Drisana Shanthan

Research Plan

A. Rationale

Throughout the study of organic molecules, it has been found that these molecules play an essential role in the biological activity and systems of many organisms. In the past, it has been found that by using molecular genetics, which involves the use of organic molecules, a protein is able to be manipulated by “eliminating” its genes in order to either change it’s activity or function. Through this, we are able to manipulate proteins involved in tumor progression or cancer cell activity and therefore, reduce their ability to function. Regarding sulfones, they are defined as a type of compound within the sulfonyl group that possess a sulfur atom with two bonds with carbon, which later may become unsaturated due to the heating of specific alcoholic solutions or by dehydrohalogenation. In past studies, it has been found that certain unsaturated sulfones may act as an efficient anticancer agent due to its ability to select and kill tumor cells, while avoiding normal cells. In respect to modern day cancer treatments, advancements in research concerning sulfones would have a major impact on society due to its ability to provide solutions for current obstacles such as minimizing normal cell death, increasing anticancer radiation and protecting normal cells from radiation.

B. Hypothesis

As (E)-4-Fluoro Styryl-4-Chlorobenzyl Sulfone is administered on cancer cells in varying concentrations, the concentration of 0.25 mM should inhibit more than 50% of cell activity.

C. Research Methods

Procedure

1. Procedure for synthesizing (E)-Fluorostyryl-4-Chlorobenzyl Sulfone:
    a. Preparation of 4- Chlorobenzyl Thioacetic acid:
        i. Using the known weights of each chemical, calculate the size of the RB (round bottom flask) needed to conduct the experiment (result: 50-100 mL). Afterwards, wash with acetone to remove any impurities or excess chemicals.
        ii. After washing, place RB in the oven for up to 5 minutes in order to dry completely. Till then, collect the following chemicals: thioglycolic acid, sodium hydroxide and methanol.
        iii. Once the RB is dried, place a clean magnetic bead inside of the flask to help combine all of the components evenly.

Next, using the appropriate sized syringe, obtain 13 mL of methanol (range from 11-15 mL).

iv. Afterwards, using a small spatula and scale, obtain about 880 mg of sodium hydroxide (exact amount obtained: 896 mg). Then, add the sodium hydroxide to the methanol.

v. Next, add 1012 mg of thioglycolic acid using the syringe (if necessary, convert mg to mL- 0.764 mL). Slowly, over the course of 10 min, add the thioglycolic acid in small portions. In between breaks, make sure to secure the nitrogen gas balloon/source.

vi. Once thioglycolic acid has been added, remove the RB from the stand and place into an oil bath located on top of a hot plate. Appropriately, set up the condenser and place the nitrogen gas source on it's top opening. In order to evaporate the methanol, using the heat plate, raise the temperature of the solution to 70 °C.

vii. Once the appropriate temperature is acquired, maintain this temperature for 2 hours in order to perform reflux.

viii. After 2 hours turn off the hot plate and allow the solution to cool to room temperature. In the meantime, measure out 1780 mg of 4- chlorobenzyl chloride using a spatula (ranges from 1770- 1780 mg). Once the solution has reached room temperature, disassemble the condenser and add the 4- chlorobenzyl chloride. While adding the substance, make sure to leave the nitrogen source on.

ix. Reassemble condenser for refluxing for another 2 hours. Afterwards, let the solution cooled.

x. Now obtain 12 g of ice in a graduated cylinder and pour the solution on top of it. Then, using a pipette and graduated cylinder, measure and add 2.2 mL of hydrochloric acid (37%), slowly.

xi. Once the solution has split into water and compound, pour the water out into a separate container. Then add ethyl acetate to the compound in order to dissolve it.

xii. Next, following Basic TLC Procedure, create 3 dots and place one drop of starting material (ethyl acetate), 4-chlorobenzyl chloride + ethyl acetate on the second dot, and synthesized compound to the dots in corresponding order. Afterwards, in a graduated cylinder add 7 mL of hexane and 3 mL of ethyl acetate (combine evenly by squeezing and releasing the pipette). Add the new solution to TLC container, alongside the plate, and wait for 5-10 minutes, prior to observation.

xiii. Once the presence of impurities has been confirmed, using separate instruments, add sodium sulfate to a funnel (no exact amount). Add compound through the funnel into a beaker.

