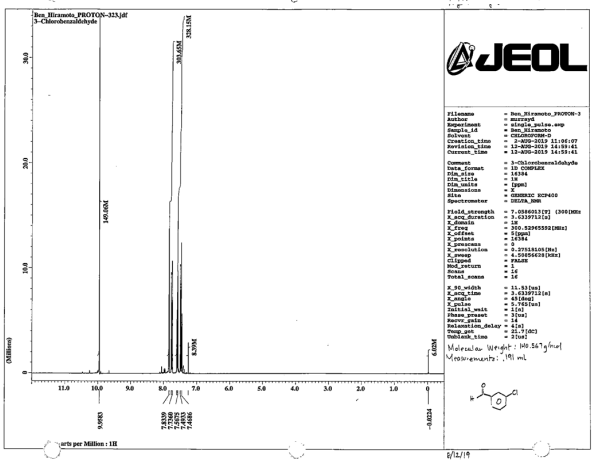
Tab. 1 List of hybrid derivatives along with starting reagents, percent, yield, and analysis of IR and NMR for presence of residual aldehydes

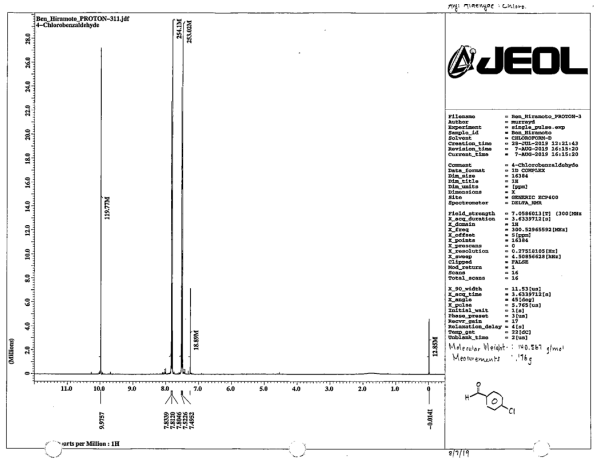
Percent yields varied depending on the type of substituent group on the compound's benzene ring as shown on this data table. Percent yields for brominated arylidenes had the highest yield ranging from 73.2% to 96.4%. Chlorinated arylidenes ranged from 66.0% to 86.6%, whereas fluorinated arylidenes ranged from 55.9% to 80.5%. Heterocyclic arylidenes containing a boronic acid substituent produced relatively poorer yields compared to their halogenated counterparts, ranging from 4.70% to 34.9%.

Percent yields also varied depending on the substituent group's position on the arylidene's benzene ring. Percent yields were higher for arylidenes containing a chlorine, fluorine, and boronic acid substituent group on the 2nd or 4th position compared to the 3rd position; brominated compounds were an exception to this trend with the 3rd position actually producing a higher yield compared to its 2nd and 4th position counterparts.

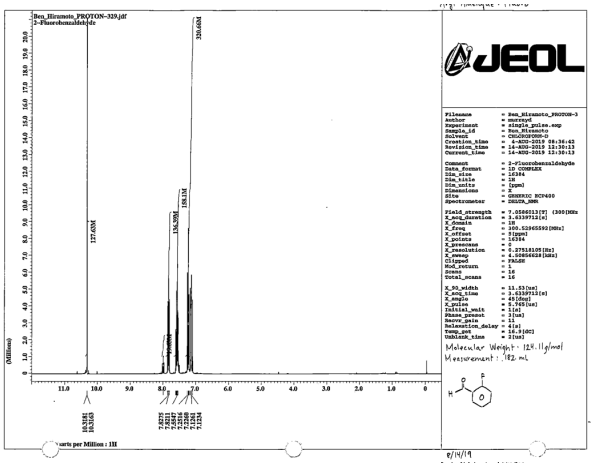








Aryl Aldehyde : Fluoro



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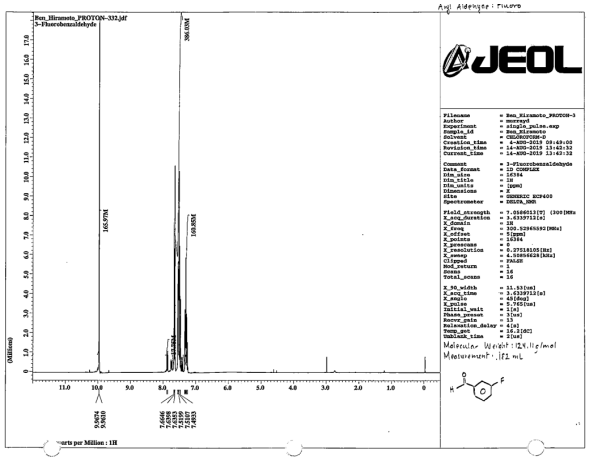

