Design, Synthesis, and Biological Activity of *O*-(4-methyl)-phenyl-*N*-(9'-acridinyl)-hydroxylamine

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Frontiers of Science 2017

Acknowledgements

This research took place in the School Chemistry at the University of Northern Colorado in Greeley, Colorado. The project was funded by Union Pacific. Matthew DeSelm was very helpful in guiding me through the research, data collection, and instrument usage in the project. Saba Wolde was also helpful in guiding us through the instrument usage and advice during this research project. Thank you to everyone who contributed to the project, including Scott Newkirk and Ben Schottler. I would also like to thank Fifteen Mile Ranch for sponsoring me with room and board for the Frontiers of Science Institute.

Table of Contents

Abstract

DNA intercalators such as 9-aminoacridine derivatives, have been researched for their effectiveness as cancer treatments. They inhibit the DNA topoisomerase II and induce apoptosis in the cell, however, they cannot differentiate between cancerous and noncancerous cells. O-(4-methyl)-phenyl-N-(9'-acridinyl)-hydroxylamine, a 9-aminoacridine derivative, attempting to be synthesized, is a novel anti tumor drug. This drug is designed to be less susceptible to hydrolysis, making smaller dosages more effective in eradicating cancer as well a wider therapeutic window. Once the synthesis of O-(4-methyl)-phenyl-N-(9'-acridinyl)-hydroxylamine is successful the drug will be researched further in order to determine its properties and how it binds to the DNA.

Introduction

Cancer, as defined by the Webster dictionary, is “a malignant tumor of potentially unlimited growth that expands locally by invasion and systemically by metastasis”(3). One of the worst diseases that mankind has ever had is cancer. Cancer defeats the patient physically and mentally and it takes thousands of dollars and various methods to treat cancer. Although there are methods to treat cancer such as chemotherapy, radiation, hormone treatments, newer targeted therapies and immunotherapies the goal of this project is to create a more effective anti-tumor drug. This drug ,O-(4-methyl)-phenyl-N-(9’acridnyl)-hydroxylamine, is a 9-aminoacridine based compound with proven anti-tumor properties. Chemotherapy drugs kill the cancer cells by inhibiting different steps of cell division. O-(4-methyl)-phenyl-N-(9’acridnyl)-hydroxylamine is a DNA intercalate which inserts itself between the DNA strands during cell division (mitosis) and arrests the cell cycle, which leads to apoptosis, cell death. O-(4-methyl)-phenyl-N-(9’acridnyl)-hydroxylamine affects both healthy and cancerous cells, however, cancer cells divide rapidly, and are therefore are more susceptible to damage from the drug than normal cells.

There have been derivatives of 9-aminoacridine, which have been synthesized and tested for their antitumor properties in vitro, but many of these DNA intercalating drugs undergo hydrolysis when used in vivo, and are therefore ineffective. The objective of this research is to synthesize the drug O-(4-methyl)-phenyl-N-(9’acridnyl)-hydroxylamine, which would be less prone to hydrolysis in vivo, and therefore more effective in small doses. So far no scholarly research has been done about synthesizing the drug, O-(4-methyl)-phenyl-N-(9’acridnyl)-hydroxylamine, so the question is can this drug be

synthesized cost effectively, will it be less prone to hydrolysis, and will it have the desired anti-tumor properties?

Lit Review

Many research articles have been published regarding the anti tumor properties of 9-aminoacridine based drugs, but to understand their effectiveness, one must look at the functions of these drugs on a molecular level and how they interact with DNA on a cellular level.

Apoptosis occurs when cells are infected or reach the end of their life cycle. In a cancer cell, apoptosis does not function properly because the cancer cell overrides the cells function to kill itself, so it can continue spreading. In a study done by Tan et. al. it was found that cancer cells can go through apoptosis if the DNA cannot replicate. This means that making out drug a catalytic inhibitor of DNA topoisomerase means that the cycle of cell replication is broken down and cancer cannot continue to metabolize and instead will kill itself (9).

