4-20-2022

Developing Novel Water-Soluble Porphyrins for Potential Use as Photosensitizers in Photodynamic Therapy

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A Senior Thesis by Kayla R. Whittington

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Acknowledgements

I wish to acknowledge the J.D. Patterson Summer Research Program at Ouachita Baptist University for their funding of this research. Secondly, I would like to thank Dr. Timothy E. Hayes, the principal investigator for the cytotoxicity testing and cell culturing. Next, I wish to acknowledge Gabe Poe, an undergraduate researcher who led the cytotoxicity testing and cell culturing and Marly Welborn, an undergraduate researcher who completed the Summer 2022 ZnTPP-TRIS trial and has continued this research effort of mine. Lastly, I would like to thank my advisor and mentor, Dr. Joseph E. Bradshaw, with whom it has been a great privilege to work alongside on this research and thesis.

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ABSTRACT

Photodynamic therapy (PDT) is a treatment modality for various illnesses, including some types of cancer. Lung cancer is the leading cause of cancer death in the United Sates. The prevalence of lung cancer in certain gender, racial, ethnic, and socioeconomic groups add to existing health disparities in the United States. For this reason, it is necessary to address the social determinants underlying lung cancer disparities, as well as improve treatment options. These treatment options should be cost effective, convenient, and increase survival rates. This research focused on synthesizing novel water-soluble porphyrin compounds for use as photosensitive agents in PDT for the treatment of lung cancer. Three novel water-soluble compounds were synthesized. The compounds were then purified and characterized prior to being tested on an A549 non-small cell lung cancer line *in vitro*.

BACKGROUND

Introduction

One in every three people in the United States is affected by cancer.[1] Cancer is caused when cells that are older or abnormal do not die as they should. As a result, the older or abnormal cell keeps making copies of itself that eventually crowd normal tissues. Certain types of cancer, such as lung cancer, begin in one part of the body and spread, or metastasize to other parts of the body. Lung cancer is a type of solid tumor cancer.[1]

Lung cancer is the second most common cancer and leading cause of cancer death in the United States.[2] Two types of lung cancer are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). They both cause similar symptoms and are capable

of metastasizing. Some patients can be diagnosed with both SCLC and NSCLC. Anatomically, the cells for NSCLC are larger under a microscope. SCLC tends to be more aggressive and spread more rapidly, but NSCLC is more common. Around 84% of all lung cancers are non-small cell lung cancer.[3] NSCLC has a five year survival rate of 23%, while SCLC has a five year survival rate of 6%.[3] Adenocarcinoma is a type of NSCLC which occurs in the outer part of the lung beginning in the mucus making cells that line the alveoli.

Lung Cancer Disparities

With the ongoing COVID-19 pandemic, disparities in healthcare have begun to gain more attention.[4] This recent gain in attention is due largely to the recognition of disparities experienced by certain racial and ethnic minority groups in regard to COVID-19 illnesses, treatment, and healthcare.[4] However, healthcare disparities are not a new problem. The COVID-19 pandemic has simply helped to bring existing healthcare disparities to light. Inequalities that exist in the social determinants of health are responsible for these disparities. Some of these determinants include neighborhood and physical environment, health and healthcare, occupation and job conditions, income, and education. Additional factors such as racial discrimination and chronic stress also influence these social determinants of health.

The disparities that exist for lung cancer vary, but factors like gender and ethnicity can put people at a health disadvantage.[4] Lung cancer is ranked first for women's related cancer deaths, and is responsible for 26% of all cancer deaths among women.[5] Many incidents of lung cancer are due to a history of smoking. Smoking is one

of the underlying determinants of health disparities that exist for lung cancer. For example, Native Americans have some of the highest prevalence of smoking and lung cancer is one of the leading causes of death for Native Americans.[6]

Lung cancer is highly prevalent in Black, Asian, and other minority groups, as well as in women. Adenocarcinomic NSCLC is the most common form of lung cancer and is diagnosed mainly in people who smoke or have smoked. It is also more commonly diagnosed in younger adults and in women.[7] For these reasons, it is important to address both the determinants that lead to these disparities and to create new and improved treatment options for adenocarcinomic NSCLC. As part of the research for this thesis, three novel photosensitive agents were synthesized, purified, and characterized for potential use in photodynamic therapy. These potential PDT agents were then tested on an adenocarcinomic non-small cell lung cancer cell line.