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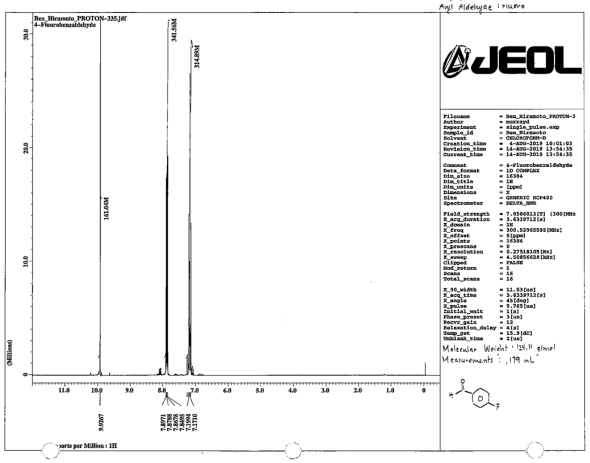
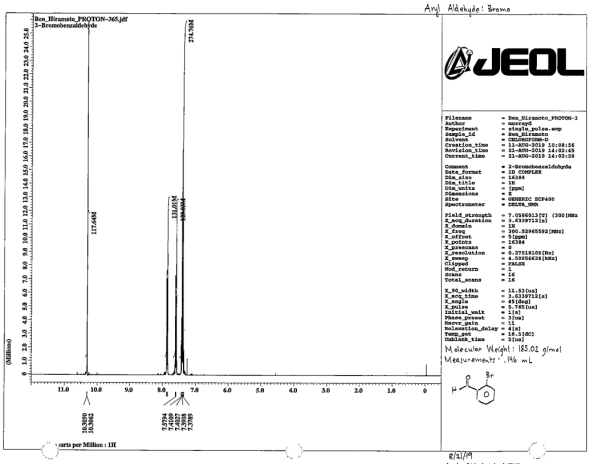
Aryl Aldehyde : Nitro



Aryl Aldehyde : Bromo



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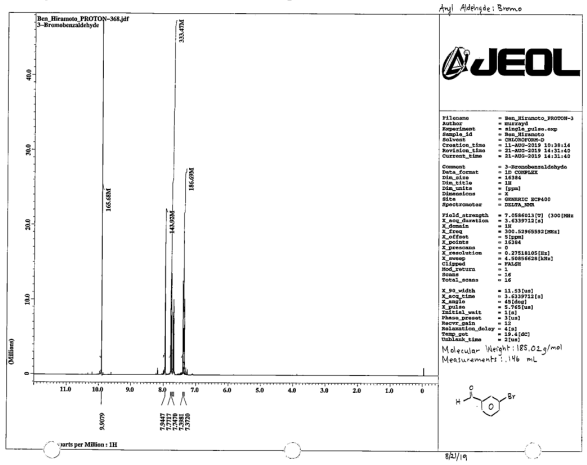
Aryl Aldehyde : Bromo



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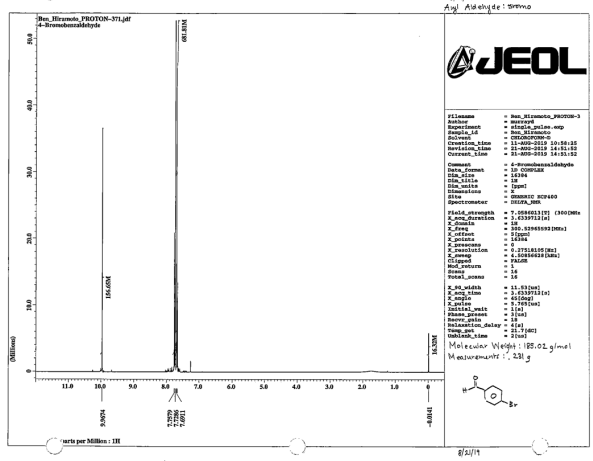
Aryl Aldehyde : Iodo



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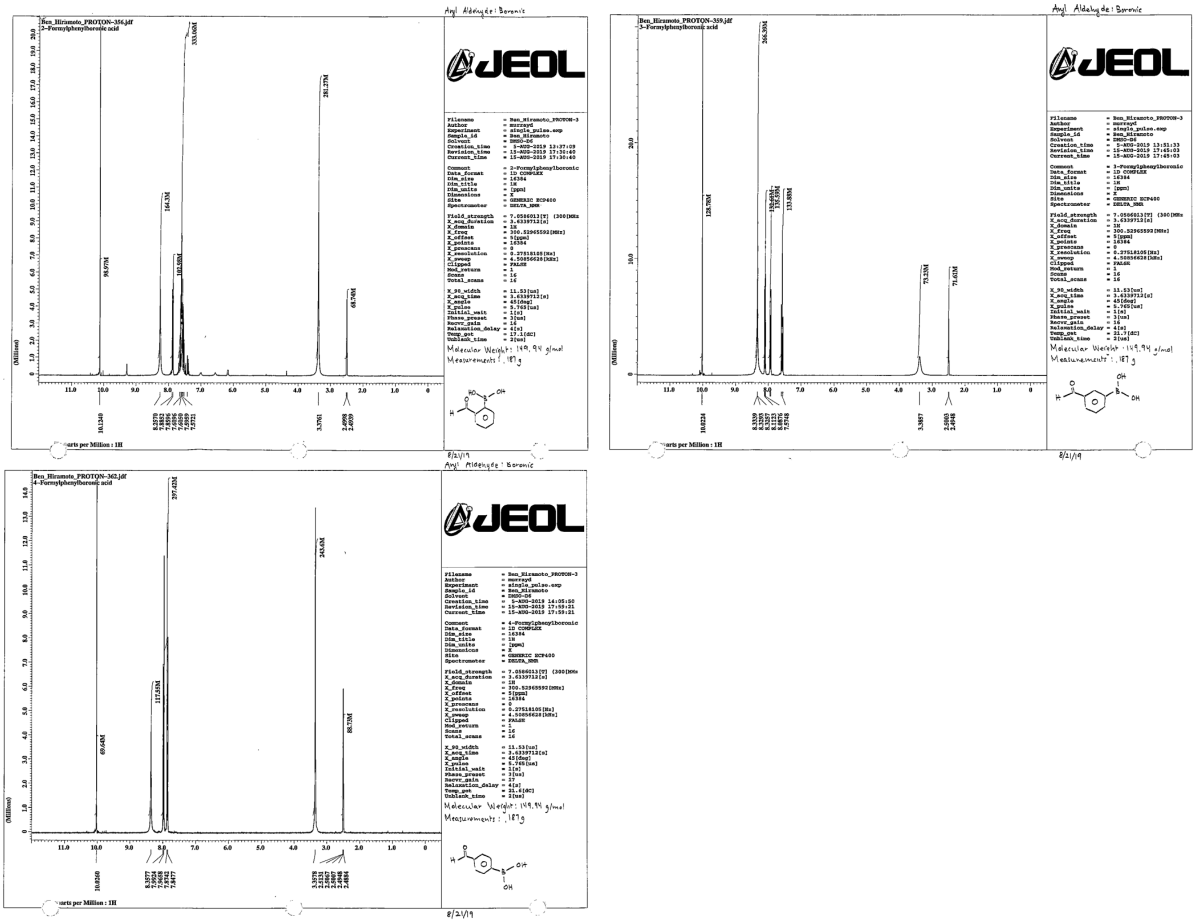
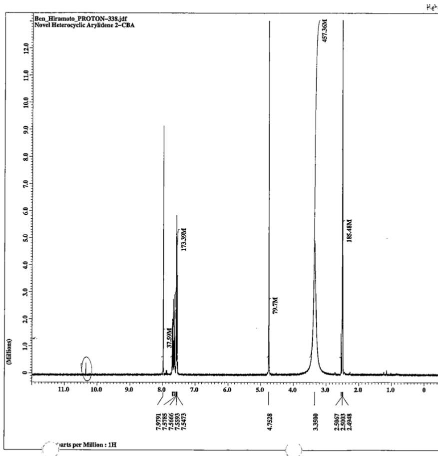