xiv. Then, following procedure, connect the compound solution to the heating bath and turn on for 10-15 minutes. Once it's finished, remove RB containing left overs from the device and add hexane. After combining, remove excess hexane and use heating bath to remove any remains.

xv. Afterwards, if the white solid still hasn't formed repeat previous step. Otherwise, store the compound in a safe location for the next procedure.

b. Preparation of 4- Chlorobenzyl Sulfonyl Acetic acid:

i. First, weigh the compound that was prepared in the previous procedure in order to acquire the "starting material" (result: 1230 mg or 1.23 g). Once finished, transfer the compound to an RB.

ii. Using this result, calculate the measurements for the other compounds (ex. 0.700 g of compound #1 requires 1.39 mL of hydrogen peroxide, therefore, 1.23 g of compound #1 requires 2.44 mL).

iii. Afterwards, wash all containers/glassware with acetone, including the nozzle used to attach the nitrogen source to the condenser or RB. Alongside this, make sure to prepare the appropriate sized RB using steps 1-3 from the previous procedure.

iv. Next, using a syringe, take 9 mL of acetic acid and place into RB containing the magnet and compound. Then, attach the nitrogen source to the opening of the RB and mix using clockwise motions.

v. Afterwards, take 3 mL of 30% hydrogen peroxide and place into the solution. Once completed, replace the opening with the nitrogen source and mix.

vi. Set up the condenser for reflux and connect the nitrogen source to the top opening. Then, place the RB into the oil bath located on top of the heat plate, the RB should be connected to the condenser with the appropriate parts. Once completed, raise the temperature of the oil bath to 120 °C (boiling point of acetic acid) and reflux for 1 hour.

vii. After the reflux procedure has been completed, remove the RB from the oil bath and allow it to cool to room temperature. Then, add 12 g of crushed ice and mix until all has melted.

viii. Once the white crystals have formed, set up a funnel lined with filter paper inside a beaker for filtration. First, pour water down the funnel to cleanse the filter paper. Then, begin pouring the compound in order to filter out the solids. While doing so, make sure to maintain a vacuum using the nitrogen source.

ix. Once the compound has been filtered out, allow it to dry before weighing (result: 704.17 mg).

c. Preparation/Analysis of (E)-4- Fluorostryl-4- Chlorobenzyl Sulfone:

i. Using the compound from procedure #2, obtain 260 mg and add it to an appropriate sized RB containing a magnet (exact amount obtained: 260.84 mg). Next, using a syringe add 2 mL of acetic acid (ranges from 1.6- 2 mL) to the compound and allow them to combine.

ii. While they are combining, add 157 mg of 4-fluorobenzaldehyde (exact amount: 157.55 mg) to 26 mL of piperidine using a 200-20 P sized micropipette (piperidine must be acquired using a micropipette).

iii. Once both solutions are combined, add 4-fluorobenzaldehyde + piperidine to the compound + acetic acid. If some compound is left behind, add some acetic acid to dissolve it and combine.

iv. Next, set up an oil bath and thermometer in order to conduct reflux. Raise the hot plate until the thermometer reads 120 °C (boiling point of acetic acid) and maintain this temperature for 2.5 hours. (Note: the condenser is not used in this set up). Once the process is completed, remove the container containing the compound and allow it to cool to room temperature.

v. Afterwards, if no solid has formed during the previous process, add some crushed ice and water as necessary. Then, allow this mixture to combine and form a white solid before proceeding to filtration.

vi. In order to conduct filtration, obtain a buchner flask and attach the nitrogen source to the flask's side opening. Next, line a clean funnel with filter paper and place inside the flask. Then, pour some water through to flush out any impurities and then follow up with the compound. Once all solid has been filtered, allow it to dry before conducting analysis.

vii. Regarding analysis, first, begin by dissolving the compound with ethyl acetate. Then, take excess product from procedure #2 and dissolve with chloroform and

methanol. Repeat this procedure for a sample of 4-Fluorobenzaldehyde.

viii. Afterwards, following Basic TLC Procedure, add a drop of 4- fluorobenzaldehyde + chloroform + methanol, chlorobenzene and finally the final compound to each respective drop. Then, place into TLC container containing 30% ethyl acetate hexane (solvent) and wait 5-10 minutes.

ix. Once the plate has absorbed the solvent, observe under UV light. If any impurities exist proceed to purification, otherwise analysis may be conducted.

d. Purification of compound:

i. First, take a funnel and fill the large opening with sodium sulfate before placing it inside of a RB. Then, pour the compound into the funnel to strip it of its impurities.