Photodynamic Therapy (PDT)

Introduction to PDT

One potential treatment option for patients with non-small cell lung cancer is Photodynamic therapy (PDT). Photodynamic therapy is a treatment where a patient is injected with a photosensitive agent that preferentially accumulates in tumor tissue. When the tumor is exposed to light for a certain amount of time, the photosensitizer is activated, and selectively destroys the tumor. The process of photodynamic therapy is thought to occur via a type II photochemical reaction. Light excites a photosensitive electron which goes through intersystem crossing to form a triplet state. This excited electron reacts with oxygen, creating singlet oxygen that is able to destroy cancer cells.[8]

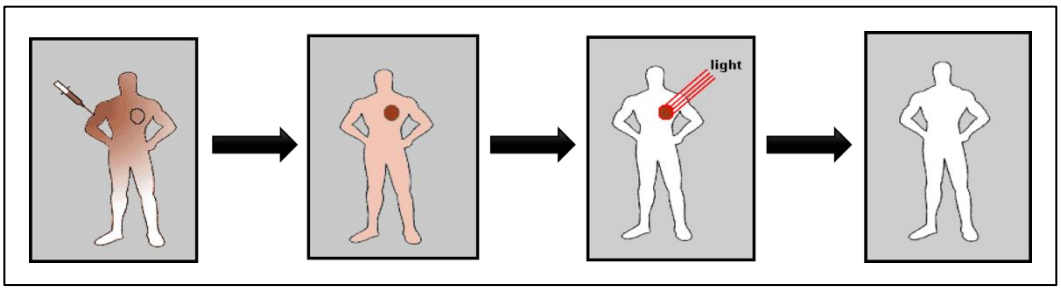

Figure 1. Photodynamic Therapy

Advantages of Photodynamic Therapy

Traditional cancer therapy, such as chemotherapy with cisplatin, has a high toxicity, undesirable side effects, and drug resistance.[9] Because traditional cancer therapy targets all rapidly dividing cells, patients often lose their hair and have gastrointestinal issues. Other traditional cancer therapy includes surgical resection.

Photodynamic therapy presents an alternative treatment option for lung cancer. It is non-invasive and would not require hospitalization. For treating lung cancer using PDT, a patient could be injected with a photosensitizer, and an endoscope with a light could be passed through the mouth to shine light on tumor tissue in the lungs.

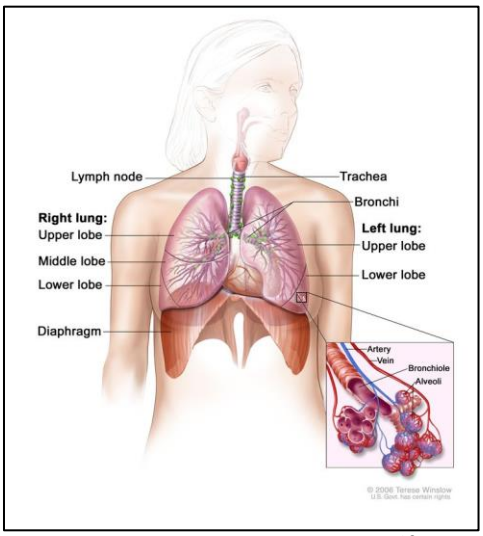

Figure 2. Lung Anatomy[10]

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Photodynamic therapy could potentially limit surgical procedures and have less burdensome side effects. This would allow for patients to return to their normal activities more quickly after treatment including returning to work.[11] Photodynamic therapy could also be used as a combination therapy for lung cancer or as a palliative treatment for patients who are not able to have surgery or radiation therapy.[12] Additionally, photodynamic therapy would be less costly. The average cost for traditional cancer therapy including surgery, chemotherapy, and radiation would be approximately $33,360.[13] In contrast, a single round of photodynamic therapy depending on the type and severity of the cancer, could be between $100 and $4,000 for a single treatment to approximately $10,000 for a series of treatments.[14] Most importantly, PDT could potentially increase the survival rates for lung cancer patients, therefore lessening the lung cancer disparities that exist in healthcare.

Photosensitive agents in PDT

Photodynamic therapy requires a photosensitive agent. There are several tetrapyrrole structures that make ideal photosensitive agents, including porphyrins. A porphyrin is a planar molecule with alternating double bonds **(Figure 3).** These double bonds allow for the absorption of light, leading to the creation of singlet oxygen which destroys tumor tissue. Additionally, porphyrins are advantageous as potential PDT agents because their core can be metallated and the peripheral functional groups can be modified. A common example of a porphyrin core is found in hemoglobin found in the blood **(Figure 4).** The heme porphyrin has an iron atom in the core and various substituents on the periphery.

