Drisana Shanthan
Research Paper

Synthesis and Cancer Cell Cytotoxic Studies of Styryl Benzylsulfone

Introduction/Background:

Cancer is defined as a disease resulting from the rapid division of cells in different parts of the body, caused by an interference or blockage in the cell's ability to perform apoptosis or differentiation. This disease can arise in many different forms such as epithelial, which focuses on the tissue of organs, or sarcomas, which focus on mesenchymal tissues. Current day treatments, such as chemotherapy, only focus on killing cells regardless of whether or not they're normal, resulting in a negative impact on the patient's health. However, over the past decade the discovery of cyclins and specific cell cycle stages has aided in the discovery and research of therapeutic or anticancer agents, such as (E)-Styrylbenzylsulfones, which possesses the ability to only target tumor cells (Reddy et al., 2007).

(E)-Styrylbenzylsulfones are known to block cells from progressing in cell cycle resulting in their death by interfering with signaling pathways, such as the MAPK signal transduction pathway. By interfering with these signaling pathways they can cause a disruption or regulation in the synthesis of proteins and transcription of genes resulting in a disturbance in functions such as cell growth, differentiation, etc. Unlike current anticancer agents, most of these compounds not only target and eliminate tumor cells, but they also protect normal cells which helps avoid toxic side effects and damage to the patient's health (Reddy et al., 2004). Alongside their efficiency in cancer, when tested it was found that (E)-Styrylbenzylsulfones also act as treatment in non-cancer proliferative disorders such as neurofibromatosis, keloid formation, restenosis, etc. (Reddy et al., 2004). An example of a (E)-Styrylbenzylsulfone would be (E)-4-Fluoro Styryl-4-Chlorobenzyl Sulfone, an anticancer agent known for inducing apoptosis in tumor cells while protecting normal ones.

As (E)-4-Fluoro Styryl-4-Chlorobenzyl Sulfone is administered to cancer cells, a negative effect on the cancer cell's activity should ensue resulting in their death. This is supported by the idea that, similar to other (E)- Styrylbenzylsulfones, this compound possesses many functions and abilities similar to the ones found by other compounds in this family, such as the regulation of cell cycle progression through phase transitions (G1/S and G2/M) and inducing mitotic arrest. Although no past studies have directly focused on this specific compound, studies regarding the overall family of compounds have helped conclude that these compounds portray a large variety of antitumor activity in many different models and assessments allowing us to assume that they are potential agents in cancer research. By synthesizing and administering this compound in different concentrations on different cell lines and performing assessments, such as IC50, more information regarding this compound's antitumor activity and its efficiency in different cell lines is obtained.

Materials and Methods:

General Procedure for (E)-Fluorostyryl-4-Chlorobenzyl Sulfone:

Preparation of 4- Chlorobenzyl Thioacetic acid (1):

Over the course of 10 minutes, 1012 mg of thioglycolic acid was added to a solution of 11 mL- 15mL of methanol and 880 mg of sodium hydroxide. This mixture was then refluxed for 2 hours at 70 °C. Then, 1771 mg of chlorobenzyl chloride is added to the solution at room temperature before being refluxed for another 2 hours at 70°C. After, it is poured into a solution of 10 g of crushed ice and 2.2 mL of hydrochloric acid (37%). Proceed to assessment (Basic TLC Procedure). Impurities were extracted with sodium sulfate before being combined with hexane and being placed into heating bath. Precipitate was filtered and washed.

Preparation of 4- Chlorobenzyl Sulfonyl Acetic acid (2):

9 mL of acetic acid was added to the resulting compound from procedure 1. Next, 3 mL of 30% hydrogen peroxide was added to the solution before being refluxed for 1 hour at 120 °C in an oil bath. Then, 12 g of crushed ice was added at room temperature. Resulting compound was filtered and dried.

Preparation of (E)-4-Fluorostryl-4-Chlorobenzyl Sulfone (3):

1.6 mL of acetic acid was added to 260 mg of 4- Chlorobenzyl Sulfonyl Acetic acid while 157 mg of 4- fluorobenzaldehyde was added to 26 mL of piperidine. Both solutions were combined before being refluxed for 2.5 hours at 120 °C. Compound is filtered out before undergoing assessment (Basic TLC Procedure).

Purification of (E)-4-Fluorostryl-4-Chlorobenzyl Sulfone:

Impurities are filtered from the compound and ethyl acetate with sodium sulfate before being placed into a hot bath at 37 °C. Once the compound is separated, silica gel is added prior to being attached to a hot bath at 37 °C. Using the CombiFlash RF 200, the compound was purified into multiple different vials. Assessment was then conducted (Basic TLC Procedure).

Analysis of (E)-Fluorostyryl-4-Chlorobenzyl Sulfone:

Basic TLC Procedure:

A pencil mark was used to indicate the location of each tested compound on a newly selected chromatography plate. A drop of each compound was placed in the appropriately marked section. Plate was soaked in the desired solvent for 5-10 minutes before assessment under UV light.

NMR Spectra:

0.6 mL of chloroform-d was added to 5 mg of (E)-4-Fluorostryl-4-Chlorobenzyl Sulfone and the solution was transferred to an NMR tube. NMR tube was placed into 600 NMR Bruker and computer system was activated using the appropriate log in. Program and new file were

selected before filling in experimental information. Codes were input according to the appropriate protocol.