ii. Next, add dry ice to the funnel of the hot bath (if necessary). Then, clean all parts with ethyl acetate and dry with oxygen. Attach the RB to the heat bath using the appropriate attachment part. Set the bath temperature to 37° C and allow it to run.

iii. Once finished, turn off and remove RB from device. Then, add a small amount of silica gel based off of how much compound is remaining. Next, add some cotton to the inside of the RB's attachment piece before attaching it to the hot bath and allowing it to run again.

iv. Afterwards, remove compound from bath and prepare for purification using CombiFlash RF 200. First, take a clean cylindrical container with the size of 25 mg and another in the size of 60 mg- 1.2 mg. Fill the 25 mg container with the compound and the 60 mg- 1.2 mg with 12 mg of silica gel. Close both containers tightly.

v. Then, attach both cylinders to the CombiFlash RF 200 and follow proper procedure.

vi. Stop the device midway in order to check the products using Basic TLC Procedure. Once set up, place in a solution of 8 mL of 20% ethyl acetate hexane and 2 mL of ethyl acetate. After about 5 minutes, remove the plate from this solution and observe under UV light. Based off these results, determine which sample is the purest and proceed to analysis.

2. Procedure for analysis of (E)-Fluorostyryl-4-Chlorobenzyl Sulfone:

a. Basic TLC Procedure:

i. Firstly, cut an appropriate sized section of chromatography paper and mark a horizontal line about 1-2 cm away from the bottom edge of the paper.
ii. Using a pencil, indicate each compound that will be assessed by placing a dot. These dots should correlate to the appropriate number of compounds.
iii. Next, using the appropriate tool, place a drop of the first compound at the first dot. Then place the second compound at the second dot and continue like so.
iv. Once each compound has been added, set the plate aside to soak in the desired solvent before assessing under UV light.

b. NMR Spectra:
i. To begin, obtain 5 mg of the final compound using a scale and vial (exact amount used: 4.89 mg).
ii. Then, using a syringe obtain 0.6 mL of the desired solvent, in this case, chloroform-d was used. Add the solvent to the compound and dissolve.
iii. Afterwards, take the solution and place it into a clean NMR tube. Make sure to seal it tightly with the appropriate cap. Place NMR tube into the correct device, in this case the 600 NMR (Bruker) is used.
iv. Once the NMR tube has been securely placed into the device, set up the computer by logging into the appropriate account, opening the program and selecting a new file. As a new file is created, make sure to input the correct information (name, experiment number, etc.).
v. After the file has been set up, proceed to input the correct commands as indicated and wait until each procedure is finished to continue:
1. Lock- proceeds to auto lock
2. Select solvent- chloroform-d
3. Gradshim- starts the automatic gradient shimming
4. Zg- starts data acquisition
5. Apk- automatic phase correction
6. Adjust peaks/ format of results using cursor
7. Indicate “show peaks” and “integrate”

3. Biological Testing of (E)-Fluorostyryl-4-Chlorobenzyl Sulfone (IC50):
a. Preparing Microplate/ Dilutions Protocol:
i. Firstly, in order to begin this process, frozen A549 (human, lung-epithelial) cells must be revived from -40 °C and plated on to a normal sized petri dish for incubation/growth. If cells become too confluent for current petri dish, cells

must be split and transferred into the appropriate sized dishes:

1. Reviving Cells Protocol:
    a. Firstly, add 10 mL of FBS (media) to 15 mL tube.
    b. Next, add 20 mL FBS to labelled plate for cells (cover and side must be labelled with media and date).
    c. Then, heat shock cells in a 37 °C water bath until 70 % thawed. Before undergoing heat shock cells should be resting on ice.
    d. Afterwards, add thawed cells to a 15 mL tube and then spin/centrifuge it for 5 minutes at 1500 rpm (1.5).
    e. Next, remove the tube from the centrifuge and remove media leaving the pellet behind.
    f. Add 5 mL of FBS (media) to the tube with cell pellet and aspirate till no clumps are remaining (avoid bubbles).
    g. Evenly spread out the cells in the plate and move in plus shaped formation. Observe under microscope before incubation.
2. Splitting Cells Protocol:
    a. Once cells have grown to maximum capacity in their current container, place trypsin in incubator for 30 minutes to activate.
    b. Next, use the pipette attached to the vacuum to remove old media from plate. Then, using the electric pipette, place PBS on to the side of the plate to avoid killing cells.
    c. Then, add trypsin to the cells using an electric pipette before setting to incubate. Meanwhile, fill a new tube with FBS (media) using an electric pipette (Note: the amount of media added should be x2 the amount of trypsin administered before hand).
    d. Afterwards, take a larger petri dish and label the sides and cover with the media and date. Add media to plate based off of how large the plate is.