DNA topoisomerase is an enzyme that is crucial in mitosis and meiosis or cell replication. DNA topoisomerase monitors topological changes in DNA allowing for replication, transcription, and decantation. It works by unwinding the DNA before the helicase unzips it. If the helicase keeps unzipping the DNA and the topoisomerase is inhibited, the DNA will not be able replicate making cell replication not possible and therefore induces apoptosis (10). DNA topoisomerase is needed to replicate and grow, making DNA topoisomerase affecting drugs very effective in the battle against cancer. Drugs affecting DNA topoisomerase can be very effective treatments for cancer (6).

According to Galvez-peralta et al, chemotherapy drugs affect the cells in two ways, they can either be a DNA topoisomerase poison or a catalytic inhibitor of DNA topoisomerase. Topoisomerase poison is when the drug prevents DNA replication after

the DNA strands separate from each other. DNA poisons occur when the "cleavable complex" is stabilized and leads to covalent links between the enzyme and DNA, therefore leading to irreversible breaks and damage along the DNA. On the other hand, catalytic inhibitors of DNA topoisomerase can prevent any other step in the seven step catalytic cycle giving catalytic inhibitors of DNA topoisomerase a longer window to function (7).

In research done by Galvez, mice are used to test the effects of the 9-aminoacridine derivative on cancer. From the research, it can be concluded that 9-aminoacridine can break down the cancerous cell and induce cell death, although it is a catalytic inhibitor of DNA topoisomerase. Although this experiment was performed on mice, most of the results can be used with human DNA as well because of the vast similarities between our DNA compositions, making O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine, a catalytic inhibitor of DNA topoisomerase in our DNA as well (7).

In another article, Goodell (9) indicates that the 9-aminoacridine derived drugs are DNA catalytic inhibitors. By examining the other amino acridine derivatives and their behavior there is strong evidence to suggest that, O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine is a DNA catalytic inhibitor as well and will cause cancer cells to go into apoptosis.

Many currently available chemotherapy drugs are DNA catalytic inhibitors of topoisomerase in the cell but have no way of targeting the cancer cells specifically. Instead, the drug can affect many other cells which are beneficial to the body and lead to many side effects. 9-aminoacridine derived drugs are effective in killing the cancer cells

but they are susceptible to hydrolysis and have to be taken in higher doses. To minimize this effect, O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine, would be less prone to hydrolysis making it more effective in smaller doses for a longer period of time (4).

Research on the synthesis for another 9-aminoacridine derivative called O-phenyl-N-(9'-acridinyl)-hydroxylamine has already been synthesized by DeSelm (4). According to Ghosh (8) the synthesis of both is very similar with O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine, the only difference is an extra methyl group compared to O-phenyl-N-(9'-acridinyl)-hydroxylamine. In the first step, N-aryloxyimides and aryloxamines are synthesized. In the first step, Gosh et. al.'s research points out that p-iodotoluene and toluene can be used to make a Diaryliodonium salt. The process in which to combine the reactants is similar to the way that DeSelm combined his first two reactants to get Diaryliodonium salt as well.

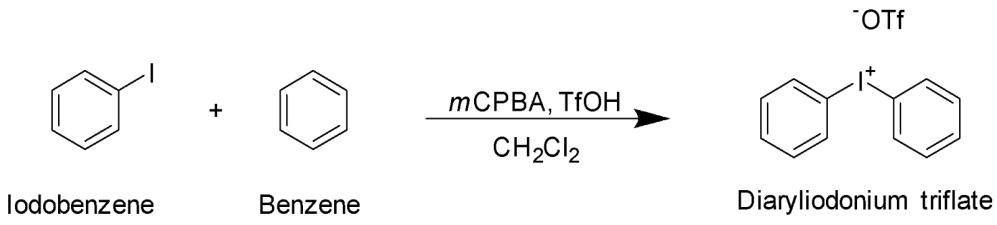


Figure 1. Step 1 in the synthesis of *O*-phenyl-*N*-(9'-acridinyl)-hydroxylamine

The second step is also very similar to the second step outlined by DeSelm in his research. The second step consists of reacting the Diaryliodonium Salt from the first step and combining it with N-hydroxyphthalimide to get N-phenyloxyphthalimide. This research differs from DeSelm's research because there is an extra methyl group attached to the compound which has properties to be synthesized more efficiently. Outlined below, in figure 2, is DeSelm's second step which he outlined for the second step in the synthesis of O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine.