Fig. 2-14 NMR spectra of the starting reagents, with each spectrum titled at the top left corner.

Heterocyclic Arylidene Synthesis : Experiment 2



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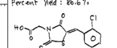
2-CBA and R-3-AA refluxed for 3 hrs at 50-55 °C.

Product Yield : .3266g

Percent Yield : 86.6%



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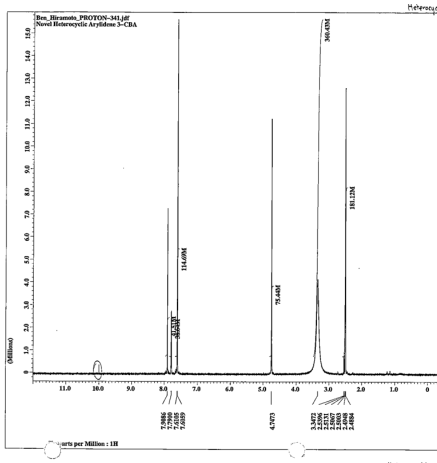
Heterocyclic Arylidene Synthesis : Experiment 2



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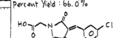
3-CBA and R-3-AA refluxed for 3 hrs at 50-55 °C.

Product Yield : .249 g

Percent Yield : 66.0%



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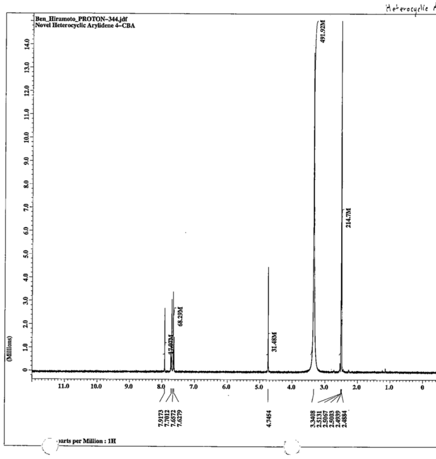
Heterocyclic Arylidene Purification : Experiment 1E



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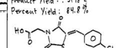
4-CBA and R-3-AA refluxed for 3 hrs at 50-55 °C. Vacuum-filtered only.

Product Yield : .3198 g

Percent Yield : 84.8%



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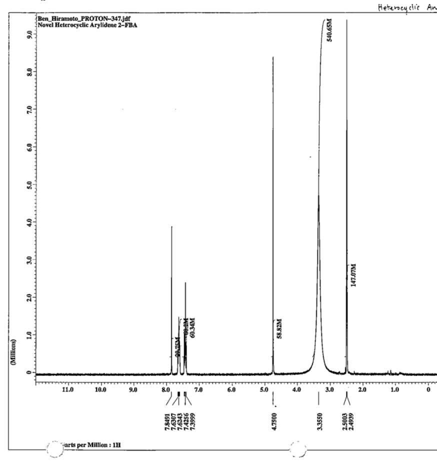
Heterocyclic Arylidene Synthesis : Experiment 3



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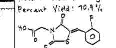
X_acq_time	= 3.6339712[s]
X_angle	= 45[deg]
X_pulse	= 5.765[us]
Initial_wait	= 1[s]
Phase_preset	= 3[us]
Recvr_gain	= 18
Relaxation_delay	= 4[s]
Temp_get	= 17.2[dC]
Unblank_time	= 2[us]

2-FBA and R-3-AA refluxed for 3 hrs at 50-55 °C.