e. After 5 minutes, using an electric pipette, remove and release cells from walls (avoid bubbles), then add media to deactivate trypsin (from tube). Continue process of removing cells from walls.
f. Once cells are detached from walls, place into tube. Then, transfer tube into centrifuge for 5 minutes and the exact same conditions as in “Reviving Cells Protocol”.
g. After the centrifuge is done, remove the media from the tube using the pipette attached to the vacuum. Then, using an electronic pipette, obtain media and use it to aspirate cells.
h. Take solution and plate the cell evenly all over the dish. Move plate in plus shaped formation and observe under microscope. Then, place in incubator.

ii. Next, once cells have grown to an appropriate size, they must be transferred to the microplate and left to incubate for a few days before administering the compounds. In order to transfer cells appropriately, we must calculate the amount of cell media and cell suspension needed for each well (in this experiment, we specifically added 7.9 mL of cell media and 72 µl of cell suspension- results will vary):
    1. Plating Cells (Microplate) Protocol:
        a. Once cells have grown to appropriate size, using a vacuum pipette isolate the cells by removing the old media.
        b. Using an electronic pipette, acquire and administer PBS. After all the cells are cleaned, remove remaining solution using vacuum pipette.
        c. Next, administer trypsin using an electric pipette before setting aside for incubation. In the meantime, using a new electric pipette add cell media + 10% FBS to a new tube. Alongside this, prepare dye by using a micropipette to obtain 10 µl of trypsin blue and adding to new container.
        d. Once cells are done incubating, using an electronic pipette, detach cells from walls by taking the solution in and out of the nozzle.

Then, add media to deactivate enzymes and repeat the previous procedure.

e. Then, add suspended cells + media to centrifugal tube and centrifuged for 5 minutes at 1500. In the meantime, prepare a new 96 black well microplate by labeling the cover and side with the name, date and cell line.
f. After the centrifugal process is completed, use a vacuum pipette to remove media from tube and administer new cell media using an electric pipette. Aspirate cells before adding 10 µl of the suspended solution to 10 µl of dye using a micropipette.
g. Take 10 µl of this solution and place inside the loading well of the hemocytometer. Observe under microscope in order to count cells in each quadrant. Conduct any necessary calculations.
h. Next, using a multipurpose pipette and a reservoir, fill cells according to proper procedure: cell media is placed into outer rows, row B-G and column 11-3 have the tested solution. When administering cell media only 10 µl is used and when using cell suspension use 72 µl.
i. Observe cells under microscope before incubating.

b. While the cells are incubating, using our stock solution concentrations are prepared accordingly:
    i. Dilution/ Microplate Preparation Protocol:
        1. Prepare centrifuge tube rack and containers by labelling and sterilizing. Make sure to distinguish the difference between containers by labelling them with concentrations (ex. 10 mM).
        2. In order to prepare the stock solution, calculate the volume of solvent needed to achieve "____ mM". For this study, the stock solution had a concentration of 10 mM and so 1.56 mL of DMSO was added to the compound. Afterwards, 50-100 µl of stock solution was transferred to the first container.