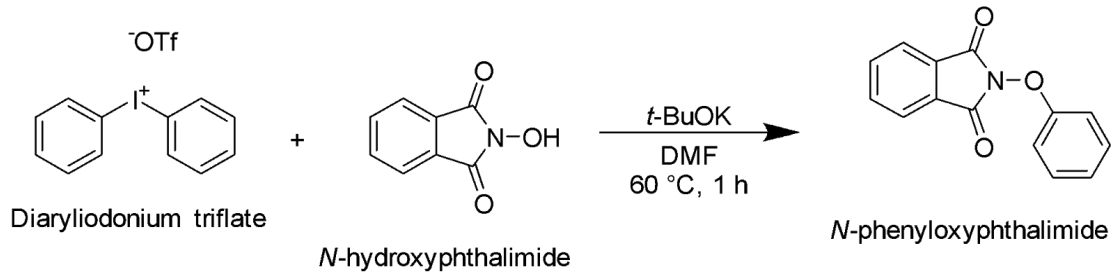


Figure 2. Step 2, Synthesis of *O*-phenyl-*N*-(9'-acridinyl)-hydroxylamine

The third step in synthesizing O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine is the hydrolysis of Hydrolysis of N-(4-methyl)-phenyloxyphthalimide. Which can be done by using the steps outlined by DeSelm once again. Once the N-(4-methyl)-phenyloxyphthalimine is created the next step begins, in which you do the hydrolysis of Hydrolysis of N-(4-methyl)-phenyloxyphthalimine to get O-(4-methyl)-phenyloxyphthalimide. Which can be done two different ways, all of them shown below. The final step is to synthesize O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine (4).For the fourth and final step of the synthesis of O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine was also outlined by DeSelm and the process is shown below in figure 4.

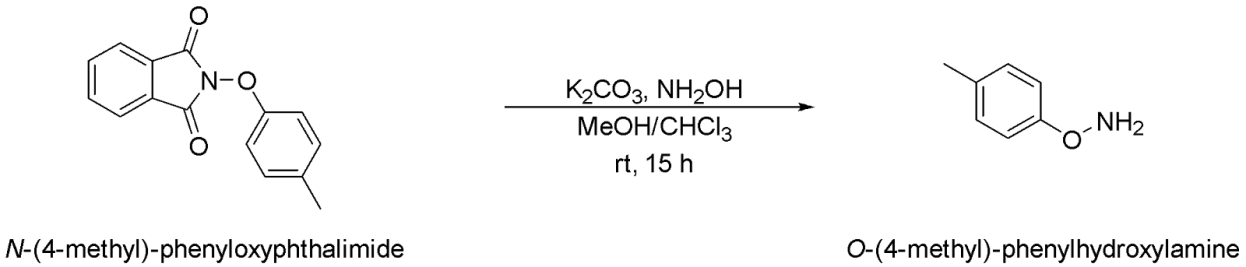
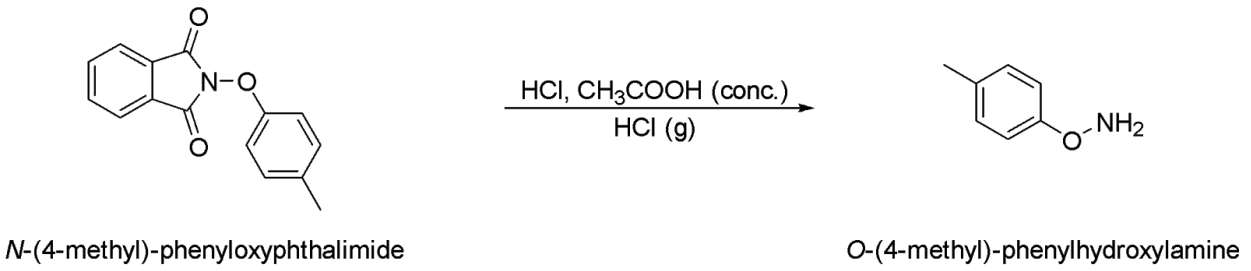
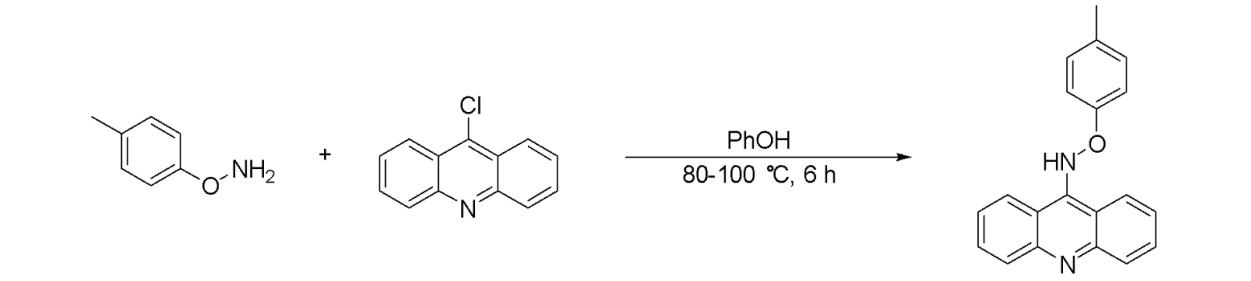