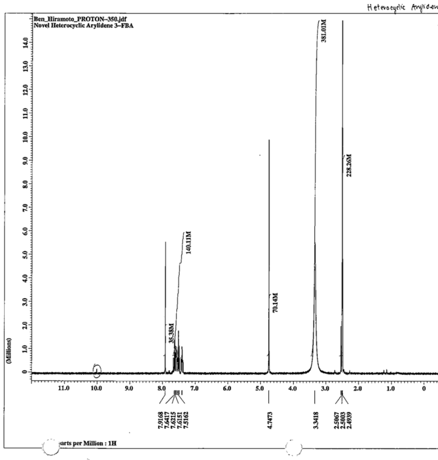
Product Yield : .2672g

Percent Yield : 70.9%



8/15/19

Heterocyclic Arylidene Synthesis : Experiment 3

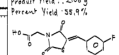


Filename	= Ben_Hiramoto_PROTON-3
Author	= murrayd
Experiment	= single_pulse.exp
Sample_id	= Ben_Hiramoto
Solvent	= DMSO-D6
Creation_time	= 5-AUG-2019 07:42:33
Revision_time	= 15-AUG-2019 11:36:05
Current_time	= 15-AUG-2019 11:36:05
Comment	= Novel Heterocyclic Ar
Data_format	= 1D COMPLEX
Dim_size	= 16384
Dim_title	= 1H
Dim_units	= [ppm]
Dimensions	= X
Site	= GENERIC ECP400
Spectrometer	= DELTA_NMR
Field_strength	= 7.0586013[T] (300[MHz
X_acq_duration	= 3.6339712[s]
X_domain	= 1H
X_freq	= 300.52965592[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 0
X_resolution	= 0.27518105[Hz]
X_sweep	= 4.50856628[kHz]
Clipped	= FALSE
Mod_return	= 1
Scans	= 16
Total_scans	= 16
X_90_width	= 11.53[us]
X_acq_time	= 3.6339712[s]
X_angle	= 45[deg]
X_pulse	= 5.765[us]
Initial_wait	= 1[s]
Phase_preset	= 3[us]
Recvr_gain	= 20
Relaxation_delay	= 4[s]
Temp_get	= 21.9[dC]
Unblank_time	= 2[us]

3-FBA and R-3-AA refluxed for 3 hrs at 50-55 °C.

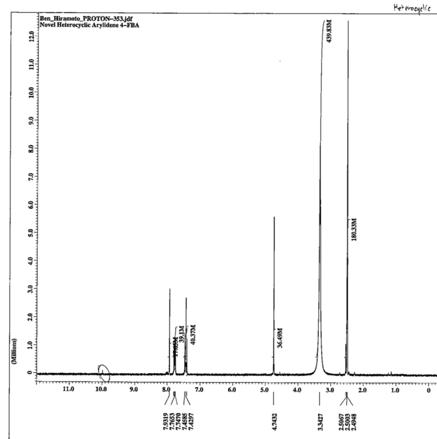
Product Yield : .2106 g

Percent Yield : 55.9%



8/15/19

Heterocyclic Arylidene Synthesis : Experiment 3.



Filename	= Ben_Hiramoto_PROTON-3
Author	= murrayd
Experiment	= single_pulse.exp
Sample_id	= Ben_Hiramoto
Solvent	= DMSO-D6
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Revision_time	= 15-AUG-2019 12:00:48
Current_time	= 15-AUG-2019 12:00:48
Comment	= Novel Heterocyclic Ar
Data_format	= 1D COMPLEX
Dim_size	= 16384
Dim_title	= 1H
Dim_units	= [ppm]
Dimensions	= X
Site	= GENERIC ECP400
Spectrometer	= DELTA_NMR
Field_strength	= 7.0586013[T] (300[MHz
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X_domain	= 1H
X_freq	= 300.52965592[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 0
X_resolution	= 0.27518105[Hz]
X_sweep	= 4.50856628[kHz]
Clipped	= FALSE
Mod_return	= 1
Scans	= 16
Total_scans	= 16
X_90_width	= 11.53[us]
X_acq_time	= 3.6339712[s]
X_angle	= 45[deg]
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Initial_wait	= 1[s]
Phase_preset	= 3[us]
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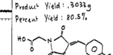
Relaxation_delay	= 4[s]
Temp_get	= 21.9[dC]
Unblank_time	= 2[us]

4-FBA and R-3-AA refluxed for 3 hrs at 50-55°C.