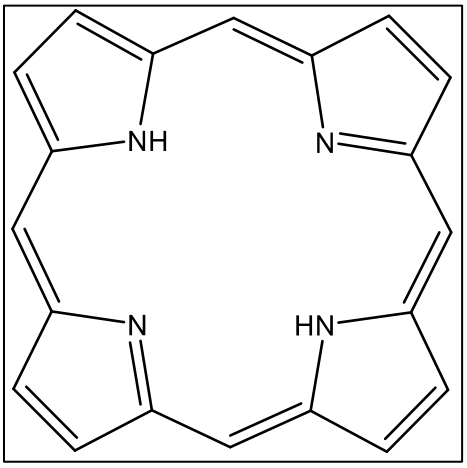

Figure 3. Porphyrin Core

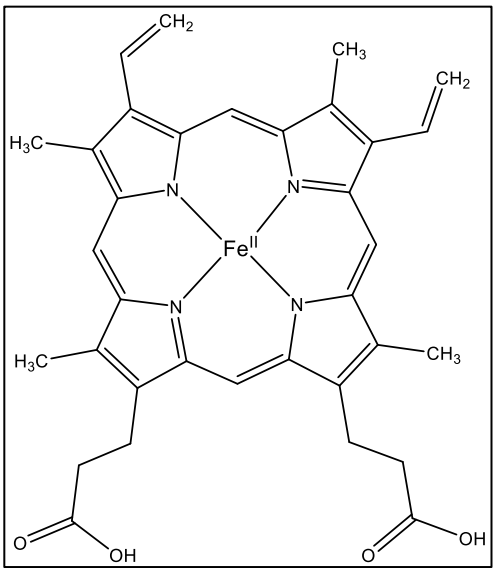

Figure 4. Hemoglobin Molecule with Porphyrin Core

Current Clinical Applications

There are several current photosensitizers currently in use. One of these photosensitizers is Photofrin, which has a porphyrin core **(Figure 5)**. It is used for endobronchial cancer. First generation photosensitizers such as Photofrin, cause side effects such as lasting skin photosensitivity because they are not rapidly cleared from the body.[12] Thus, the creation of new second generation photosensitizers is necessary. One example of a second-generation photosensitizer is taloporfin **(Figure 6)**. It has a chlorin core and has been approved for use in Japan for the treatment of lung cancer.

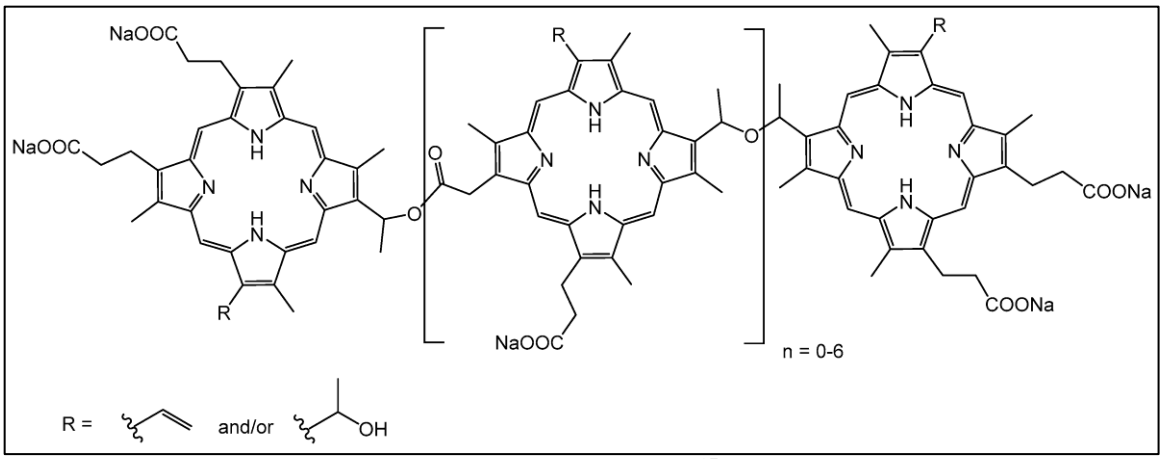

Figure 5. Photofrin[15]

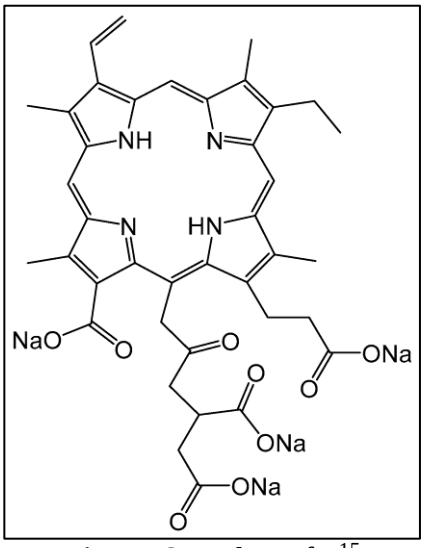

Figure 6. Taloporfin[15]

INTRODUCTION TO PROJECT

This research focused on synthesizing novel water-soluble porphyrin compounds for use as photosensitive agents in PDT for the treatment of lung cancer. The periphery of the porphyrin core can be modified with various substituents to assist with water-solubility and cytotoxicity. In this research, the core of 5, 10, 15, 20-tetrakis(4-carboxyphenyl)porphyrin, H_2TPPC, was modified with the attachment of L-threoninol to create the novel H_2TPP-Thr. The core was also modified with

tris(hydroxymethyl)aminomethane, TRIS, to synthesize H_2TPP-TRIS. Additionally, ZnTPPC was modified using TRIS to form the novel ZnTPP-TRIS.

