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Synthesis, Characterization and Cell Viability of Novel Tripodal Amines

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Synthesis, Characterization and Cell Viability of Novel Tripodal Amines

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Chemistry and Biochemistry

By
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Abstract

Cancer, over the years, has become a much more prevalent focus for the scientific community. Organizations and laboratories all over the world have spent countless hours searching for a cure, trying to learn more about what makes cancer so powerful and what is the best way to stop its growth. Iron-chelation drugs were already on the market, and it was shown that they did have the ability to act as both iron-chelators and anticancer drugs. Most of these iron-chelating drugs are not as effective at killing cancer cells as the medical field desires. Novel iron-chelating tris-indolyl derivatives, GSO2, GSO4 and GSO6, were synthesized and tested for their potential anti-cancer properties. These compounds were characterized using both melting points and NMR. Apoptosis was the chosen method for cell death of the PC3 cells. The compounds, GSO2 and GSO4 did show promising results when tested on human prostate cancer cell lines. The success of these compounds does sanction further research into apoptosis of other human cancer cell lines.

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Introduction

Cancer is one of the most feared words for most families in today's world. Cancer, by definition, is the continuous division and replication of cells. These cells lack the signals needed to tell them when to go through the process of cell division and when to stop, due to a mutation in their DNA. The mutated cells can quickly spread throughout the entire body, which can lead to different variations of cancer. Some forms of cancer, such as, liver, colorectal and breast cancer have been linked to increased levels of iron in the body. The correlation between these increased iron levels and cancer cells has been proven using biomarkers that analyze iron-binding proteins.[1] Hemochromatosis is a disease caused by a gene mutation, which leads to excess iron building up in the body, causing damage to the liver and heart. Those suffering from hemochromatosis are also at an increased risk of developing cancer.[1] Despite the problems excess iron can cause, it is an important element in the body. Iron is key to the function of the protein hemoglobin, which transports oxygen from the lungs throughout the rest of the body. Myoglobin, which is similar to hemoglobin, relies on iron to help transport oxygen to the muscles. Iron also plays an essential role in electron transfer and redox reactions and cell's metabolic activity, such as growth and metabolism.[2]

Present day cancer treatments are very draining for the patient, and they do not guarantee that the cancer will not return. Under ideal circumstances, there would be a treatment plan that would cure all cancers, once and for all. This is rather difficult due to the different variations in the types of cancers and their cells. By focusing on the commonality of increased iron levels in most cancer types, it was thought that it may be possible to create a treatment that could target this problem specifically. Novel tripodal

amines have shown the ability to act as iron-chelators and anticancer drugs.[3] Tripodal amines have the ability to grab the excess iron from the cells and assist the body in removing it. Currently there are two drugs approved for iron-chelator cancer therapy by the United States Food and Drug Administration (USFDA); deferoxamine (DFO) and deferasirox (DFX) (Figure 1), but both come with risks for the patient. DFO requires constant subcutaneous injections due to its short plasma half-life and DFX is needed in high concentrations in order to be effective even to a small degree.[4] DFX has the ability to induce reactive oxygen species (ROS) production, which is becoming an effective way to kill cancer cells. ROS are used for maintaining redox homeostasis within the cells. Designing a new iron-chelating drug that comes with limited side effects and a high cell death rate, would be ideal for targeting the cancer cells in iron-overloaded areas.

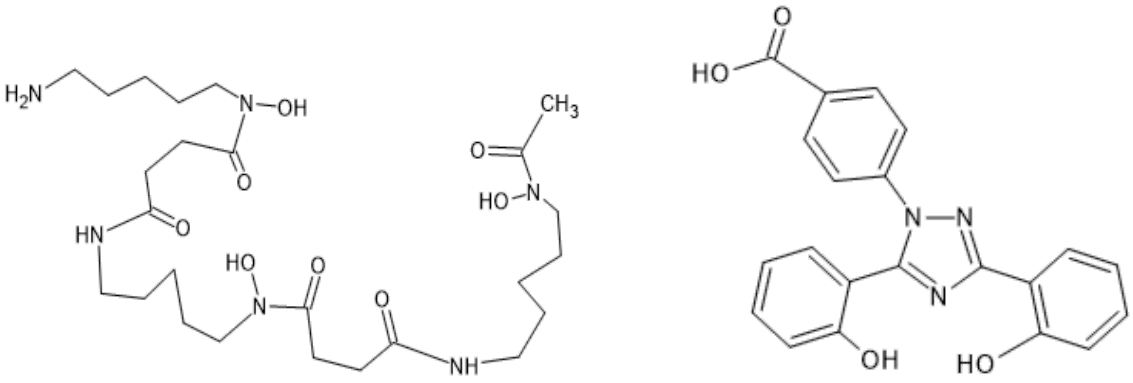

Figure 1. Deferoxamine (left) and deferasirox (right) are the two USDA approved iron-chelating drugs.