Product Yield : .3033g

Percent Yield : 80.5%



8/15/19

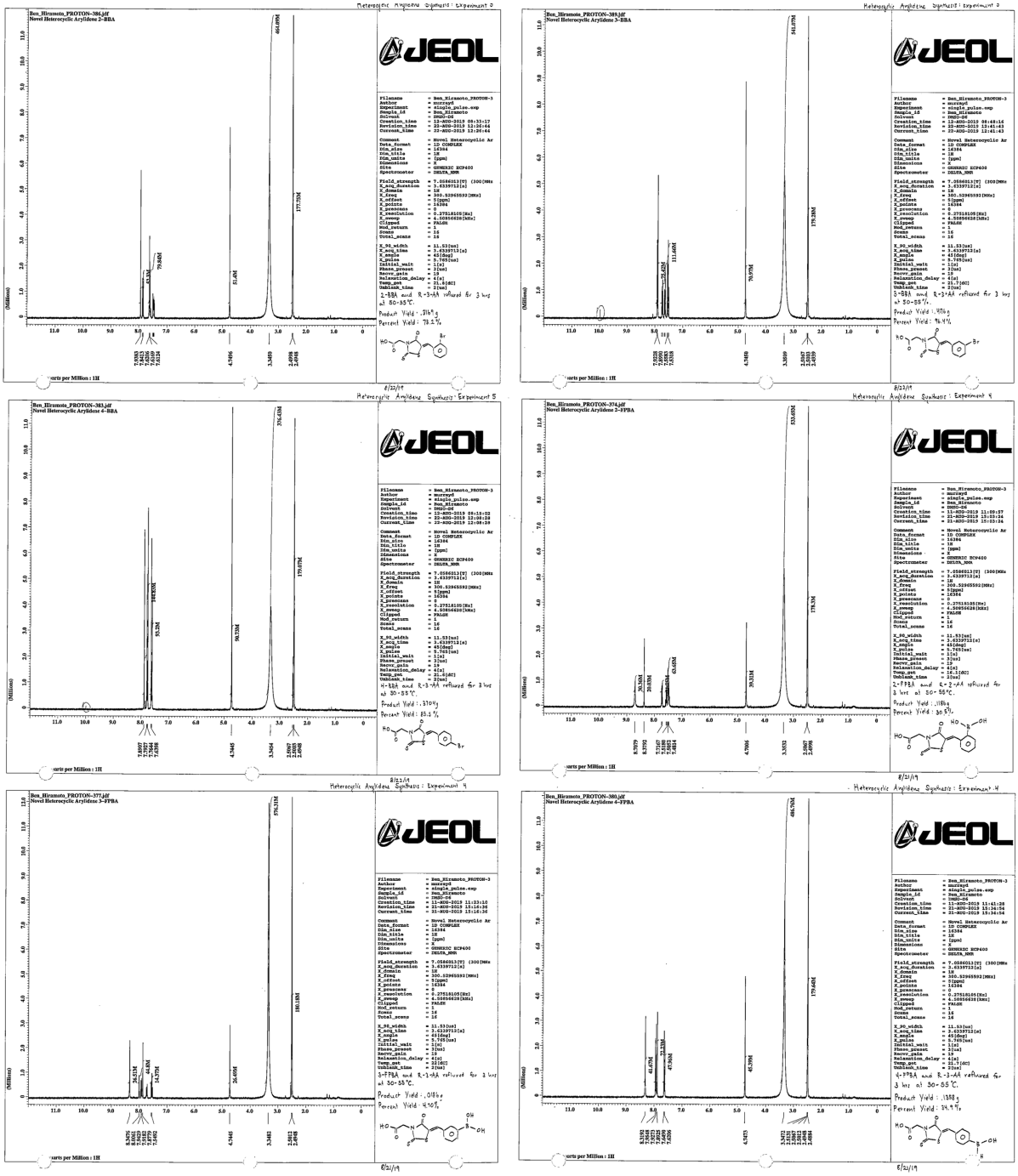


Fig. 15-26 NMR spectra of the starting reagents, with each spectrum titled at the top left corner.

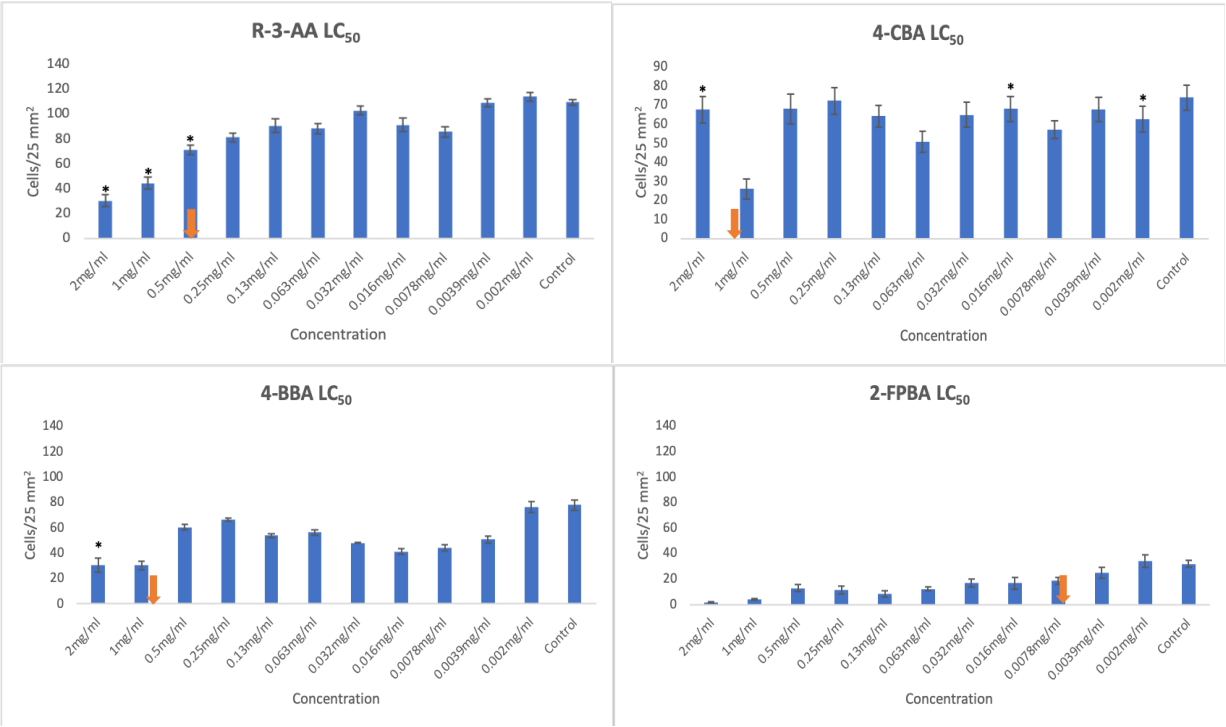
RESULTS: BIOLOGY

The purpose of investigating these novel compounds and administering different dilutions in each well is to determine the compound's LC_{50}. As mentioned before, the LC_{50} is the lethal dose at which 50% of the sample population dies. A smaller dose that causes 50% lethality is ideal because it requires less of the compound in order to elicit its lethal effects on glioblastoma cancer cells. In other words, the smaller the dose, the more potent the drug.