3. Next, in order to achieve the concentration 7.5 mM, transfer 37.5 µl of the first solution (10 mM) to its correlating container and add 12.5 µl of DMSO for dilution. Then, to achieve the concentration of 5 mM, add 25 µl of the solution in the first container (10 mM) to its correlating container and add 25 µl of DMSO for dilution. Lastly, in order to synthesize the 2.5 mM, repeat the same steps for the 5 mM, however, start with the 5 mM solution instead of the 10 mM.
4. In order to achieve the rest of the concentrations, the solutions of 10 mM, 5 mM and 2.5 mM will be diluted by a 10th. For example, in order to synthesize 0.5 mM solution, 5 µl of the 5 mM concentration will be taken and combined with 45 µl of DMSO in its correlating container. In summary, the solutions made in step three will now be diluted using these measurements to formulate the other concentrations. These new concentrations will be diluted to formulate the next set of concentrations and so on and so forth. Repeat these steps with the exact same measurements for the following concentrations: 0.05 mM, 0.005 mM, 1 mM, 0.1 mM, 0.01 mM, 0.001 mM, 0.25 mM, 0.025 mM, 0.0025 mM.
5. Afterwards, obtain separate containers and label with micromolar concentrations. Using an electric pipette, add 1 mL of cell media to each container.
6. In order to administer the compound onto the cells, the solutions made prior to this must now be combined with the cell media in the new containers. This will result in the concentrations changing from “mM” to “µM”. To prepare these solutions, transfer 1 µl of the “mM” solution to the containers containing cell media using a micropipette. Mix and repeat for each concentration.
7. Unlike the other concentrations, in order to prepare 25 µm, transfer 2.5 µl of stock solution to a container containing cell media and mix.
8. Once all the “µm” concentrations have been prepared, using an electric pipette transfer 1 mL of each solution to the appropriate well in the transfer plate. Once completed, label the cover of the

microplate in correlation to the transfer plate's set up.

9. Next, using a multipurpose pipette, remove the old cell media from the microplate and pipette new solutions according to the labels indicated on its cover. Observe under microscope before incubating for 96 hours.

c. Microplate Reading Protocol:
    1. Firstly, all procedures involving cell titer blue must be conducted in the dark or under indirect light. Then, using an electrical pipette, add 4-5 mL of cell titer blue to the reservoir.
    2. Next, using a multipurpose pipette, pipette 20 µl of dye into each cell wall, including the blanks. Afterwards, incubate the cells for 3 hours.
    3. Once cell samples have been incubated, turn on microplate reader and select: "Protocol, Fluorescence, Cell titer blue, Optical/ blue, Select plate (controls, blanks, etc.), Open door"
    4. Place the microplate into the door and allow the plate to be read. Once the plate has been read, input the USB and select "copy results"
    5. Input results into computer and proceed to graph using the appropriate program.
d. Preserving Cells Protocol:
    i. First, set a new cryovial on top of dry ice before preparing any cells. While that is at rest, using a vacuum pipette remove any old media from the cell sample. Once removed, using an electric pipette administer PBS, indirectly. Remove the solution afterwards using a vacuum pipette.
    ii. Next, using an electrical pipette, administer trypsin to cells and incubate for 5 minutes. Meanwhile, label new tubes and add new media. Depending on the amount of media needed, the concentration of it should consist of 20% FBS and 10% DMSO. All media should be added using a micropipette.
    iii. Once the cells have finished incubating, using an electric pipette, detach cells from walls by taking the solution in and out. Add the desired cell media and continue this process.
    iv. After all the cells have been detached, add cell solution to tube and place inside of centrifuge for 5 minutes at 1500

rpm. Once this procedure is done, remove the media using a vacuum pipette. Then, using a micropipette, administer the cell media and aspirate the cells until no clumps are remaining.

v. Add cell suspension to cryovial and place back into dry ice for 5 minutes before transferring to -40 °C.

Risk and Safety

- Potential Risks: exposure to biological hazards (ex. laboratory cell cultures, pathogenic microorganisms, etc.), chemical hazards (ex. skin irritants, respiratory sensitisers, etc.), chemical related injuries (skin contact with corrosive chemicals, eye damage, etc.)
- Safety Precautions: proper laboratory training, use of safety precautions (gloves, safety goggles, lab coat, etc.), knowledge of fire instructions, medical emergencies, professional staff and their responsibilities, etc.

Data Analysis

1. Regarding the observational set of data, once the chemical has been created and administered on to the stained cancer cell sample, using a regular microscope the dead cancer cells will be viewed and any visual observation of activity, state, etc. will be recorded. Photos of cell samples will be taken as well.
2. Regarding the numerical set of data, a microplate reader, which is used to detect chemical, biological or physical reactions, will be used to obtain and record the percentages of cell survival. (Proper instruction of laboratory devices will be provided during orientation/training)

3. Potential Hazardous Biological Agents Research:

- **Give source of agent, source of specific cell line, etc.**
  - Cancer cell lines (Cell Line: A549, Bought from ATCC #CCL-185)
- **Describe Biosafety Level Assessment process and resultant BSL determination**
  - Research risks associated with chemicals on suppliers website (ATCC)
  - Discussed appropriate procedures and rules with mentor and designated supervisor
  - Reviewed safety procedures during training
- **Results:**
  - **Risk Group/BSL:** BSL-2: Involving human diseases, moderate health hazard, restricted access, low risk
  - **Laboratory Safety:** always follow basic safety procedures (wear protective clothing, never reuse syringes/needles, etc.), always dispose of hazardous materials in the appropriate fashion (ex. Used syringes must be deposited in the “sharps” container after being recapped to avoid injury).
  - **Evaluation:** All staff members are aware of safety procedures and how to handle emergencies (spills, fires, etc.)