L-threoninol and TRIS both contain primary amine groups, -NH_2, which allow for their attachment with the porphyrin core via an acid chloride intermediate. TRIS contains three alcohol, -OH, groups, while L-threoninol contains two alcohol, -OH groups. It was thought that the addition of -OH groups may allow for greater water solubility and increase the efficacy of the porphyrin product in killing cells. In addition, TRIS is biologically friendly and used for buffering,[16] so it was thought that the attachment of TRIS would not increase the cytotoxicity of the porphyrin product without the presence of light.

Each compound was purified by column chromatography using both Sephadex LH-20 and Sephadex G-50. Additionally, nuclear magnetic resonance (NMR), infrared (IR), and UV-visible spectroscopies (UV-vis) were used to characterize each compound. Purity of the final products was determined using high performance liquid chromatography (HPLC). Finally, compounds were tested using MTT assays to determine cell viability in both light and dark conditions on an A549 non-small cell lung cancer cell line. It is important that photosensitive agents, such as these porphyrin products, cause minimal to no cytotoxicity in the absence of light.

METHODS

Synthesis of Porphyrin Compounds

Formation of H_2TPPC (3)

H_2TPPC, 5, 10, 15, 20-tetrakis(4-carboxyphenyl)porphyrin, served as the starting material for each reaction. A 3.00 g amount of 4-carboxybenzaldehyde **(2)** and 1.5 mL of pyrrole **(1)** were added to a 500 mL round bottom flask with approximately 200 mL of propionic acid and a stir bar **(Figure 7)**. The flask was wrapped in aluminum foil to prevent side products. The reaction was then heated to reflux and refluxed for one hour. The reaction was cooled to room temperature, before being covered with parafilm and put in a -20 °C freezer overnight.

The product of the reaction was then vacuum filtered using a medium sintered glass fritted filter. Dichloromethane, DCM, was used to wash the product three times to remove impurities. The H_2TPPC final product was then transferred into a vial and dried for 30 minutes in the oven.

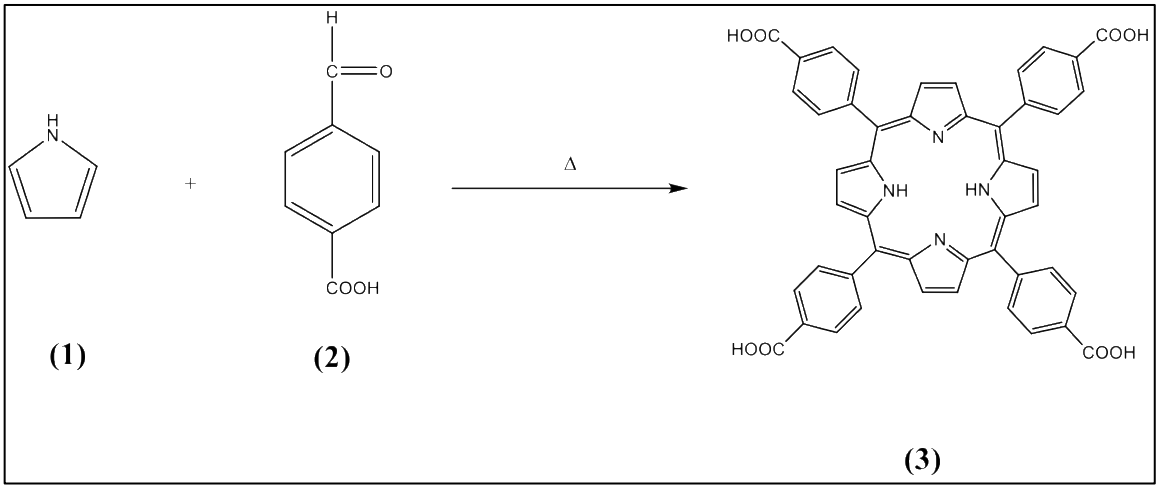

Figure 7. Reaction 1. 4-carboxybenzaldehyde **(2)** reacted with pyrrole **(1)** in propionic acid solution to form H_2TPPC **(3)**.

Formation of the Acid-Chloride Intermediate (4)

To form the acid-chloride intermediate, 0.15 g of the starting material, H_2TPPC **(3)**, was dissolved in 20 mL of dried dimethylformamide (DMF) in a clean, oven dried, 100 mL round bottom flask. A syringe was used to add 0.2 mL of thionyl chloride, $SOCl_2$, to the flask before the flask was capped with a rubber septum **(Figure 8)**. The reaction was stirred under a constant flow of nitrogen for one hour. Afterward, the DMF was evaporated off using a rotovap, and the flask was placed under vacuum overnight.