Although there was some decrease in U87 glioblastoma cell viability with the administration of these heterocyclic arylidenes, their generally high LC_{50} and their t-tests indicate that this decrease is not statistically significant. Despite this, there was some variance in anticancer activity among heterocyclic arylidenes containing different substituent groups. Fluorinated and brominated arylidenes exhibited the lowest LC_{50} compared to other compounds, ranging between 0.063 mg/ml to 0.25 mg/ml. Chlorinated compounds' LC_{50} revolved around 0.25 mg/ml. Arylidenes containing a boronic acid substituent group exhibited the highest LC_{50} ranging from 0.25 mg/ml to 1 mg/ml.

Comparing the LC_{50} of heterocyclic arylidenes with the same substituent functional group but different substituent position, substituent placement had no significant effect on the novel compound's anticancer activity. Although there was a differential in LC_{50} values among compounds with different placement, these differences were minimal.

When comparing these heterocyclic arylidenes to their starting materials, some starting reagents, especially benzaldehydes containing a boronic acid substituent, actually had a lower LC_{50} compared to that of their respective heterocyclic arylidene. This is shown in the bar graphs between these boronated benzaldehydes and their respective heterocyclic counterparts. This comparison between the starting reagents and hybrid drugs' LC_{50} suggests that the addition of a rhodanine-3-acetic acid counteracts or antagonizes the benzaldehyde's anticancer activity.



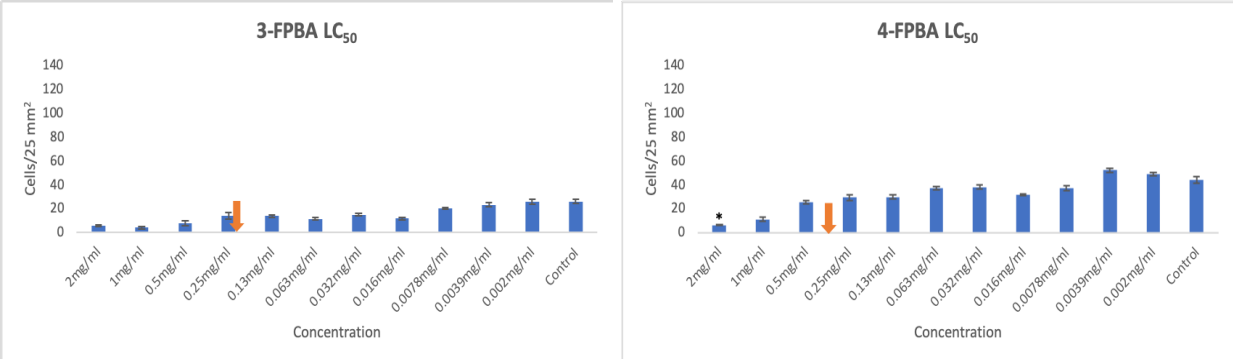
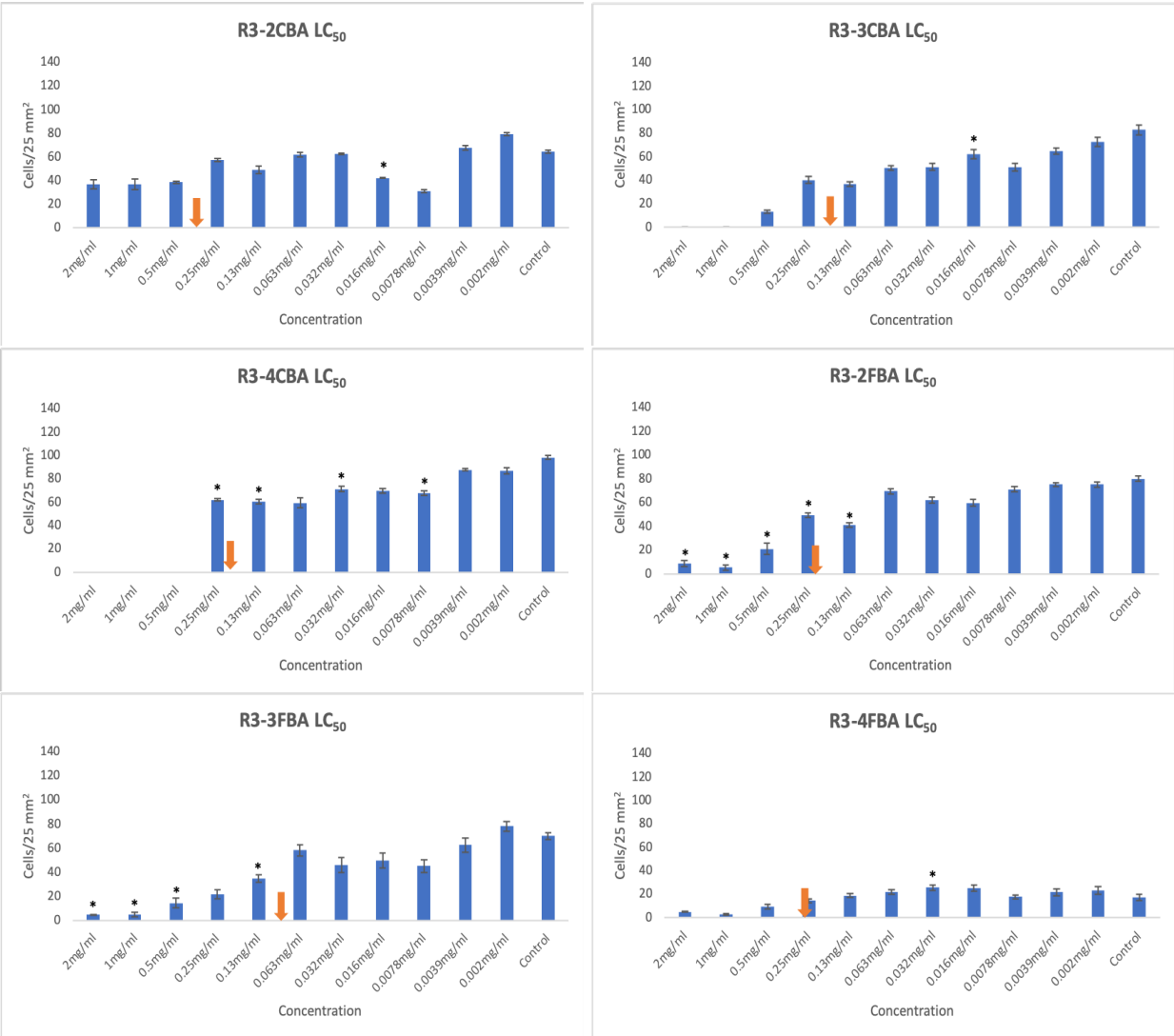


