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Using Light to Kill Cancer: Development of a Novel Photodynamic Therapy

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SENIOR THESIS APPROVAL

This Honors thesis entitled

"Using Light to Kill Cancer: Development of a Novel Photodynamic Therapy"

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Using Light to Kill Cancer-Development of a Novel Photodynamic Therapy

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Abstract:

Photodynamic therapy (PDT) is a treatment that uses special drugs called photosensitizing agents along with light to kill cancer cells. The specialized drugs only work after they have been activated or "turned on" by light. Photodynamic therapy may also be called photoradiation therapy, phototherapy, or photochemotherapy. In this research, I focused on the addition of four separate hydroxyl-amines to the unsubstituted porphyrin core, H_2TPPC. The hydroxyl-amines attached to the porphyrin core were 5-amino-1-pentanol, 2-amino-2-ethyl-1,3-propanediol,3-amino-propanediol, and 5-aminovaleric acid. The novel water soluble PDT agents, H_2TPP-A5OH, H_2TPP-2ET, H_2TPP-3NH, and H_2TPP-5AV, which were synthesized and purified, was structurally characterized by infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy(NMR), and UV-vis spectroscopy. The purity of the compounds were confirmed through analyzing using high performance liquid chromatography (HPLC). Finally, the cytotoxicity of the novel PDT porphyrins were determined in the presence and absence of light using MTT assay on MDA-MB-231 triple negative breast cancer cells and Ewing's Sarcoma cells.

Introduction

Although PTD is a relatively new treatment, a method of treating diseases with light can be traced far back in history. The ancient Egyptians utilized a combination of orally ingested plants containing light-activated psoralens and sunlight to treat diseases over 4,000 years ago. Contemporary photodynamic therapy began in 1900 with Oscar Raab and Hermann von Tappeiner. While in research, the two men noticed paramecia, that had been incubated with the dye, died when exposed to sunlight from an adjacent window.[1] From the time of this discovery, investigations were made to develop light therapy that would aid in killing cancer cells. Photodynamic therapy requires photosensitizing agents paired with specific amounts of light to decrease cancerous cells. The photosensitizing agents are molecules that produce reactive oxygen species that are activated when illuminated with light. When the molecules are exposed to the specific light source, they release oxygen that kills nearby cells. These are activated by specific wavelengths of light. The wavelength of light determines the depth the light penetrates

into the tissue. The photosynthesizing agents are injected directly into the bloodstream and the photosensitizing agents are absorbed into most cells throughout the body, but remain absorbed in only the cancer cells after a period between twenty-four and seventy-two hours. Therefore, photosensitizers have a higher cellular uptake in cancer cells over normal cells. When most of the agent has exited normal cells but remains in cancerous cells, the tumor is then exposed to the correct amount of light. The photosensitizer in the tumor absorbs the light and produces oxygen that destroys the nearby cancer cells. Along with directly killing the cancer cells, the photosensitizer may also damage nearby blood vessels preventing the cancerous tumor from receiving essential nutrients. Therefore PDT does not have the harmful side effects often encountered with chemotherapy or radiation therapy.

Porphyrin derivatives are the most common photosensitizing agent used in PDT. A porphyrin consists of four interconnected pyrrole rings making it an aromatic structure. Many porphyrins are naturally occurring. A well-known porphyrin is heme (Figure 1) which is a cofactor in hemoglobin. The ability of red light to penetrate tissue better than light of other wavelengths is a desired characteristic of photosensitizing agents for targeting cancer cells.

Breast cancer is divided into subgroups and distinguished by presence and appearance of the tumor based on a histological classification system. This histological classification system includes subgroups based on lymph node involvement, tumor size, and distant metastasis occurrence. Characteristics such as stage, grade, and receptor status help identify the type of breast cancer. The type of breast cancer used throughout this research was the subgroup MDA-MB-231 triple negative breast cancer. This

subgroup lacks the presence of three main receptors: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER2).[2] Unfortunately, treatments such as chemotherapy and radiotherapy target a combination of these receptors making triple negative breast cancer a difficult subgroup to treat. About fifteen percent of breast cancer patients are diagnosed with triple negative breast cancer. This subgroup proves to be more common in younger women.[1] The specific prognosis depends on the stage of the cancer, but the cancer is aggressive and highly likely to reoccur throughout the body. Because of the percentage rate, aggression, and reoccurrence, triple negative breast cancer was chosen for this research. Photodynamic therapy does not target specific receptors; the therapy pairs with light to diminish the cancerous cells making this a great method of treatment for triple negative breast cancer. Triple negative breast cancer was also chosen for this research because of its location on the body. Photodynamic therapy is known to be limited to areas where light can reach. This treatment option is prominently used to treat affected areas on the skin or in the case of this research, just under the skin. To determine if PTD was strictly limited to areas easily penetrated by light, this research also included trial runs with Ewing's Sarcoma.

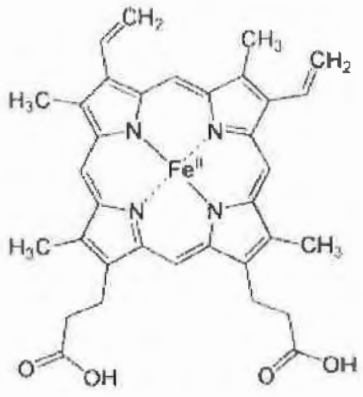


Figure 1.

Methods

H_2TPPC (Figure 2) was synthesized by combining a pyrrole with 4-formyl benzoic acid then undergoing an acid chloride formation (Figure 3). Once the acid chloride of H_2TPPC was synthesized, an additional side-chain was added to create the desired porphyrin (Figure 4). The side chain determined which porphyrin would be synthesized. In this research, all porphyrins were synthesized alike with the exception of the different side chains being adding onto the H_2TPPC. Figures 4-6 display the different porphyrins synthesized in this research. Before applying the porphyrin to either type of cancer cells within this research, a series of characterization steps were completed to correctly identify the desired porphyrin. After synthesizing the novel water-soluble porphyrin, before characterization, the porphyrins were purified through a series of two columns, Sephadex G-50 and Sephadex LH-20. The brown liquid exiting the column was discarded until it turned purple. After this moment, the eluent was collected then dried by using a roto-vap within the laboratory. This procedure was done two different times to ensure purity. The characterization steps were critical in this research. The porphyrin was first characterized by infrared spectroscopy. Infrared spectroscopy (IR spectroscopy) was used to identify certain peaks found within the IR spectrum. Prior to running the porphyrin using the IR spectrometer, the original amine was characterized to serve as a parallel example. The amine and the porphyrin, because of correct characterization, portrayed similar peaks on each absorption spectrum.