Figure 3. Step 3, Method 1 for the synthesis of O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine



N-(4-methyl)-phenyloxyphthalimide *O*-(4-methyl)-phenylhydroxylamine

Figure 4. Step 3, Method 2 for the synthesis of O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine



O-(4-methyl)-phenylhydroxylamine 9-chloroacridine *O*-(4-methyl)-phenyl-*N*-(9'-acridinyl)-hydroxylamine

Figure 5. Step 4, Synthesis of O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine

Cancer is an epidemic that affects everyone differently, so there is a need for a large variety of cancer treatments that not only eradicate cancer but also are cost-effective for patients. Although some of the drugs in the steps can be purchased, the objective of this research is also to be cost effective and intern easy and cost effective to manufacture. O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine hopefully has the potential to be less susceptible to hydrolysis, an effective antitumor agent, and also cost effective.

Methods

The synthesis of *O*-(4-methyl)-phenyl-*N*-(9'-acridinyl)-hydroxylamine is a multiple step product that consists of four steps. The first step, Synthesis of Diaryliodonium Triflate, is synthesized from p-iodotoluene and toluene as demonstrated in figure (6). Next *N*-(4-methyl)-phenyloxyphthalimide is synthesized by diaryliodonium salt and *N*-hydroxyphthalmine as shown in figure(insert number here). The next step, shown in figure(insert number here), is the Hydrolysis of O-(4-methyl)-phenylhydroxylamine. The final step of the reaction is the synthesis of *O*-(4-methyl)-phenyl-*N*-(9'-acridinyl)-hydroxylamine is outlined in figure(insert number here). Most of the steps used in the methods are outlined in DeSelm's paper as well as other sources (4).