Fig. 27-32 Cell viability count per 25 mm² per well at progressively lower concentrations of various starting reagents. Some starting reagents were not tested because they were insoluble in DMSO. Arrow indicates LC_{50}, and asterisks indicate statistical significance. $p \leq 0.05$



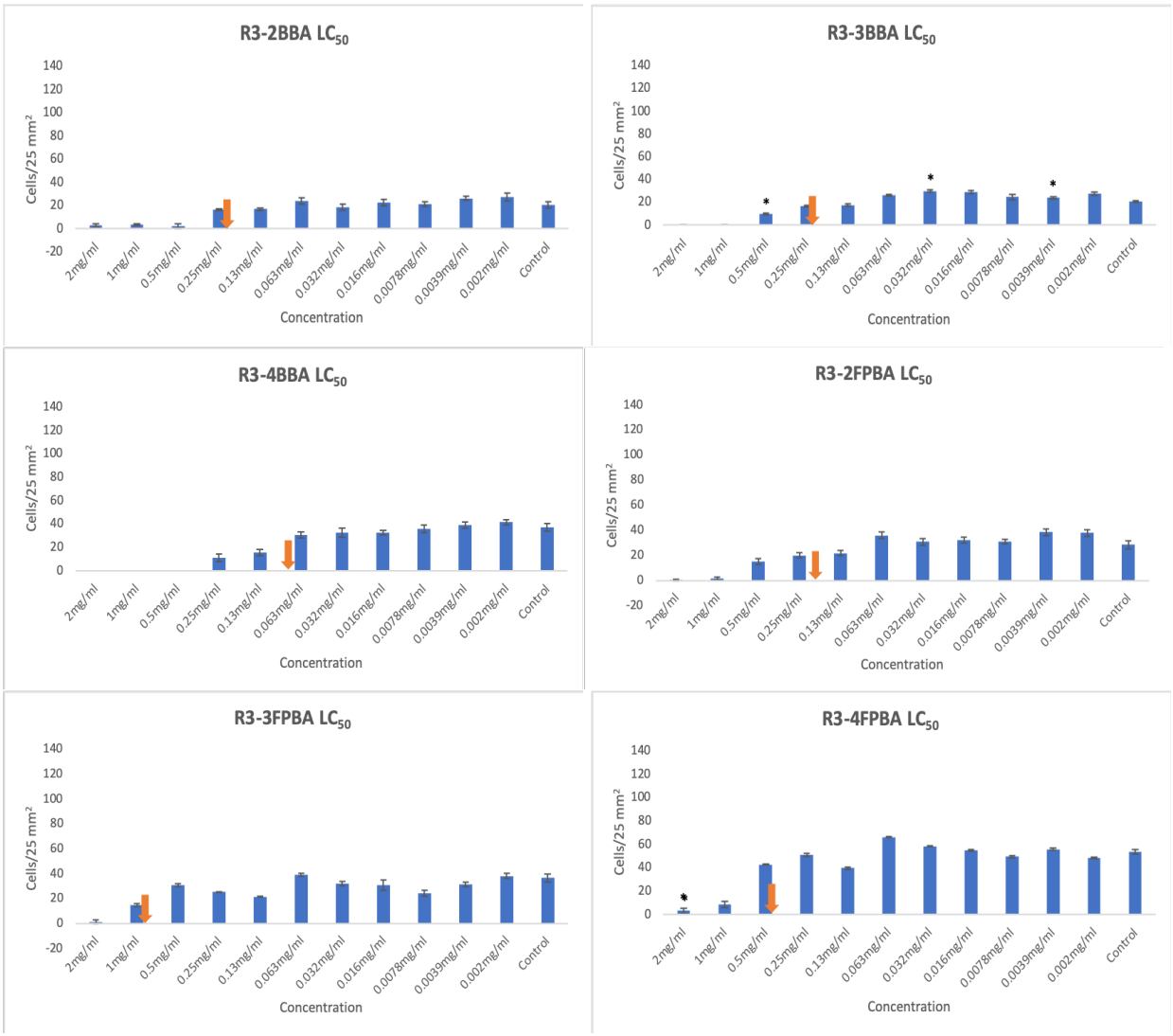


Fig. 33-44 Cell viability count per 25 mm² per well at progressively lower concentrations of all 12 heterocyclic arylidenes. Higher concentrations for some products were excluded because solid precipitates obscured visualization of cancer cells. Arrow indicates LC_{50}, and asterisks indicate statistical significance. $p \leq 0.05$