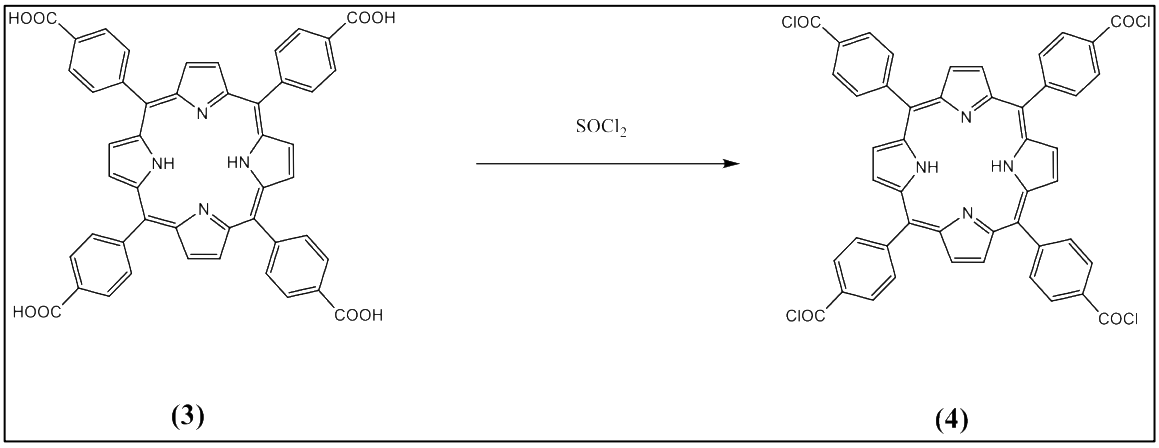

Figure 8. Reaction 2. H_2TPPC **(3)** reacts with $SOCl_2$ in DMF, forming an acid chloride porphyrin **(4)**.

Formation of the Final Products
H_2TPP-Thr (5)

Following the initial formation of the acid-chloride intermediate, the final product, H_2TPP-Thr **(5)**, was synthesized by using the amine L-threoninol **(Figure 9).** L-Threoninol was dried overnight and then 0.3 g of L-threoninol was dissolved in freshly distilled methanol and this solution was transferred to the round bottom flask containing the acid-chloride intermediate. The reaction was placed back under nitrogen and stirred for one hour. The methanol was then evaporated to yield the final H_2TPP-Thr **(5)** product. A small amount of product was placed in MilliQ H_2O to test for water solubility.

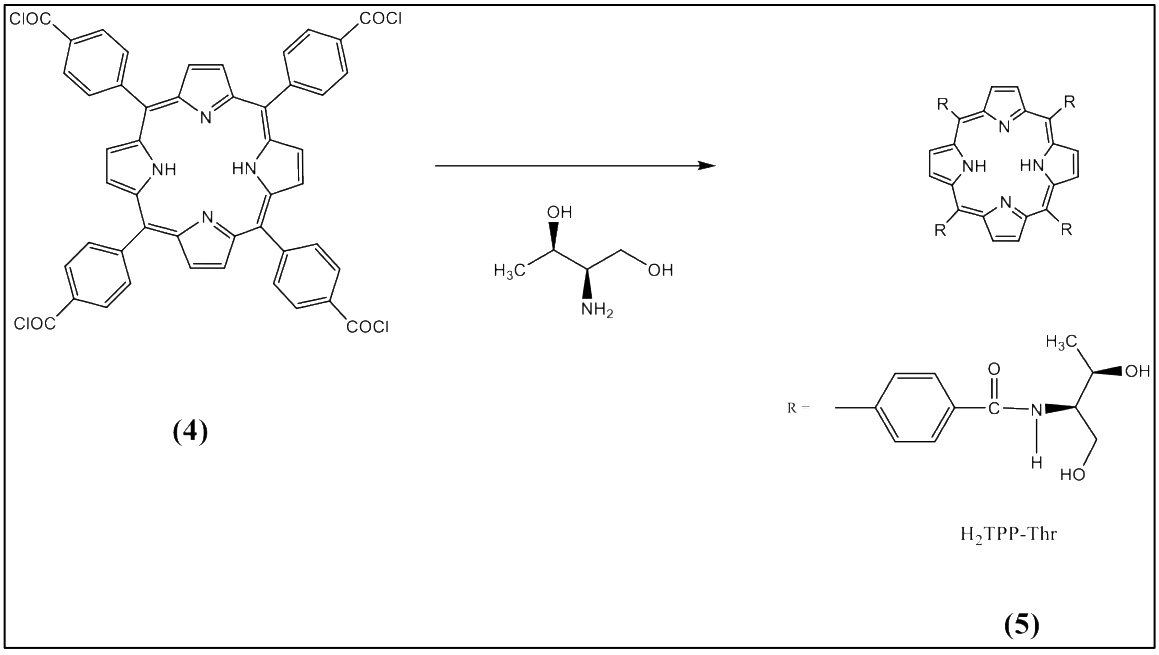

Figure 9. Reaction 4. The acid chloride porphyrin (4) reacts with L-threoninol in methanol to form H_2TPP-Thr, the final product (5).