A calculated concentration of the porphyrin was placed into an Ultraviolet-visible spectrometer. Ultraviolet-visible spectroscopy (UV-vis) refers to the absorptions

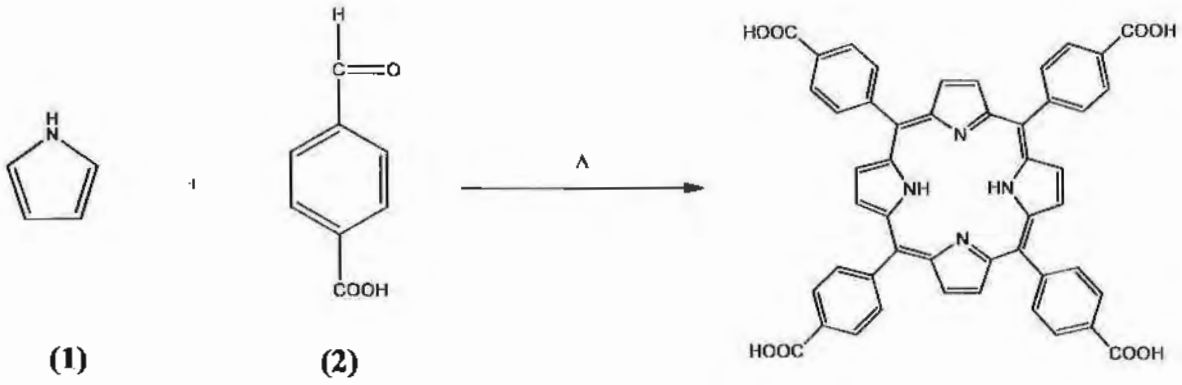
spectroscopy in the ultraviolet-visible spectral region. Porphyrins are characterized by the presence of an intense UV-vis band at approximately 415nm called the Soret band. Using Beer's Law, the specific absorption of the porphyrin was calculated. Beer's Law also aided in calculating the appropriate concentration of the porphyrin needed to put into the cell plate for MTT assay.

The porphyrin was then characterized by nuclear magnetic resonance spectroscopy. Nuclear magnetic resonance spectroscopy (NMR spectroscopy) is a technique that allows characterizations of organic molecules. Again, the original amine was tested to provide a comparison to that of the desired porphyrin material. The graphs of the porphyrin displayed properties of the original amine proving that the porphyrin was correctly characterized.

After each characterization of the porphyrin was complete, the purity of the compound was analyzed through high performance liquid chromatography. High performance liquid chromatography (HPLC) is a highly improved form of column chromatography like previously used in this research. The solvent is forced through the column under high pressures making this technique much faster than column chromatography. A much smaller particle size for the column packing material is utilized which allows a greater surface area for interactions between the stationary phase and the molecules moving through it. This technique gives a much better separation of the components, which is why the HPLC technique was used to determine the purity of the compound. Having a porphyrin purity no less than 97% pure, the porphyrin was now ready to be placed into the cell plate.

Prior to applying the prepared porphyrin into the cell plate, the cell plate needed preparation. After the cancer cells were cultured in medium, the cells were injected into two parallel 96-well plates. Appropriate concentrations of the porphyrins were injected into the cells approximately 72 hours after plating. The calculated concentrations were 1μM, 3μM, 10μM, 30μM, and 100μM. The plates were prepared in duplicated to test under both light and dark conditions. One plate was exposed to light for approximately 15 minutes 24 hours after the porphyrin was added. During this time, the parallel plate was kept in complete darkness. Three days after the exposure to light, the cytotoxicity of the porphyrins were assayed using MTT assay.

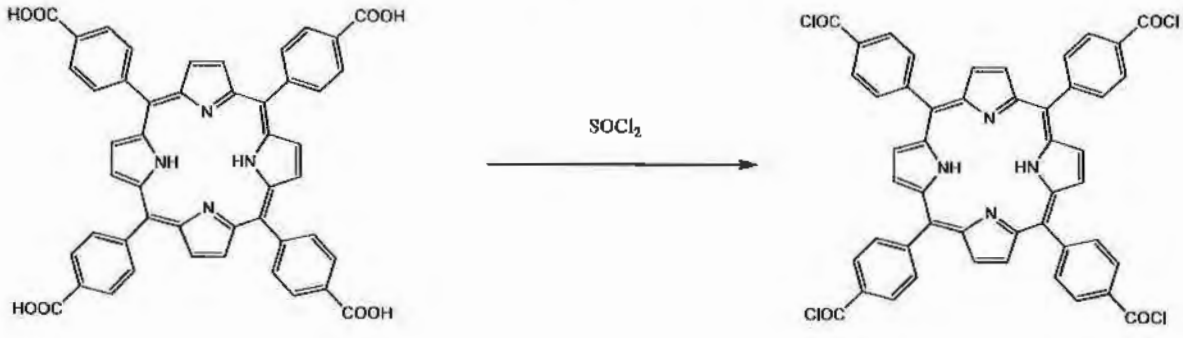
Reaction 1



(1) **(2)** **(3)**

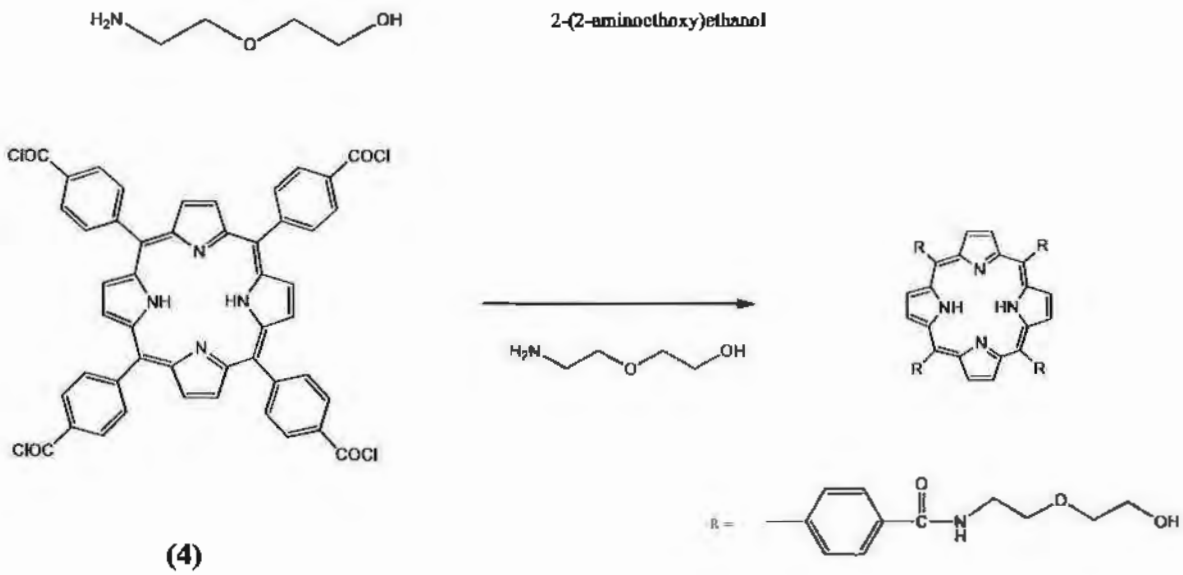
Figure 2.

Reaction 2



(3) **(4)**

Figure 3.



(4) **(5)**

Figure 4.