Of the various ways cells can die, apoptosis is the most beneficial for the surrounding cells. Apoptosis is also known as programmed cell death. It is characterized by the collapse of the cell along with cell shrinkage, irregular lumps in the cell membrane, known as membrane blebbing, the condensation of chromatin and DNA fragmentation.[5] When the cell undergoes these different processes, the cell will die and

then be consumed by the surrounding cells. Apoptosis is more beneficial because protein capsules within the cell assist in breaking it down, which in turn prevents any of the defective parts of the cell from contaminating the other healthy cells.[6] The goal of many cancer treatments is to induce apoptosis either through radiation or medicinal purposes. Cancer cells are usually able to avoid apoptosis prior to treatments because they have mutations in their DNA that does not allow the cell to recognize they have anomalies.

Previously, tris-indolyl compounds have been synthesized in the lab and have proven to be effective against cancer cells.[7] The aim of this project is to synthesize new derivatives of the first tris-indolyl created that are more effective against the cancer cells and still maintain the ability to act as iron-chelators in the body. Iodine and bromine were chosen as the substituents on the indoles. They were chosen in order to determine if adding electronegative halogens to the indole would have any effect on the activity of the compounds with the cancer cells. All three compounds synthesized are based on a TRENSOX derivative that relies on indoles instead of quinolones.[8]

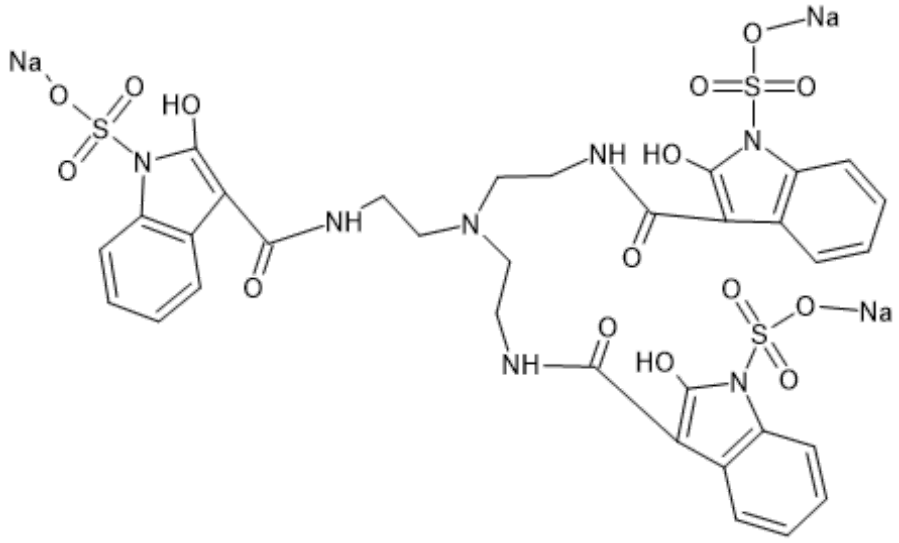

Figure 2. TRENSOX molecules used for the GSO derivatives

Methods and Materials

Synthesis of GSO2: The procedure followed was based on a previously established method for the synthesis of a general tris-indole compound.[8] Indole (2.52g) was added to ether (40mL) in a 250 mL round bottom flask. The solution was stirred for 5 minutes, then oxalyl chloride (2.74g) was added dropwise. The solution was stirred over ice for 30 minutes. The solution was filtered and a yellow precipitate was collected. A second solution of approximately 70 mL of Tetrahydrofuran (THF), 2.14g triethylamine (TEA) and 0.94g Tris-(2-aminoethyl) amine (TREN) was prepared. The precipitate was added to this second solution and left to stir for two days at 0℃. After the two days, methanol (30mL) was added to recrystallize the mixture. The solid did not recrystallize, so it was left for four days to continue stirring. The solution was then evaporated using the rotary vapor instrument for approximately 1.5 hours. The solid was then able to be collected.