H_2TPP-TRIS (6)

To create the final product, H_2TPP-TRIS **(6)**, tris (hydroxymethyl)aminomethane, TRIS, was first oven dried overnight. The TRIS, 0.37 g, was dissolved in freshly distilled methanol, and transferred to the round bottom flask containing the acid chloride intermediate **(Figure 10)**. The reaction was kept under nitrogen and stirred for one hour. The methanol was evaporated, and the final product was tested for water solubility in MilliQ H_2O.

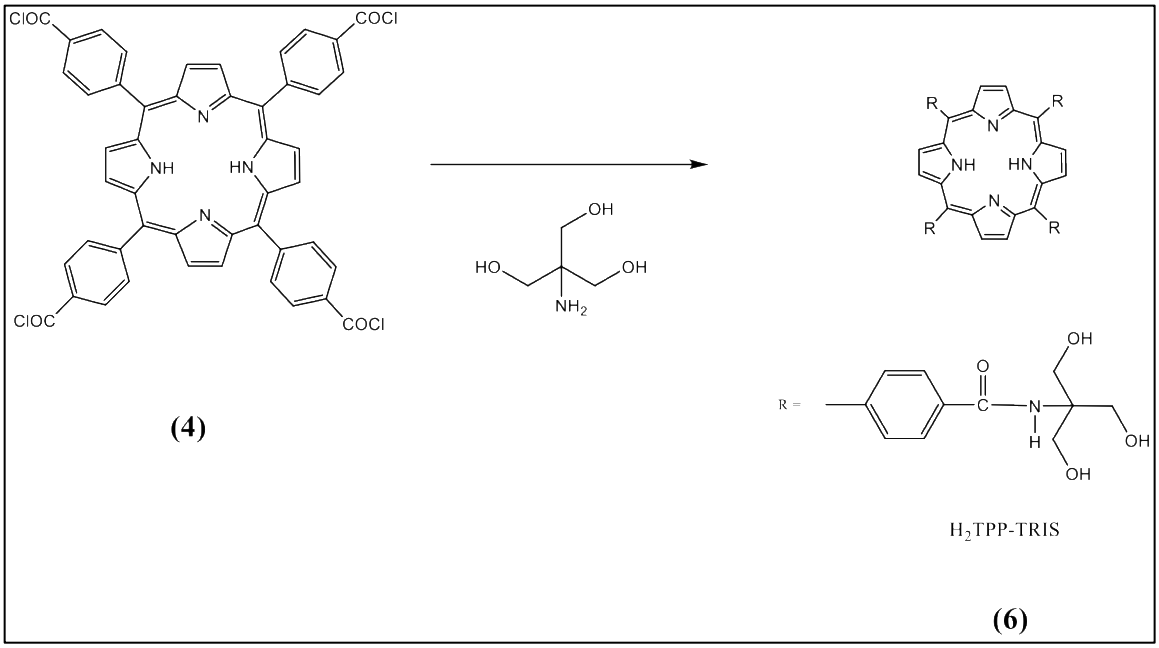

Figure 10. Reaction 3. The acid chloride porphyrin **(4)** reacts with TRIS in methanol to form H_2TPP-TRIS, the final product **(6)**.

ZnTPP-TRIS (8)

To create the ZnTPP-TRIS, 1.00 g of the H_2TPPC starting material **(3)** and 0.36 g of $ZnCl_2$ were put in a flask. DMF was added and the reaction was heated to reflux and refluxed for 3 hours while being covered in aluminum foil. The DMF was evaporated. The remaining product was dissolved in methanol, MeOH, and then filtered using a sintered glass filter. The filtrate was rotovapped. The product was filtered and washed with DCM to obtain the ZnTPPC solid **(7) (Figure 11)**.