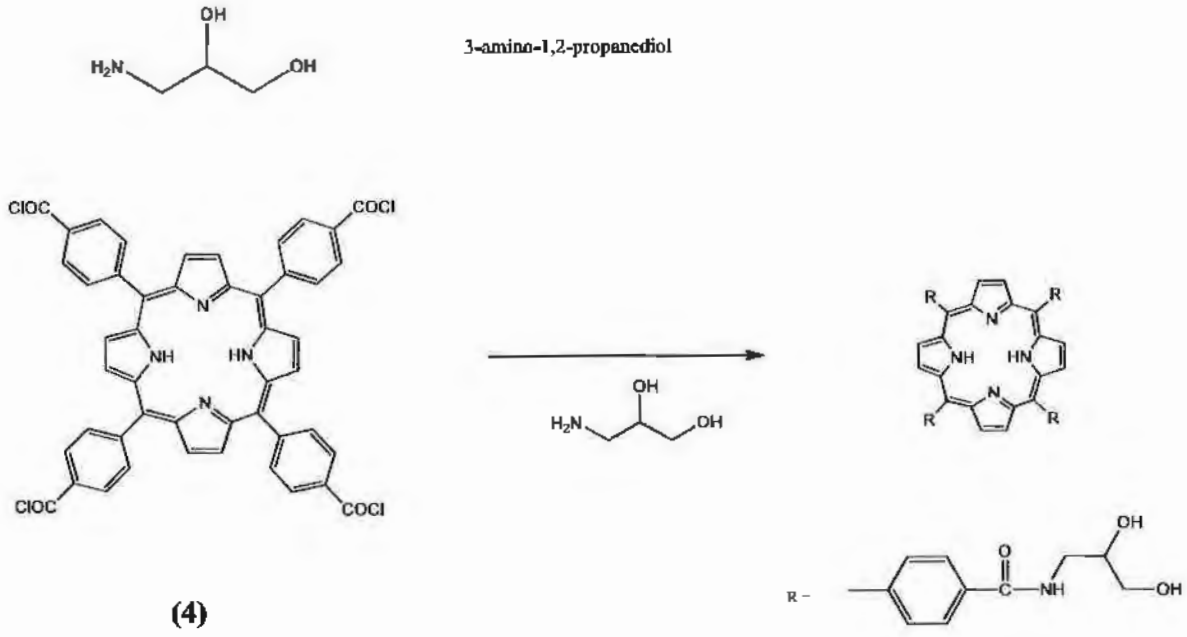


Figure 5.

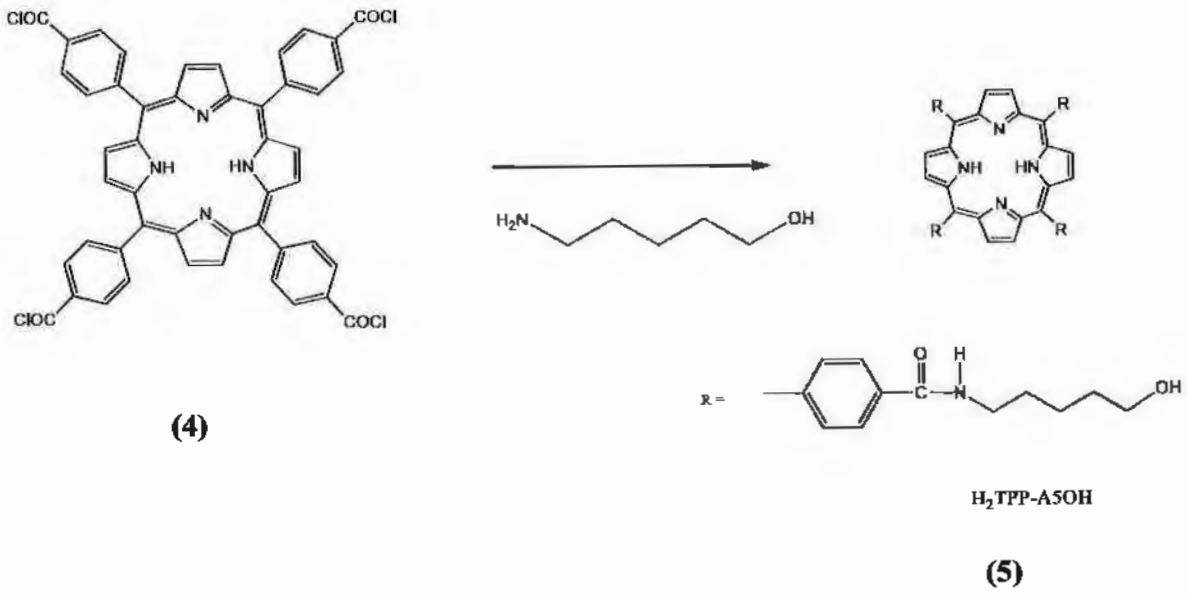
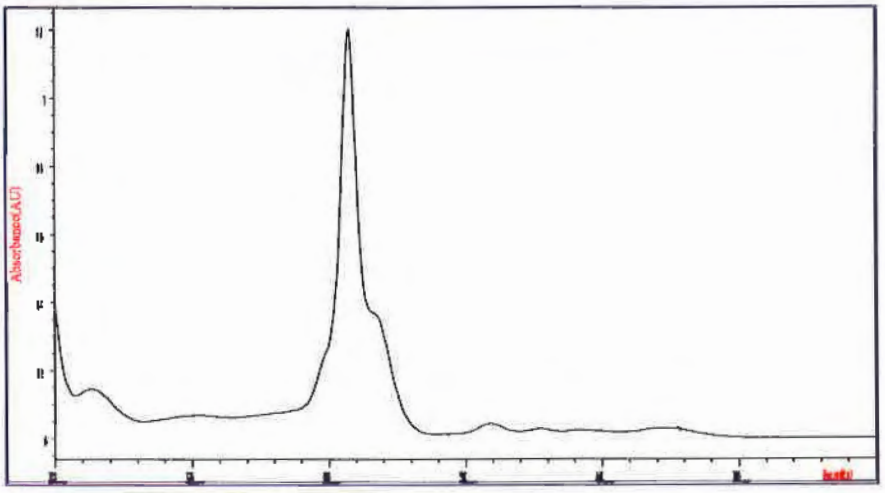


Figure 6.

Results



Peaks (nm)	(mM cm)
415	303.99
434	1.84
518	0.213
655	0.154

Figure 7.

Figure 7 is the UV-vis Spectroscopy graph of the porphyrin H_2TPP-A5OH in H_2O. This graph showed the absorbance of a typical porphyrin at the peak located at 415nm. Beside the graph is the table used to conduct Beer's law in order to calculate the desired concentration of porphyrin to be injected into the different cancer cells.

Figure 8.

Figure 8 is the UV-vis spectroscopy graph of the porphyrin H_2TPP-2ET in H_2O.