- **Detail safety precautions**
    - Practice all forms of personal hygiene:
        - Wash hands after working with biohazard, removing contaminated clothing, before eating/drinking, etc.
        - Do not make direct contact with skin after working with biological material
        - Never eat, drink, smoke, etc. in work areas
    - Wear appropriate clothing:
        - Wear masks, gloves, scrubs, lab coats, etc. when working with agents
        - Avoid wearing contact lenses when working
        - Do not wear any contaminated clothing outside of laboratory
            - Remove clothing in an appropriate manner (ex. remove gloves by taking them off from the inside out)
    - Handling Procedures/ Syringes
        - Use pipetting devices when handling agents
        - Always use secondary leak-proof containers when transporting biohazardous materials
    - Work Area
        - Keep laboratory sealed when experiments are being conducted (helps limit access)
        - Have warning signs available
        - Have vacuum lines with a filter trap somewhere present in the lab
        - Decontaminate work areas and equipment daily, especially after spills
        - Transport contaminated materials in leak-proof containers
        - Remove any miscellaneous materials from work space (ex. phones, books, etc.)
- **Discuss methods of disposal**
    - Solids:
        - Must be placed in autoclavable waste bags before being disposed into biohazardous waste containers (All bags must be placed into a secondary container incase of spillage, leakage, etc.)
    - Liquids:
        - If necessary, treat all liquids with disinfectant before disposing down lab sink.
        - Regarding autoclave treatment, place all liquids in a closed collection vessel and place into a secondary container for transport. Use a liquids cycle and discharge when cooled down a sanitary sewer.
    - Pathological Waste
        - Must be double bagged by biohazard bags and stored safely to reduce the risk of spills or contamination.

4. Use of Potentially Hazardous Chemicals

- **Chemical Name Concentrations**
    - **Thioglycolic Acid:** 1012 mg (0.1 moles)

- **4- Chlorobenzyl Chloride:** 1771 mg (0.1 moles)
- **4- Fluorobenzaldehyde:** 157 mg (0.01 moles)
- **Sodium Hydroxide:** 880 mg (0.05 moles)
- **Methanol:** 11 mL
- **Hydrochloric Acid:** 36% in 2.2 mL
- **Hydrogen Peroxide:** 1.39 mL (30%)
- **Glacial Acetic Acid:** 4.20 mL

- **Chemical Risk Assessment Procedure:**
  - Research risks associated with chemicals on suppliers website (ATCC)
  - Discussed appropriate procedures and rules with mentor and designated supervisor
  - Reviewed safety procedures during training
- **Chemical Risk Assessment Results**
  - **Thioglycolic Acid:**
    - A corrosive acid that may cause irritation in the nose, lungs, skin, etc. when in contact.
    - Minimum interaction on a day to day basis, minimum exposure should be about 1 ppm, unless an air respirator is supplied. Recommended exposure time for 1 ppm is 8-10 hours.
    - Overall the chemical is ranked at a class 8 on the DOT Hazard Class due to its severe corrosive effects.
  - **4-Chlorobenzyl Chloride:**
    - A laboratory chemical that can cause irritation or damage to eyes, skin, etc.
    - Since the chemical doesn't possess any hazardous side effects there is no recommended exposure time or level.
    - Overall the chemical is ranked at a class 6.1 on the DOT Hazard Class due its ability to be toxic when consumed.
  - **4- Fluorobenzaldehyde:**
    - A chemical that can cause irritation when in contact with skin, ingestion, etc. when in contact.
    - Since the chemical doesn't possess any hazardous side effects it can be used at 100% concentration in a laboratory setting, there is no recommended time.
    - Overall the chemical is ranked at a 3 in the DOT Hazard Class due to its ability to be flammable.
  - **Sodium Hydroxide:**
    - A corrosive chemical that can result in severe irritation when in contact with skin, eyes, etc., can be toxic if ingested.
    - Since the chemical possesses some side effects, it is recommended that exposure levels are around 2 mg/m^3 to avoid any irritation in the nose and throat.