DISCUSSION

Although this line of research has not necessarily yielded any promising results, there have been significant developments in enhancing our knowledge of novel hybrid derivatives and improving our experimental protocol. Unlike previous undergraduate and graduate studies conducted years before, this research is the first to study the effects of brominated heterocyclic arylidenes as well as compounds containing rhodanine-3-acetic acid in Dr. Smith's lab.

Along with researching the biological activity of this new class of heterocyclic arylidenes, this study has also explored enzymes as potential biocatalysts in condensation reactions. Previous undergraduate and graduate researchers have relied on calcium oxide as a catalyst in their organic syntheses. However, this inorganic catalyst can be difficult to remove during purification and has a deleterious effect on cancer cell viability; this inadvertently skews cell counts, resulting in inaccurate data. This method employed an alternative biocatalyst that does not have any known adverse effects on cell survival and be easily denatured or deactivated with high salt solutions and extremely high temperatures.

In terms of improving experimental protocol, this study is also one of the first this year to use a digital microscope to count fixed glioblastoma cells. Use of this digital microscope has enhanced the accuracy of my results as compared to the original method of individually counting cells with a phase contrast light microscope. Unlike the new digital method, the other method was prone to inaccurate counts, leading to enlarged errors bars on the graphs. Additionally, my study is one of the first to make a cross comparative analysis between the starting reagents and their corresponding heterocyclic arylidenes to observe any differences in biological behavior and anticancer activity.

Despite the developments that were made in my research, there are still some weaknesses that need to be addressed. Although the biocatalyst, pancreatin, shows some promise as an alternative catalyst, NMR analyses reveal that 50% of the final product are impure. Although the presence of residual aldehyde peaks in the NMR are very small, this still places doubt on pancreatin's reliability as a biocatalyst.

In terms of the compounds investigated during this study, some starting reagents, more specifically 2-chlorobenzaldehyde, 3- chlorobenzaldehyde, 4- fluorobenzaldehyde, 2-bromobenzaldehyde, and 3- bromobenzaldehyde did not completely dissolve in DMSO while making primary stock solutions. As a result, some compounds were unable to administer them in a 12-well cell plate, and a comparison between these starting materials and their corresponding arylidene products. Additionally, 2-fluorobenzaldehyde and 3-fluorobenzaldehyde were not tested because there was not enough in stock to conduct an assay. Some heterocyclic arylidenes, namely R3-3CBA, R3-4CBA, R3-3BBA, R3-4BBA, precipitated out of solution when added with media at a high concentration, resulting in solids obscuring any possible visualization of that particular well.

Although improvements to the experimental protocol have been made, there are still some issues. In terms of the counting method, it is difficult to distinguish cells. Although the resolution is significantly enhanced, neurospheres, large clumps of cancer cells, make it difficult to accurately count each cell individually. As a result, the number of cells were approximated within each neurosphere, counting only cells that I can visibly distinguish. Another possible source of error was inappropriate technique or use of equipment. For example, washing away any residual crystal violet stain too vigorously or mishandling of micropipettes during plate set-up, may inadvertently reduce the number of cells that should be there. However, a major

weakness in this study is the fungal contamination that had plagued the lab's incubator. Unfortunately, due to improper handling or neglect, fungal spores have infiltrated many of the cell culture plates, completely decimating entire cell populations and compromising many cell assay plates. Because of their invasive and deleterious nature, these fungal spores pose an immense threat to the integrity of this study since the entire premise of this research is to assess cancer cell viability when exposed to graded concentration of a potential anticancer drug.

For future studies, these class of heterocyclic arylidenes and their reagents could be tested on other cancer types and lines to observe their biological activity in response to the drug. These studies would involve revising the synthesis or purification method of the experimental protocol to completely remove any residual aldehydes in the final product. However, a more promising inquiry that is investigating another class of heterocyclic arylidenes synthesized by Jemma McLeish. McLeish previously synthesized a set of chlorinated heterocyclic arylidenes that were structurally similar my own; however, instead of using rhodanine-3-acetic acid, she synthesized these compounds with simple rhodanine, which lacks an acetic acid group. In this comparative study, a set of chlorinated heterocyclic arylidenes will be recreated and tested on U87 glioblastoma cells to observe any similarities or differences that the absence of an acetyl group has in the arylidene's anticancer activity.

CONCLUSION

In conclusion, a class of heterocyclic arylidenes were synthesized from rhodanine-3-acetic acid and a select set of benzaldehydes via a 3-hour, pancreatin-catalyzed controlled reflux reaction with limited success, as 50% of these products still contain residual benzaldehydes. In terms of their biological activity, their high LC_{50} indicate that this class of heterocyclic arylidenes are not strong candidates as anticancer agents against GBM. Furthermore, the addition of a rhodanine-3-acetic acid may counteract the anticancer effects of its benzaldehyde partner.

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