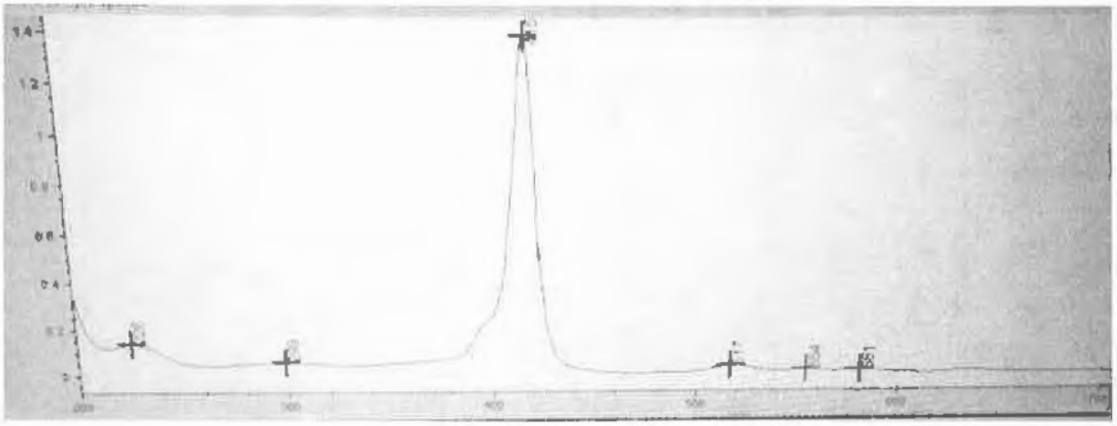


Figure 9.

Figure 9 is the UV-vis spectroscopy graph of the porphyrin H_2TPP-3NH.

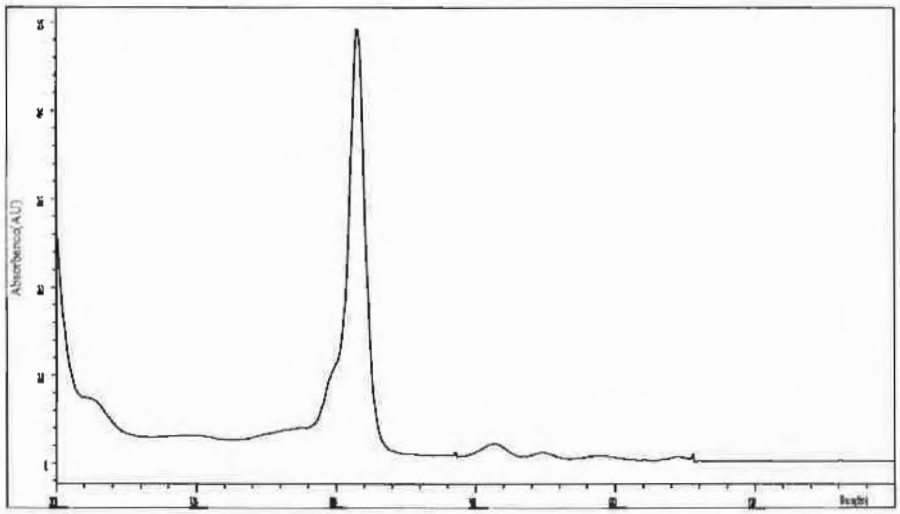


Figure 10.

Figure 10 is the UV-vis spectroscopy graph of the porphyrin H_2TPP-5AV.

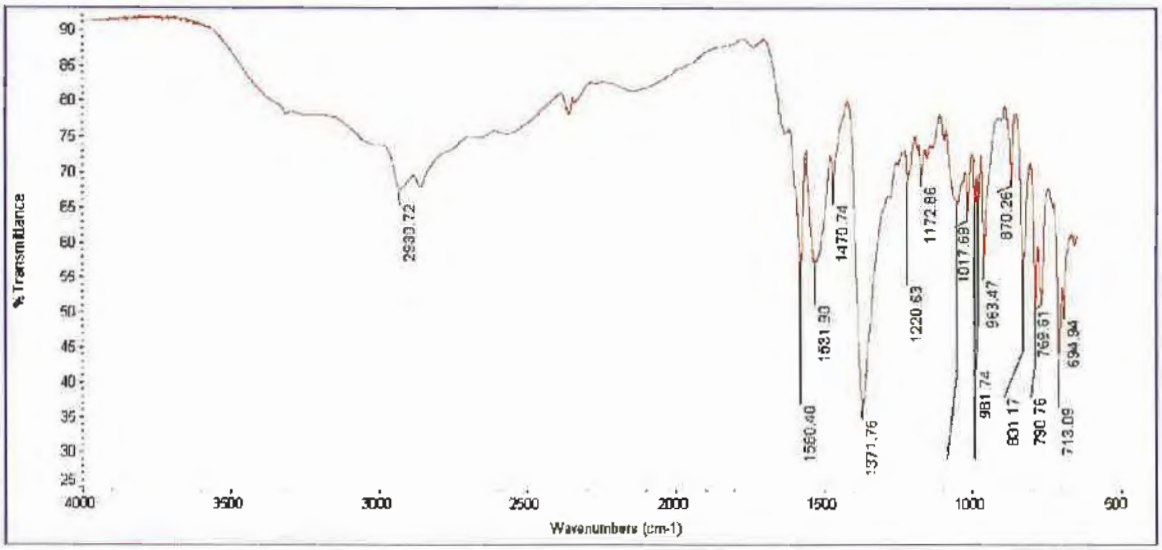


Figure 11.

Figure 11 is the IR Spectroscopy graph of the porphyrin H_2TPP-A5OH. This graph shows the peaks of both the original amine as well as the porphyrin core which characterized the porphyrin as H_2TPP-A5OH.

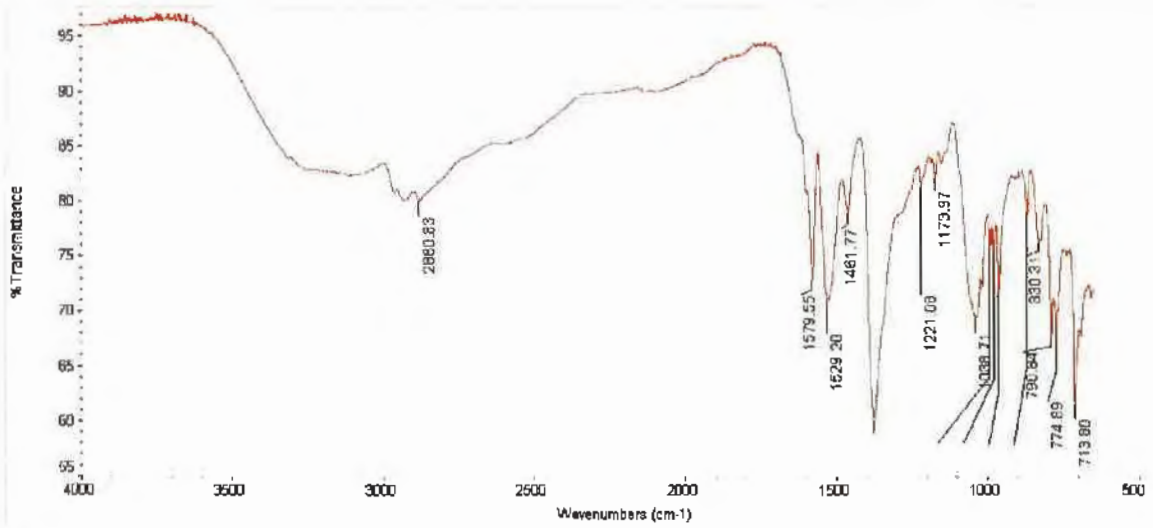


Figure 12.