Basic Protocol for Reviving Cells:

10 mL of FBS (Gibco) is added to a 15 mL tube and 20 mL of FBS is added to a plate. Heat shocked at 37 °C until it was 70% thawed after resting on ice. Cells were added to a 15 mL tube and centrifuged for 5 minutes at 1500 rpm. Afterwards, media is removed and 5 mL of FBS is added to aspirate cells. Administered to plate evenly and observed before incubation.

Basic Protocol for Splitting Cells:

When cells become confluent, trypsin (Gibco) is activated in an incubator for 30 minutes. Old media is removed and PBS (Gibco) is administered to cells. Trypsin is added to cells and incubated. Meanwhile, a new tube is filled with FBS (x2 amount of trypsin). After incubation, cells are detached from the walls and media is added to deactivate enzymes. Cells were centrifuged for 5 minutes before removing media and aspirating cells with new FBS. Finally, cells were plated evenly on a larger plate before observation and incubation.

Basic Protocol for Plating Cells (Microplate):

Cells are isolated and cleansed using PBS before trypsin is administered and incubated. After incubation, cells were detached from walls and media was added to deactivate enzymes. Cells were centrifuged for 5 minutes before media was removed and cells were aspirated with cell media. Afterwards, a micropipette was used to combine 10 µl of cells and 10 µl of trypsin blue dye. 10 µl of solution was placed into loading well of hemocytometer and cells were counted in quadrants. Volume of cell suspension and cell media was calculated before a multipurpose pipette was used to fill the microplate. Finally, cells were observed before being incubated.

Basic Protocol for Preserving Cells:

A new cryovial was prepared by resting it on top of dry ice. In the meantime, cells were prepared by being washed with PBS and trypsin was administered before being incubated for 5 minutes. Next, a cell media of 20% FBS (Gibco) and 10% DMSO was prepared. After cells had finished incubating, they were detached and cell media was administered before being added to centrifugal tube for centrifugation for 5 minutes at 1500 rpm. After the procedure is finished, media was removed and cells were aspirated using the new media. After aspiration, cell suspension was transferred to cryovial and set back into dry ice for 5 minutes before being transferred to -40 °C.

Basic Protocol for Dilutions and Microplate Preparation:

A 10 mM stock solution was prepared by diluting 4.86 mg of ONO1060 with 1.56 mL of DMSO. A container was prepared by having 50-100 µl of original stock solution pipetted into it. The next three concentrations (7.5 mM, 5 mM, 2.5 mM) were formulated by diluting the starting concentration based off its proportion with the desired concentration (ex. 7.5 is ¾ of 10, so 50µl x ¾ equals 37.5 µl of stock solution and 50 - 37.5 equals 12.5 µl of DMSO). To formulate the

rest of the concentrations, 45 µl of DMSO was added to 5 µl of the starting concentration (5 mM, 2.5 mM, 7.5 mM). This process was repeated with the new concentrations until all desired concentrations are synthesized. Next, in separate containers, 1 mL of cell media was combined with 1 µl of "mM" solutions. This was repeated for each and every concentration, however, for 25 µm, 2.5 µl of stock solution was added instead. 1 mL of each solution was transferred to the wells of the "transfer" plate, following basic microplate set up. Old cell media was removed from wells before the compound was administered to the new cells. Was then incubated for 96 hours. Before reading, 20 µl of cell titer blue was administered and incubated for 3 hours. Basic protocol for reading using the Promega Glomax Multi was followed.

Results:

Efficiency:

The least effective concentration, in regards to activity inhibition, is .001 µM. It's administration resulted in a 64,435.17 ± 1,396.74 **λ** of cell activity. Concerning the strongest concentration, the administration of 25 µM resulted in a 45,371.37 ± 2,294.79 λ of detectable cell activity, with disregard towards background activity (3,857.91 λ). Although the compound has shown to decrease cell activity, no concentration has effectively reduced 50%.

IC50:

In regards to the IC50 reading, it was indicated that no concentration has efficiently achieved 50% inhibition.

Discussion:

Concerning the results, it is concluded that the hypothesis is rejected due to the compounds' inability to achieve 50% inhibition of cell activity at its strongest concentration (25 µM). Due to this, it is suggested that the compound itself is inefficient within the A549 cell line, as suggested by the presence and absence of specific signaling pathways. It is suggested that this compound cannot be utilized in patients possessing a similar cell line due to the inability to administer a concentration that exceeds over 25 µM, as it violates protocol and may or may not be dangerous towards the patients' health.

Citations:

Reddy, M. R., Mallireddigari, M. R., Cosenza, S. C., Pallela, V. R., Iqbal, N. M., Robell, K. A., ... & Reddy, E. P. (2007). Design, synthesis, and biological evaluation of (E)-styrylbenzylsulfones as novel anticancer agents. *Journal of medicinal chemistry*, *51*(1), 86-100.

Reddy, E. P., & Reddy, M. R. (2002). *U.S. Patent No. 6,359,013*. Washington, DC: U.S. Patent and Trademark Office.

Sever, R., & Brugge, J. S. (2015). Signal transduction in cancer. *Cold Spring Harbor perspectives in medicine*, *5*(4), a006098.

Reddy, E. P., & Reddy, M. R. (2004). U.S. Patent No. 6,762,207. Washington, DC: U.S. Patent and Trademark Office.

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.