Step 1: Synthesis of Diaryliodonium Triflate

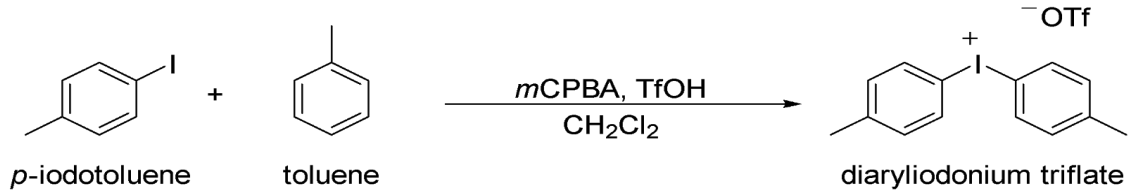


Figure 6.. Synthesis of Diaryliodonium Triflate

Step 2: Synthesis of *N*-(4-methyl)-phenyloxyphthalimide

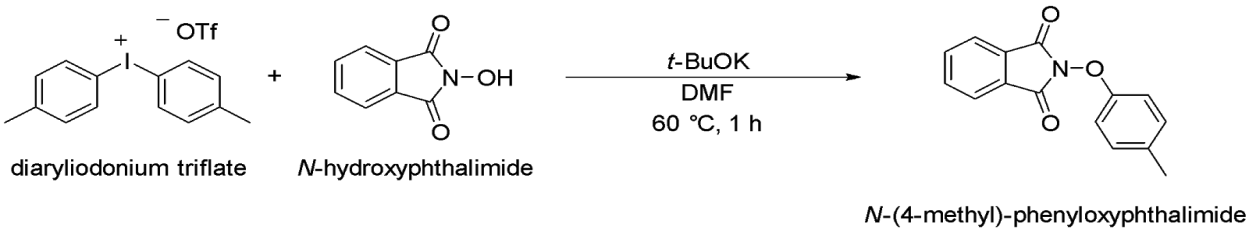
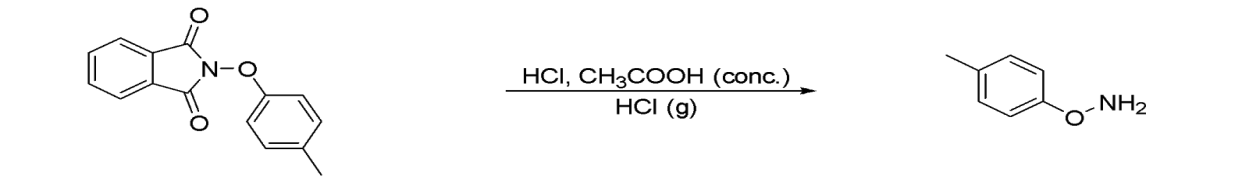


Figure 7. Synthesis of *N*-(4-methyl)-phenyloxyphthalimide

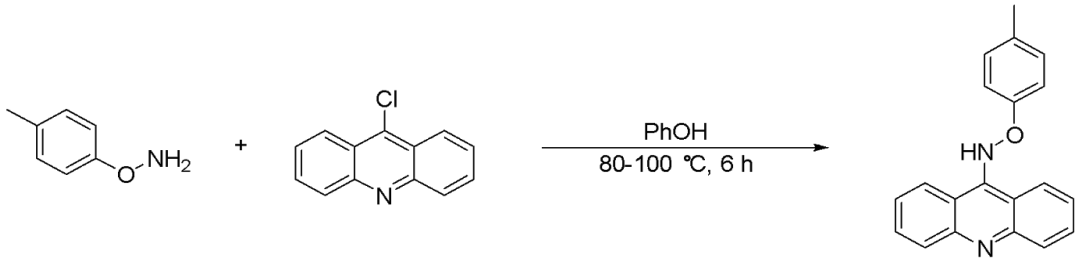
Step 3: Hydrolysis of O-(4-methyl)-phenylhydroxylamine



N-(4-methyl)-phenyloxyphthalimide *O*-(4-methyl)-phenylhydroxylamine

Figure 8 . Hydrolysis of O-(4-methyl)-phenylhydroxylamine

Step 4: Synthesis of *O*-(4-methyl)-phenyl-*N*-(9'-acridinyl)-hydroxylamine



O-(4-methyl)-phenylhydroxylamine 9-chloroacridine *O*-(4-methyl)-phenyl-*N*-(9'-acridinyl)-hydroxylamine