Figure 12 is the IR spectroscopy graph of H_2TPP-2ET.

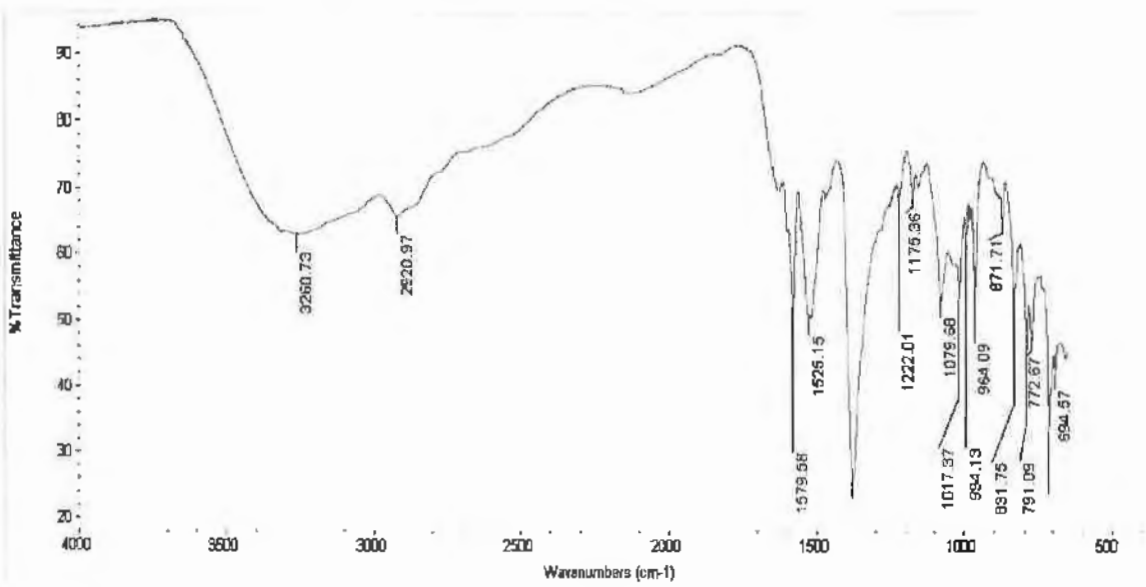


Figure 13.

Figure 13 is the IR spectroscopy graph of H_2TPP-3NH.

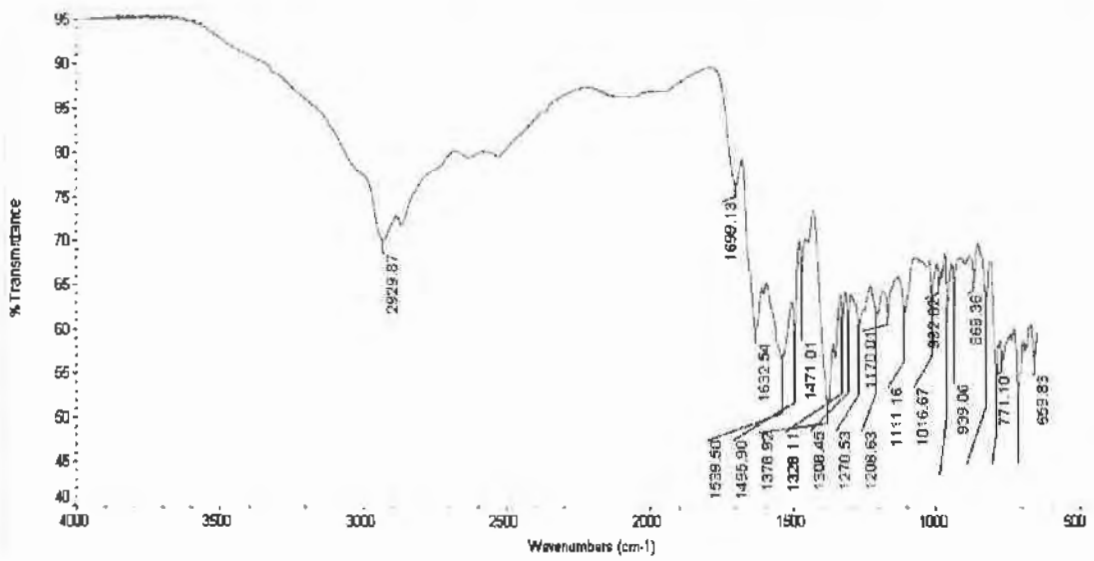


Figure 14.

Figure 14 is the graph of the IR spectroscopy of H_2TPP-5AV.

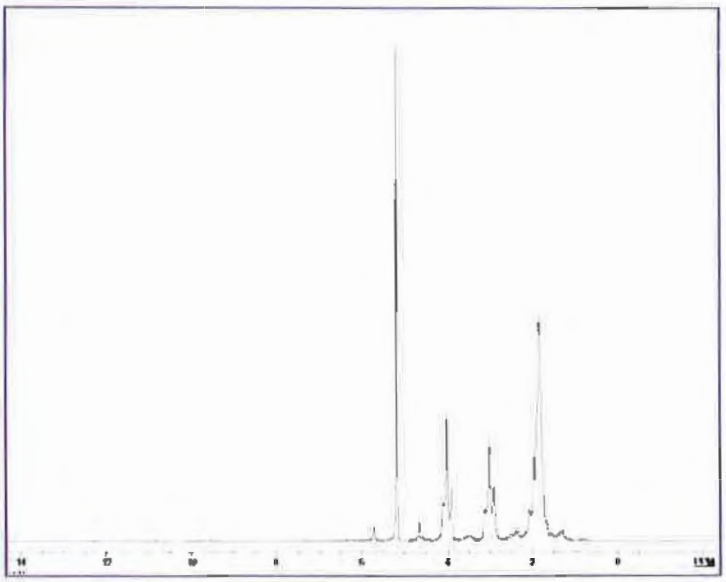


Figure 15.

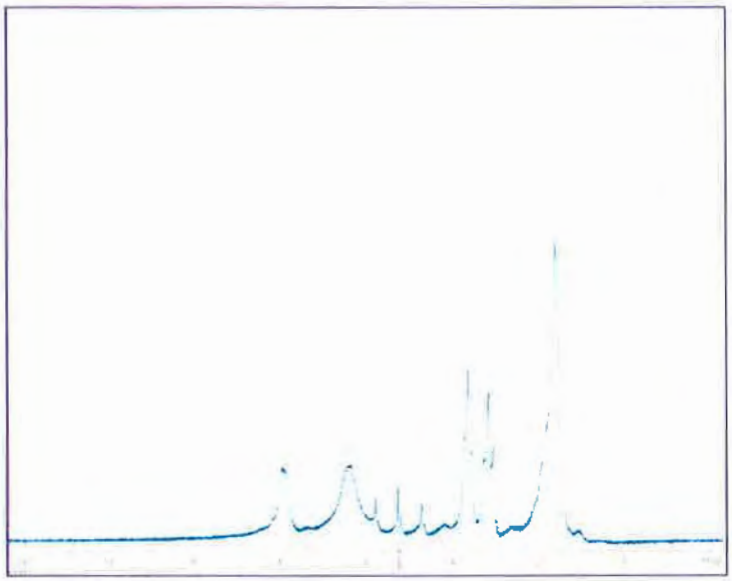


Figure 16.