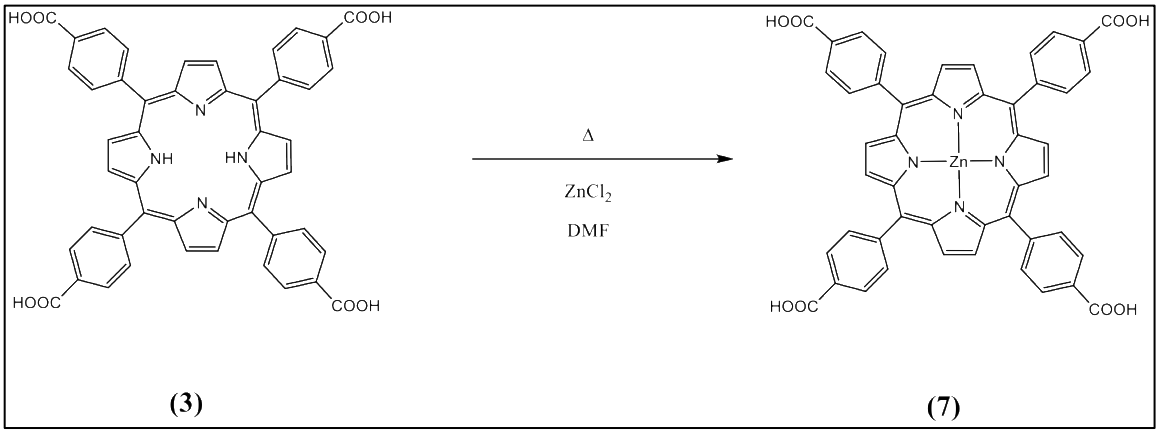

Figure 11. Reaction 5. H_2TPPC **(3)** reacts with $ZnCl_2$ in DMF to form ZnTPPC **(7)**.

ZnTPPC **(7)** in the amount of 0.15 g was dissolved in 50 mL of tetrahydrofuran, THF. Then, 0.32 g of TRIS was dissolved in DI H_2O and added to the flask along with 0.23 g of HOBT. The flask was placed in an ice bath and stirred for 15 minutes. A 0.36 g amount of DCC was added to the flask and then the flask was removed from the ice bath and allowed to warm to room temperature. The reaction was then stirred for 3 hours at room temperature. The solvent was then removed by rotovap. The final product **(8)** was placed in MilliQ H_2O to test for water solubility.

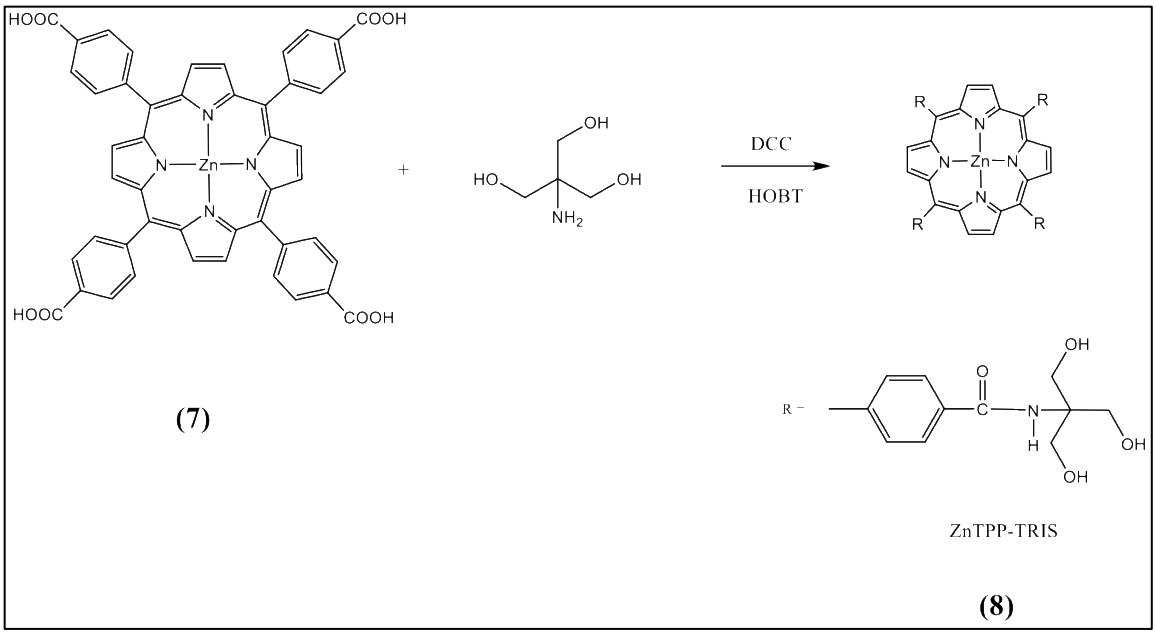

Figure 12. Reaction 6. ZnTPPC **(7)** reacts with TRIS in THF/DI H_2O/HOBT/DCC forming ZnTPP-TRIS, the final product **(8)**.

Each reaction was done three times to ensure repeatability, and obtain enough working product.