Figure 9. Synthesis of *O*-(4-methyl)-phenyl-*N*-(9'-acridinyl)-h

Synthesis of Diaryliodonium Triflate

This step is based on the procedure outlined by DeSelm as well as Bielwaski et al (2). The first step in the synthesis of Diaryliodonium Triflate will begin with a 25 mL round bottom flask which is stoppered. In order to perform the reaction under inert conditions, argon gas was inserted into the flask as well. Then 121 mg of *m*-chloroperbenzoic acid (*m*CPBA) and 2 mL of dichloromethane were added into the flask. Next 100.4 mg of p-iodotoluene to the flask and 0.047913 mL of toluene were added to the flask and the stir magnet was turned on. 120µL (3 equivalents) of trifluoromethanesulfonic acid (TfOH) was added into the flask via needle, after that the mixture turned dark green. Once TfOH was added into the flask, the mixture was left to stir for ten minutes. After the reaction was completed, the rotary evaporator was used to

separate the solvent. Then, diethyl ether was measured to 2 mL and was added to the concentrated solution and stirred for ten minutes once again. The flask was then placed in the refrigerator overnight. This reaction is illustrated in figure 7.

Synthesis of *N*-(4-methyl)-phenyloxyphthalimide

The second step in the synthesis of *O*-(4-methyl)-phenyl-*N*-(9'-acridinyl)-hydroxylamine starts with a 25 mL round bottom flask inserted with argon gas in order to mimic inert atmosphere conditions. These steps are detailed by Ghosh et. al. 2 mL of anhydrous dimethylformamide (DMF) and 61.7 mg of Potassium *t*-butoxide (*t*-BuOK) were added to the flask (8). Next, 82 mg of Hydroxyphthalimide was dissolved in the round bottom flask. The flask was then left to stir for ten minutes so the reaction could occur. Then the diaryliodonium salt from step one was added into the mixture. In order to get all the diaryliodonium salt out, the flask was rinsed with DMF. The solution was then heated to 60 °C and left to heat for one hour. After an hour the mixture was cooled to room temperature and added into a separatory funnel in order to separate the aqueous layer from the organic layer. 20 mL of deionized water and 20 mL of ethyl acetate were added to the separatory funnel to aid in the separation of the two layer. Once the two liquids were added, the separatory funnel was shaken then left to separate into two layers. Once the two layers were split, the aqueous layer was removed. 20 mL of water was once again added and shaken inside of the separatory funnel. After draining the aqueous layer once again. The solution was once again washed with 20 mL of concentrated brine solution in order to separate the rest of the aqueous layer from the organic layer. Once the aqueous layer is drained, the organic layer was poured into an Erlenmeyer flask and was dried using anhydrous sodium sulfate. Once the anhydrous sodium sulfate could not

retain any more water, it was filtered out using a gravity filtration system. In order remove the solution stuck in the anhydrous sulfate, ethyl acetate can be rinsed through the anhydrous sulfate. Ethyl acetate can be used in order to get the solution stuck in the anhydrous sulfate out. Next, the mixture is placed in the rotary evaporator in order to remove the ethyl acetate. After this step, there is an orange powder in the flask. In order to make sure the compound was formed an NMR was taken and can be seen in Figure _.

Hydrolysis of O-(4-methyl)-phenylhydroxylamine

In order to do step three shown in figure 8, N-(4-methyl)-phenyloxyphthalimide was added to concentrated hydrochloric acid (HCL, 12.1 M) and 15 mL of glacial acid. This process was done by the method outlined by Carlson (). The mixture was then allowed to reflux for two hours. In order to remove the rest of the solvent, the rotary evaporator is once again used. Next, the powder is scraped into a 50mL Erlenmeyer Flask. The residue in the flask is washed out using ionized water and poured into the Erlenmeyer flask. Then sodium hydroxide solution was added using a pipette in dropwise addition. Then the whole solution was poured into a separatory funnel, and 30 mL of dichloromethane and 20 mL of deionized water was used to separate the liquid into two layers. The solution was dried using anhydrous sodium sulfate once again and a gravity filter was used to separate the anhydrous sodium sulfate from the rest of the solution. Next HCl was bubbled through the mixture and left to stir for 30 minutes. Next, a suction filter was used to separate the solvent and the mixture was left to drive overnight.