Both figure 15 and figure 16 are NMR spectroscopy graphs. Figure 15 represents the NMR spectroscopy graph of the attached amine, 5-amino-1-pentanol. Figure 16 represents the NMR spectroscopy graph of the synthesized porphyrin, H_2TPP-A5OH. Having the figures parallel to one another presents a clear representation that the correct porphyrin was synthesized. The peaks of each graph are identical as desired. In figure 16, there are two new peaks which are characteristic of a porphyrin.

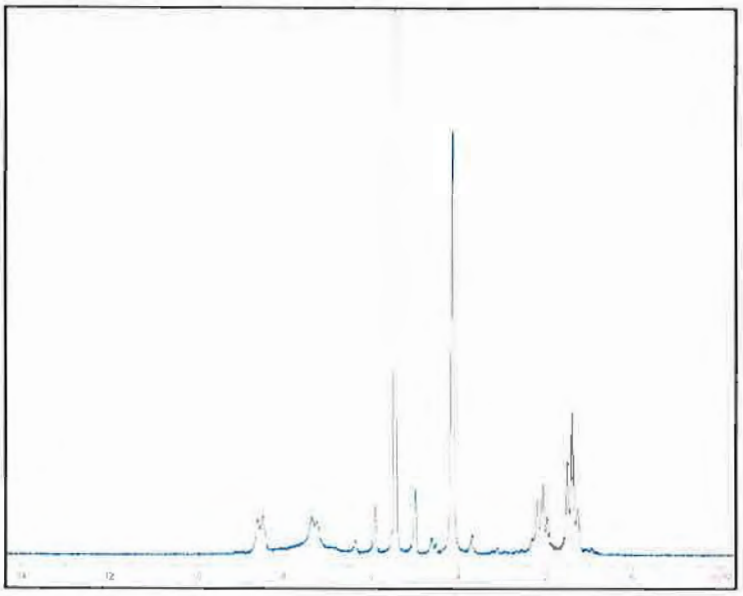


Figure 17.

Figure 17 is the NMR spectroscopy graph of H_2TPP-2ET.

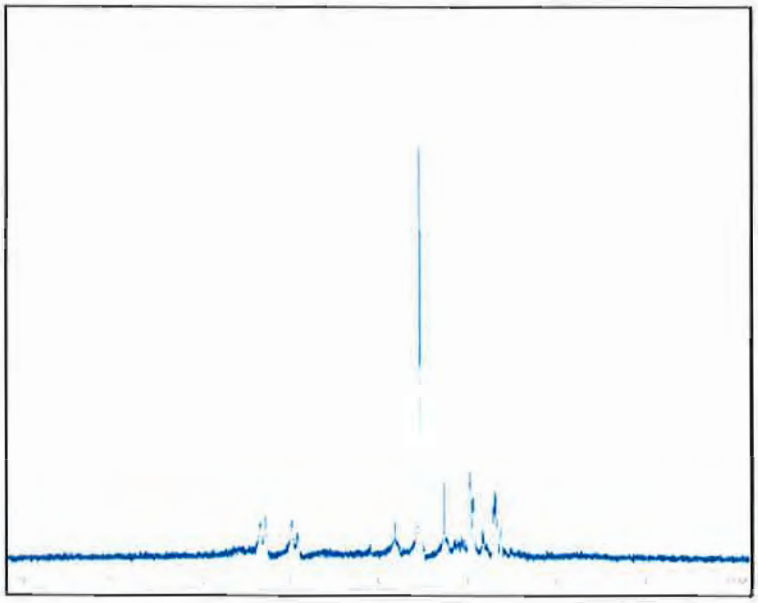


Figure 18.

Figure 18 is the NMR spectroscopy graph of H_2TPP-3NH.

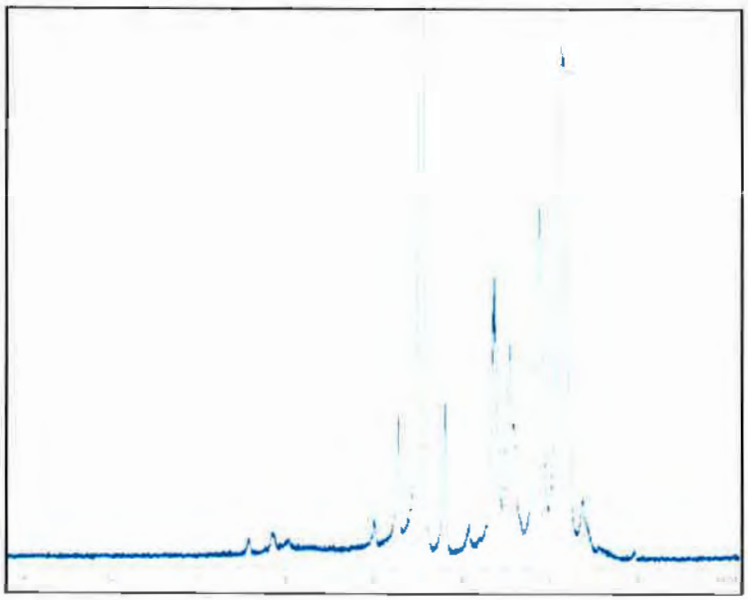
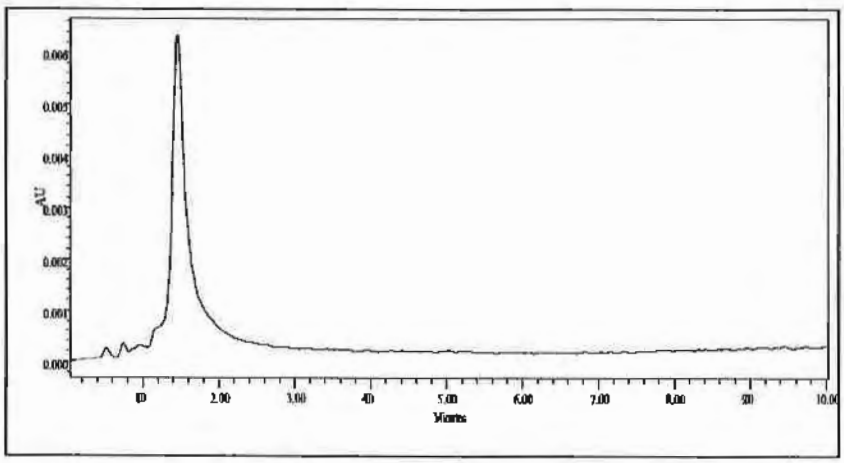


Figure 19.

Figure 19 is the NMR spectroscopy graph of H_2TPP-5AV.