Synthesis of GSO4: For the synthesis of GSO4, ether (20mL) was added to 5-bromoindole (2.5g) in a 250mL round bottom flask. The solution was stirred over ice for 5 minutes. Oxalyl chloride (1.38g) was added dropwise and then the reaction was left to stir for another 30 minutes at 0℃. A bright yellow precipitate was filtered out. While the reaction was stirring, a solution of TREN (0.45g), and TEA (1.095g) was stirred over ice, along with a separate flask of THF (25mL). The THF, TREN and TEA solution was combined with the filtered yellow precipitate and stirred for two days at 0℃. After the two days, methanol (30mL) was added to aid in recrystallizing the precipitate. The solution was filtered again, and 0.40g of light yellow solid was collected. The solid was left to dry for a week.

Synthesis of GSO6: The same procedures for GSO4 were followed for the synthesis of GSO6. 2.5g of 5-iodoindole was used in replacement of the 5-bromoindole. After the methanol was added to the THF, TREN, TEA and precipitate solution, no solid was formed. Ice was added to the solution in an attempt to speed up the recrystallization process. This was successful as crystals were formed and the precipitate was filtered out. 0.3g of GSO6 precipitate was collected.

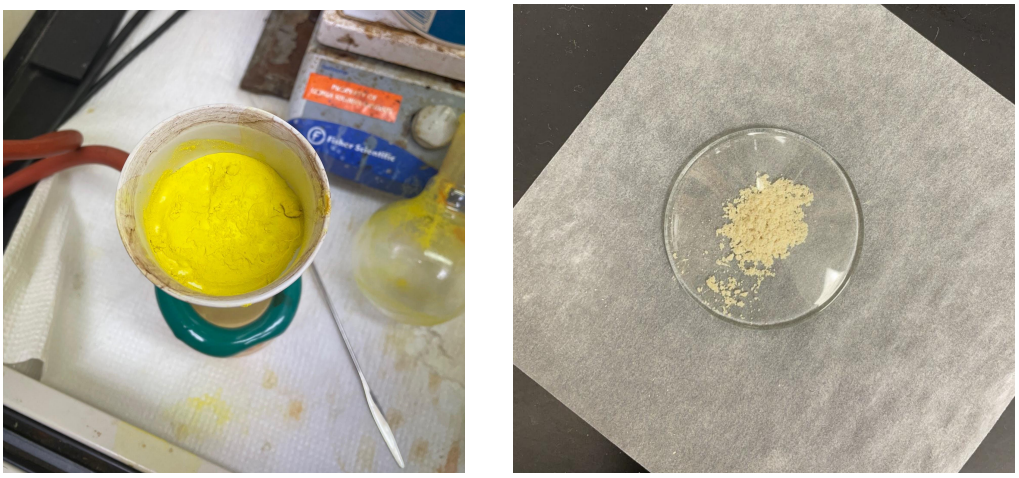

Figure 3-4: The precipitate formed during the reaction of 5-Iodoindole, Ether and Oxalyl Chloride (Left) and the final product, GSO6 (Right).

Apoptosis Assay: The PC3 cells were plated in a 96-well plate format. Each was treated with a 50 μM dose of the selected compound and 0.1% dimethyl sulfoxide (DMSO). They were treated for 48 hours. The viability of the cells was then determined using a colorimetric MTS assay and the formazan dye that is detectable when NAD(P)H-dependent enzymes are released. The plates were then scanned using an AccuSkan FC plate reader at 492 nm and the SkanIt Software. Each compound was tested in replicates of 6.

Data and Results

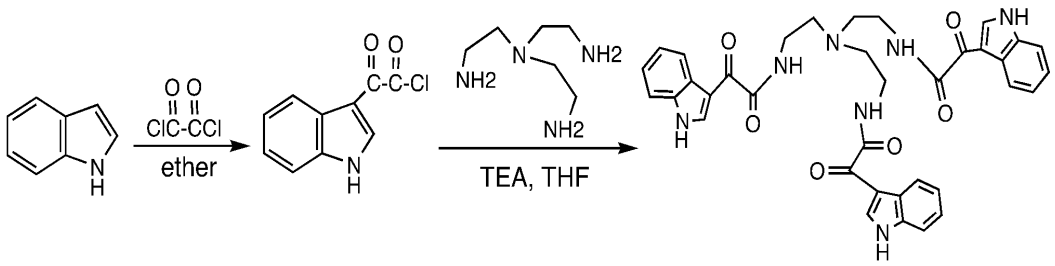

Figure 5. Synthesis of GSO2. Image representation of the synthesis of the indolylglyoxalyl derivative.