Synthesis of *O*-(4-methyl)-phenyl-*N*-(9'-acridinyl)-hydroxylamine

The processes in this step are described by DeSelm (5). The final step of the reaction begins with 3.3 g of molten phenol (PhOH) and 9-chloroacridine was added to a

round bottom flask with a stir bar. O-(4-methyl)-phenylhydroxylamine is also added into this flask. Then, this flask is then heated to 80-90 °C and left for six hours. After six hours the flask is cooled to room temperature and added to 50 mL of CH_2Cl_2. Next, the mixture and 100 mL of sodium hydroxide solution are added to the separatory funnel and the excess phenol is removed. Once the excess phenol is removed, the organic mixture will be dried using anhydrous $MgSO_4$ and then gravity filtered to remove the anhydrous $MgSO_4$. In order to purify the substance, the mixture will be placed in the rotary evaporator.

Results and Discussion

N-phenyloxyphthalimide, from step two, was successfully synthesized after the second trial. After the first attempt in the synthesis of *N*-phenyloxyphthalimide a H-NMR was taken in order to determine if the right compound was created. The H-NMR scan in figure 10 demonstrates that the compound is present but in very low yields. After the second trial, a H-NMR was once again conducted. This H-NMR had more distinctive peaks. The second trial also had a product yield at 80%. In order to take the H-NMR, chloroform-d was added into the mixture to prepare a H-NMR sample. The mixture of the chloroform-d and our compound was then tested in the H-NMR machine. The H-NMR results can be seen in figure _. The rotary evaporator was then used at a temperature around 60 °C in order to remove the chloroform. Once the chloroform was removed, the compound and the round bottom flask were measured and were about 74.48 grams. The compound was then scraped out of the flask, the flask was washed and weighed again after it was dried. The amount of compound was then found by subtracting the weight of the compound and flask from the weight of the flask. It was derived that 0.2057 grams of the *N*-phenyloxyphthalimide were created.

Although we got an 80% yield, after trial two, the H-NMR spectroscopy scan reveals that very little of *N*-phenyloxyphthalimide was actually created. The peaks on the H-NMR that were taken demonstrate that there are other compounds besides *N*-phenyloxyphthalimide in the mixture. This means that the compound that was desired from this step, *N*-phenyloxyphthalimide, was created but was impure . The H-NMR from trial two is shown below in figure (10).