Purity: 98%
Waters Nova-Pak C18
3.9 x 150 mm column
Solvent: 100% Acetonitrile
Flow Rate: 1.00 mL/min

Figure 20.

Figure 20 is a graph of the HPLC test. After characterization of the porphyrin, the purity was analyzed through high performance liquid chromatography. The purity of $H_2TPPA5OH$ was 98% pure which allowed the porphyrin to be prepared to be injected into the cancer cells.

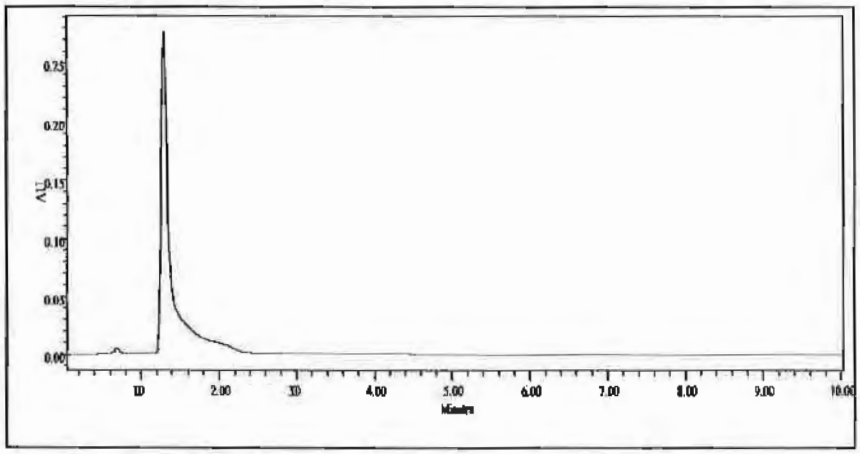


Figure 21.

Figure 21 is a graph of the HPLC test conducted on H_2TPP-3NH.

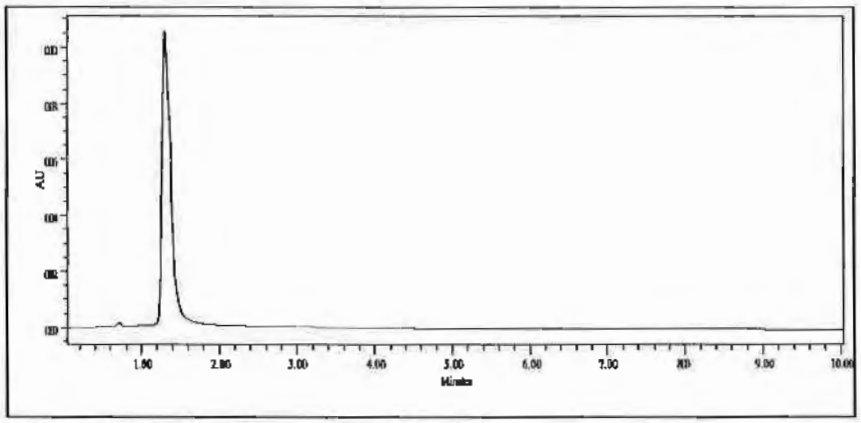


Figure 22.

Figure 22 is a graph of the HPLC test conducted on H_2TPP-5AV.

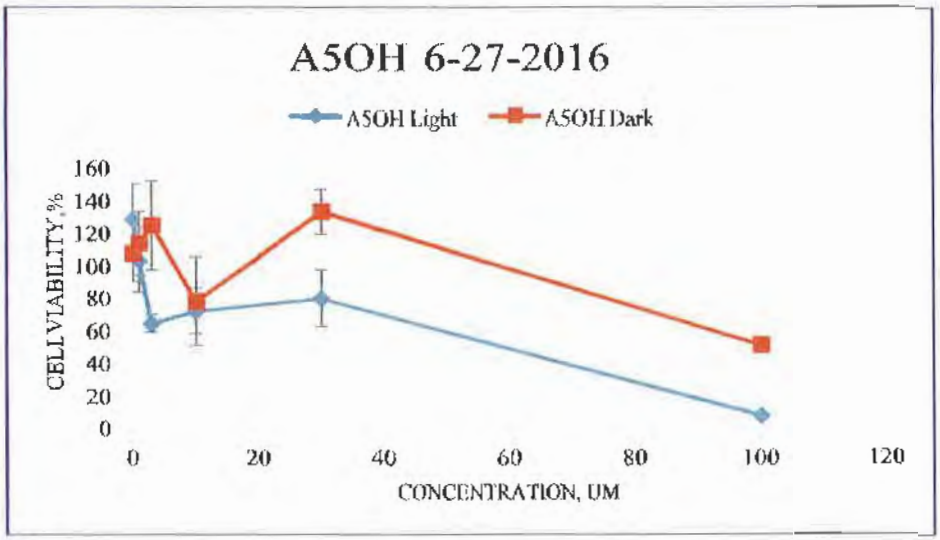


Figure 23.

Figure 23 is a graph of the MTT assay results of H_2TPP-A5OH injected into MD-MB-231 triple-negative breast cancer cells. The graph includes the results from both the 96-well plate exposed to light and the 96-well plate kept in darkness. The results show a greater cell death in those cells exposed to light.

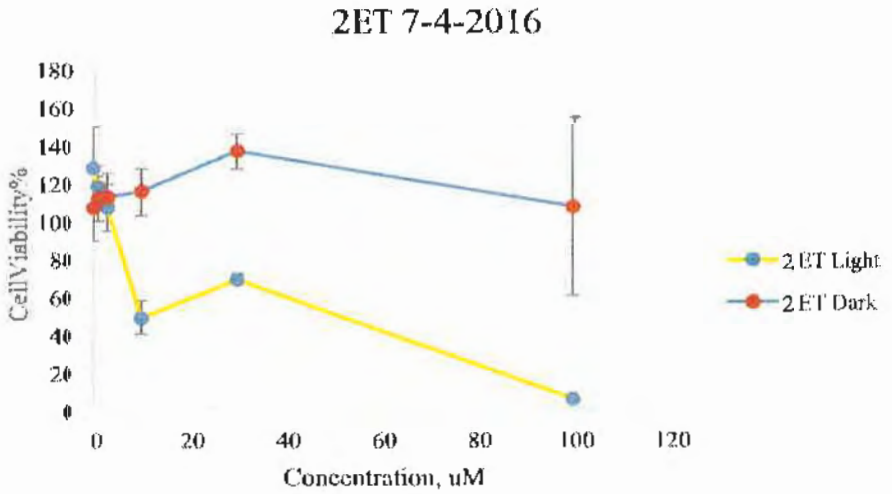


Figure 24.

Figure 24 is a graph of the MTT assay results of H_2TPP-2ET into MD-MB-231 triple breast cancer cells.