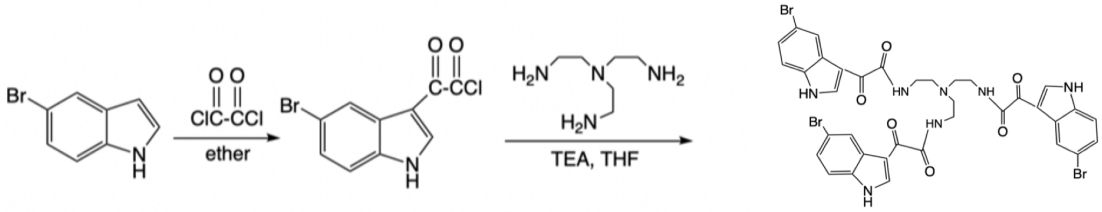

Figure 6. Synthesis of GSO4. Image representation of the synthesis of the 5-bromoindolylglyoxal derivative.

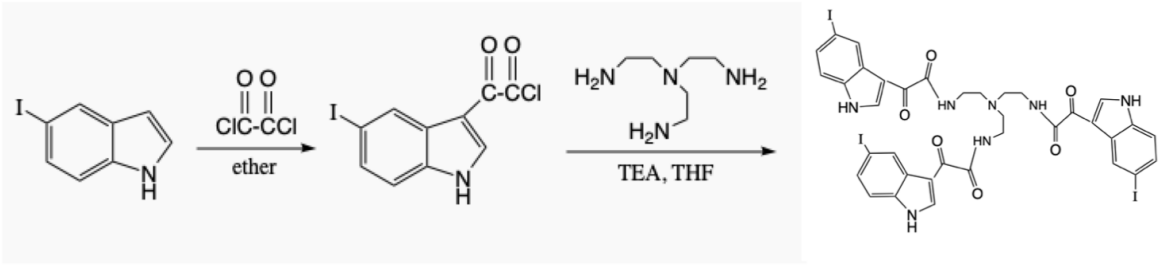

Figure 7. Synthesis of GSO6. Image representation of the synthesis of the 5-iodoindolylglyoxalyl derivative.

	Actual Yield	Percent Yield	Actual Yield	Percent Yield	Actual Yield	Percent Yield
GSO4	0.4 g	14.4%	0.2 g	7.2%	1.5 g	53.9%
GSO6	0.3 g	9.3%	0.4 g	12.5%		

Figure 8. Yields for five syntheses.

Characterization of Product:

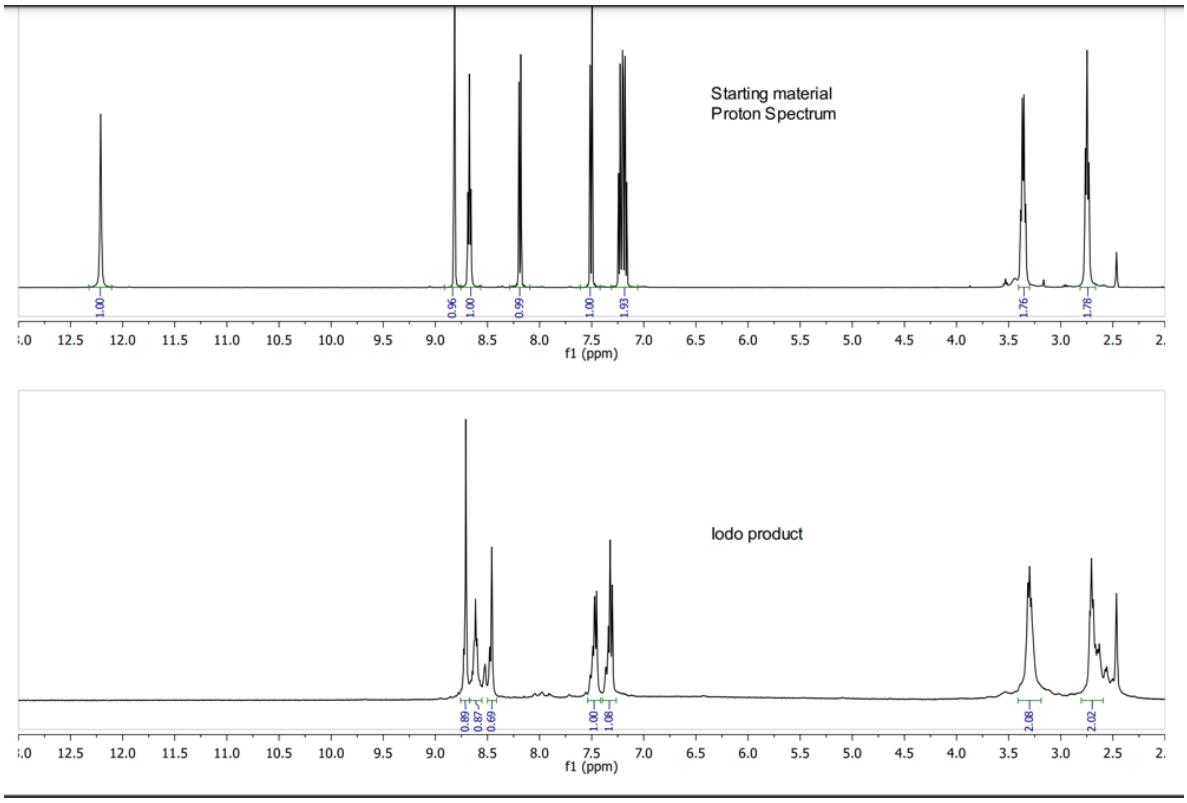

Figure 9. Proton NMR for the GSO6 compound. The spectra on the bottom is for the final product, and it does match to what was predicted. Since these compounds are symmetric, only the spectra for one arm of the compound will appear on the results.

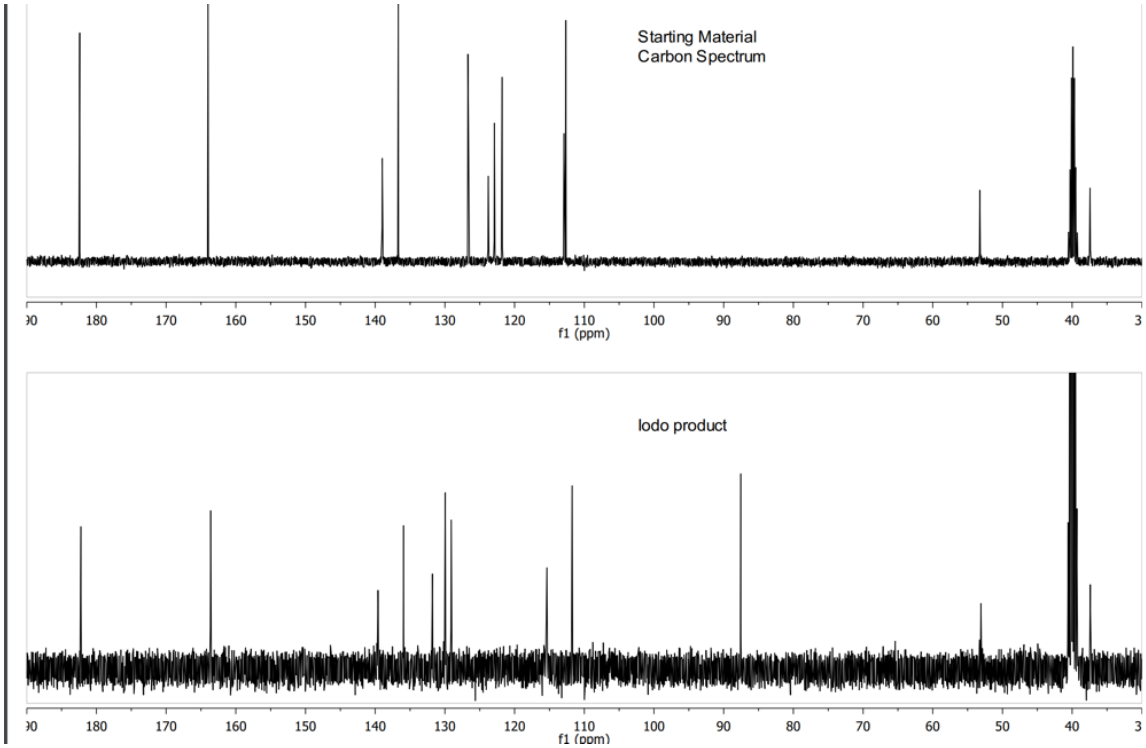

Figure 10. Carbon-13 NMR spectra for the GSO6 compound. The bottom spectra displays the C-13 NMR for the final product.