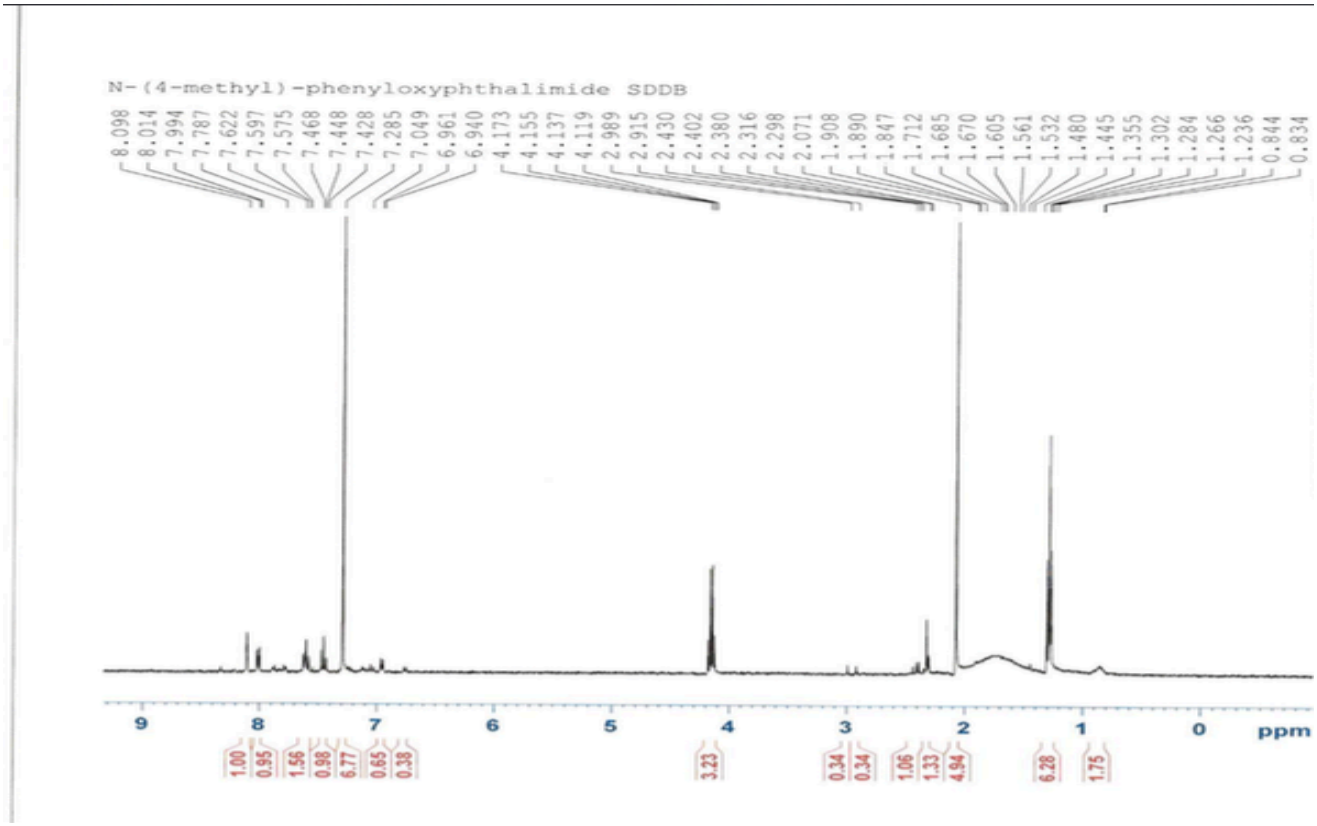


Figure 10. H-NMR after Step 2

In the H-NMR above, the peaks between 7.4 and 6.8 correlate with our compound but compared to the other peaks the peaks between 7.4 and 6.8 are significantly smaller revealing that very little of was synthesized in step two. Step three and four were not conducted because of time and material limitations.

Although we did not conduct step three and four, the data gathered from steps one and two prove that the synthesis of O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine will be extremely difficult because of the unwanted compounds also included in the mixture. The low yields from step one and two could also indicate that a new process in step two is needed to get rid of the DMF and other impurities. In addition, step three and four will need to be tested in order to determine their efficiency. More research proves that there is more than one process to synthesize the compound needed for step three. The

processes are further outlined in the DeSelm's paper. Although the reaction was incomplete, it is predicted that the synthesis of the compound is possible under inert and specific conditions.

Conclusion

Future research will involve new methods for the synthesis of the product in step three and more effective ways to synthesize *N*-phenyloxyphthalimide, from step two, more purely. This research will also include the synthesis of the products of step three and four, that was not able to be conducted. Once the drug, O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine, is synthesized, further research will be done to find new, more effective ways of synthesizing it. In addition, more research would have to be done to find the effect of the drug on DNA, to see whether the drug is truly effective in stopping DNA replication. The purity of the compound in each step would also have to be tested in order to make the drug in high yields. Research would also have to be conducted in vivo and vitro to see the effects of the drug and to determine whether the drug is truly less susceptible to hydrolysis and can last in vitro and vivo for a longer period of time than other chemotherapy drugs. Once the drug is tested in both vitro and vivo it will move on to see the effects in mice and other animals. Then the drug would have to be tested in humans and the side effects would be evaluated. Although O-(4-methyl)-phenyl-N-(9'acridnyl)-hydroxylamine was not synthesized completely in this six week period, research proves that the drug could be synthesized with specific and inert conditions.

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