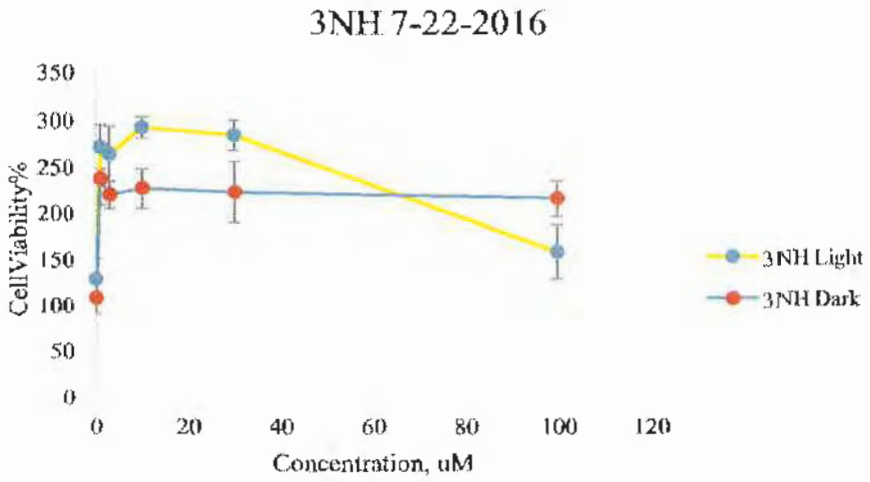


Figure 25.

Figure 25 is a graph of the MTT assay results of H_2TPP-3NH injected into MD-MB-231 triple negative breast cancer.

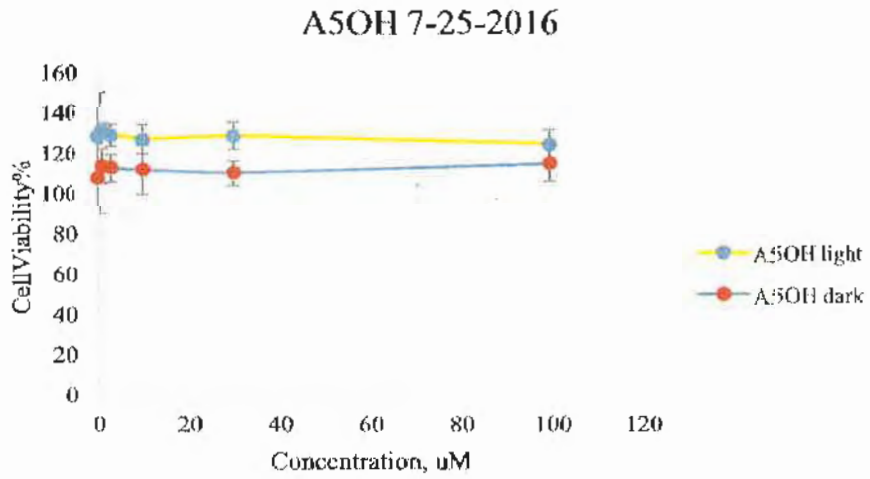


Figure 26.

Figure 26 is a graph of the MTT assay results of H_2TPP-A5OH injected into Ewings sarcoma cells.

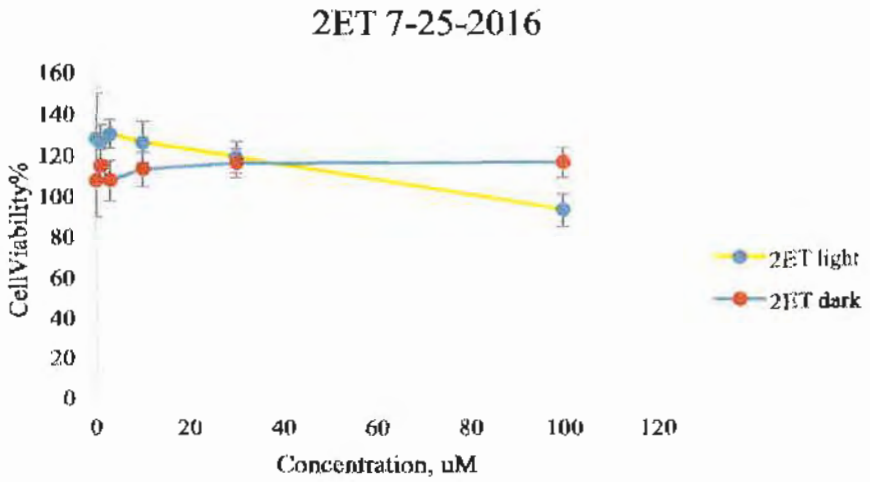


Figure 27.

Figure 27 is a graph of the MTT assay results of H_2TPP-2ET injected into Ewings sarcoma cells.

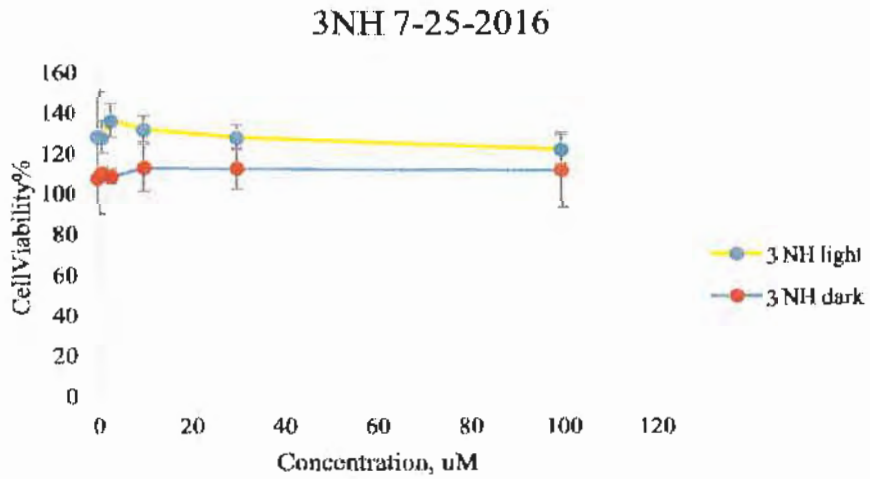


Figure 28.

Figure 28 is a graph of the MTT assay results of H_2TPP-3NH injected into Ewings sarcoma cells.

Conclusion

Throughout this research, a series of novel water-soluble porphyrins were synthesized and purified. Characterizations of the compounds were determined through infrared spectroscopy, nuclear magnetic spectroscopy, and ultraviolet-visible spectroscopy. The spectrums not only identified the correct compound, but also exhibited the purity of the compound. At greater than 98% pure, the porphyrin identified was then used as a photosynthesizing agent with MDA-MB-231 triple-negative breast cancer cells and Ewings Sarcoma cells. MTT assay was preformed to achieve the results. At a concentration of 60μM to 100μM, cytotoxicity becomes evident. This research proves that photodynamic therapy could treat triple negative breast cancer cells at a high concentration of 60μM to 100μM. Unfortunately, this concentration is rather high and could be detrimental to the human body. To improve this portion of the research, a different hydroxyl amine should be added onto the porphyrin core structure in order to alter the characteristics of the compound. Cytotoxicity at a low concentration is desired for the best possible results with triple negative breast cancer cells. With Ewings sarcoma cells, this research would need further advancement because the cell viability percentage was high no matter the concentration of the synthesized porphyrins. With further research, photodynamic therapy has the potential to diminish tumors in the body without harmful side effects.

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