        - Overall, the chemical is ranked at 8 in the DOT hazard class due to its ability to be corrosive.
    - **Methanol:**
        - A highly flammable chemical that can be toxic if ingested, inhaled or having contact with skin.
        - Since the chemical possesses some side effects, it is recommended that the exposure levels be about 200-250 ppm.
        - Overall, the chemical is ranked at a 3 in the DOT hazard class due to its ability to be flammable and combustible.
    - **Hydrochloric Acid:**
        - A corrosive chemical that can result in irritation to organ, serious eye injuries, corrosion of skin, etc.
        - Since the chemical possesses some side effects, it is recommended that the exposure levels remain around 2-5 ppm (ceiling).
        - Overall, the chemical is ranked at 8 in the DOT hazard class due to its ability to be corrosive.
    - **Hydrogen Peroxide:**
        - A chemical that can result in irritation if inhaled or consumed, its side effects are highly influenced by concentration.
        - Since the chemical possess some severe side effects based off of concentration, it is recommended that hydrogen peroxide, mainly in consumer form, is limited to 12% or less.
        - Overall, the chemical is ranked at a 5.1 on the DOT hazard class due to its ability to oxidize.
    - **Acetic Acid:**
        - A flammable chemical that can result in severe damage to eyes and skin if in contact, may be toxic if inhaled or consumed.
        - Since the chemical possesses some severe side effects, it is recommended that its concentration remains around 25 mg/m^3 or less.
        - Overall, the chemical is ranked at a 3 on the DOT hazard class due to its ability to be flammable.
- **Safety Precautions**
    - Only use the chemicals in locations with local exhaust ventilation or enclosures, otherwise respirators should be used.
    - Wear long sleeves, gloves, lab goggles, etc.
    - Once you are finished working with the chemical, wash thoroughly to areas that have made contact.
        - If at any event there is contact between skin, eyes, etc. and chemicals seek the proper medical attention immediately.
    - Follow all lab safety rules (no drinking/eating, handle with care, etc.)
    - Avoid breathing in any fumes, vapors, etc.
    - If clothing is contaminated, was it before reusing it at all times
- **Methods of Disposal**

- Always dispose all chemicals into the proper waste container/plant
    - If it is considered hazardous make sure to dispose of it as hazardous waste, in which the state Department of Environmental Protection (DEP) or the federal Environmental Protection Agency (EPA) must be contacted for special instructions.
    - In the case of an accidental spill, absorb all liquids in dry sand, earth or similar material and deposit in sealed containers.
- All workers must be properly trained and equipped to clean-up accidents.
- Avoid dumping chemicals down drains, sewers, waterways, etc. to avoid environmental contamination

Bibliography

Reddy, E. P., & Reddy, M. R. (2003). *U.S. Patent No. 6,541,475*. Washington, DC: U.S. Patent and Trademark Office. (Title: ot, B-UNSATURATED SULFONES FOR TREATING PROLIFERATIVE DISORDERS)

Reddy, E. P., Reddy, M. R., Cosenza, S. C., & Helson, L. (2003). *U.S. Patent No. 6,667,346*. Washington, DC: U.S. Patent and Trademark Office. (Title: METHOD FOR PROTECTING CELLS TISSUES FROM IONIZING RADIATION TOXICITY WITH ot, B UNSATURATED ARYL SULFONES)

Reddy, D. B., Babu, N. C., Padmavathi, V., & Sumathi, R. P. (1999). A novel route for the synthesis of unsaturated oxo sulfones and bissulfones. *Synthesis*, *1999*(03), 491-494.

Reddy, E. P., & Reddy, M. R. (2004). *U.S. Patent No. 6,762,207*. Washington, DC: U.S. Patent and Trademark Office. (Title: (E)-STYRYL SULFONE ANTICANCER AGENTS)

Cosenza, S. C., Reddy, M. R., & Reddy, E. P. (2004). *U.S. Patent No. 6,767,926*. Washington, DC: U.S. Patent and Trademark Office. (Title: METHOD FOR PROTECTING NORMAL CELLS FROM CYTOTOXICITY OF CHEMOTHERAPEUTIC AGENTS)

THERE ARE NO ADDENDUMS TO THIS RESEARCH PLAN