Cell Viability:

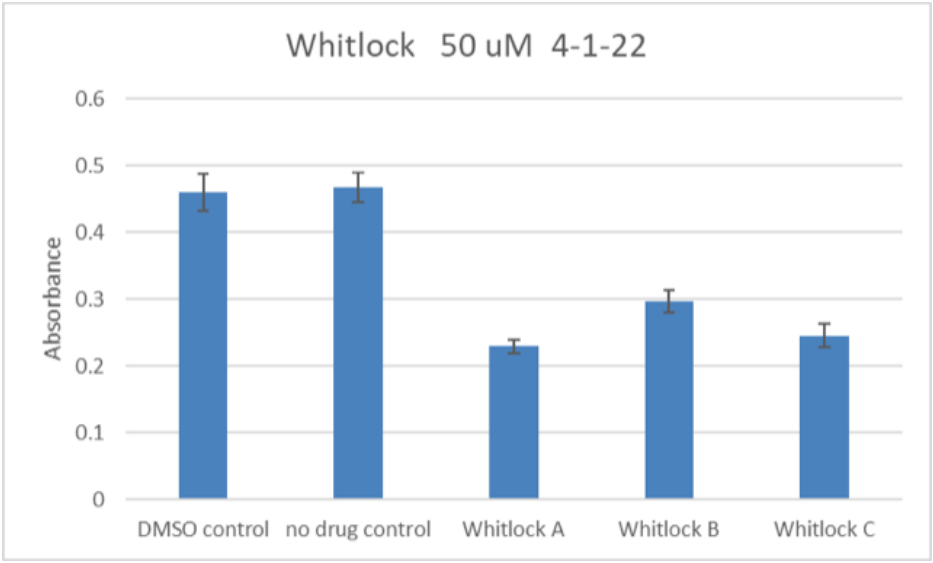

Figure 11. This is the raw average absorbance values for three compounds produced in the lab. Compound A is GSO2, compound B is GSO4 and compound C was produced by

a previous lab group. GSO2 showed to be the most effective against the prostate cancer cell line, PC3.

Discussion

Synthesis of all three compounds, GSO2, GSO4 and GSO6 was successful overall. Some synthesis trials did not produce a high yield, which is something that would need to be refined for future trials. Refining synthesis would include ensuring the TREN was over ice for a long enough time period. It is believed that some reactions did not synthesize as clean of a product as expected because the TREN was not allowed to cool down enough.

GSO6 was confirmed with both ^{1}H-NMR and ^{13}C-NMR spectroscopy to ensure the correct product was obtained. Since the product is symmetrical, the NMR spectrums do not show a peak for every proton or carbon of the molecule as a whole, instead it only displays peaks for one of the "arms". The peaks farthest upfield on the ^{1}H-NMR spectra (Figure 9) are representative of the two CH_2 groups between the center nitrogen and amine group of one arm. Moving downfield on the spectrum, the next three peaks, ranging from 7.4 ppm to 8.5 ppm, come from the hydrogens on the benzene ring of the indole. The peaks downfield from 8.5 ppm are believed to be from the hydrogen on the nitrogen of the indole and the remaining CH of the indole. On the ^{13}C-NMR iodo product spectrum (Figure 10), there are 12 distinct carbons. The peaks farthest upfield, around 37 ppm and 53 ppm, are the two CH_2 groups coming off the central nitrogen atom. The peak at 87 ppm is the carbon on the indole with the iodine attached. The electronegativity of iodine causes that carbon to be more shielded than the other carbons of the ring. The peak at around 113 ppm is more than likely the carbon that attaches the indole to the ketone

group. The next 6 peaks in the range of 115 ppm to 140 ppm are from the remaining carbons of the indole portion. The two peaks farthest downfield at 163 ppm and 183 ppm are the carbons of the ketone groups.

Two of the compounds, GSO2 and GSO4, were tested at 50 μM against PC3 cell lines. The death rates for both compounds is considered significant. The overall percent change for GSO2 was 0.23. The overall percent change for the GSO4 compound was 0.15. GSO2 caused a higher cell death rate compared to GSO4. Overall both compounds were effective at killing the PC3 cancer cells. GSO6 has not yet at this time been tested on any cancer cell cultures. Based on the cell culture data, it does not appear that the bromine substituent aided in killing more of the cancer cells. Bromine is the middle mark for halogens in regards to electronegativity. It would be interesting to see if using a less electronegative halogen, such as iodine, is more potent or if using one that is more electronegative, such as chlorine, is what is needed to see a difference in potency. Testing of all three compounds should also be conducted on other cancer cell lines to determine their effectiveness.

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