Purification of Porphyrin Compounds

LH-20 and G-50 column chromatography

A 50:50 MeOH:H_2O mixture was added to the flask to dissolve the final product. Syringe filtration was performed using a 0.45 μm nylon syringe filter and the filtrate was added to a chromatography column packed with Sephadex LH-20. The desired product was eluded with a 50:50 MeOH:H_2O mixture. Only the desired purple product coming off the column was collected. The MeOH:H_2O mixture was then rotovapped to obtain the purified, solid porphyrin product. Following LH-20 purification, the solid porphyrin product was dissolved in Milli-Q water. The solution was then run through a chromatography column containing Sephadex G-50. Each of the final purified products were collected and the H_2O was evaporated. This purification process was done for each of the desired final products.

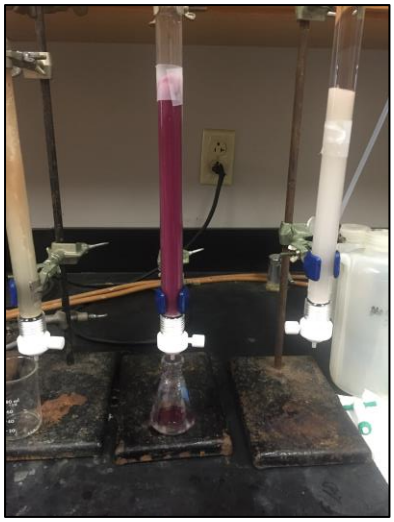

Figure 13. LH-20 and G-50 Chromatography Columns

Characterization

Infrared Spectroscopy (IR)

Background

The bonds in compounds can stretch and bend when excited by infrared radiation, which will cause them to vibrate. This is analogous to a spring that can be stretched or compressed and vibrates when it is released. Upon excitation with infrared radiation, the bonds in compounds will absorb differing frequencies due to their energetic properties. The IR spectrum measures these frequencies in wavenumbers, cm^{-1}. The energy differs depending on the type of bond, as some bonds require more energy to be stretched or compressed. This allows for the determination of functional groups within a compound.[17]

Results

IR spectra was run on both the free amines and final porphyrin products **(Figures 14-18)**. Spectra for the free amines show a sharp N-H stretch around 3300 cm^{-1}. The absorption bands are characteristic of primary amines. In coupling to the amine porphyrin core for H_2TPP-Thr, H_2TPP-TRIS, and ZnTPP-TRIS, several other absorption bands appear. There is a broad O-H stretch around 3300 cm^{-1}, a C=O stretch around 1700 cm^{-1}, and amide I, II, and III bands. The appearance of the amide bands is one of the strongest demonstrations that the free amine has been attached to the porphyrin core. The amide I band appears between 1600-1500 cm^{-1}. The amide II band is between 1650-1515 cm^{-1} due to N-H bending. The amide III band appears at around 1379 cm^{-1} due to a C-N stretch. In the final porphyrin product, the N-H stretch is not seen due to the broad O-H stretch, further confirming the attachment of the free amine to the porphyrin core.

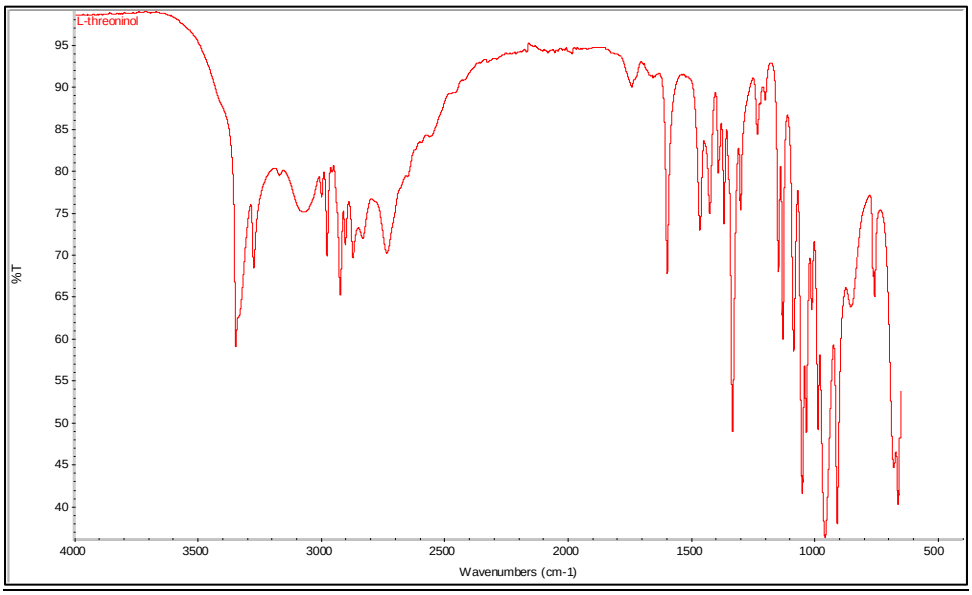

Figure 14. IR Spectrum for L-Threoninol

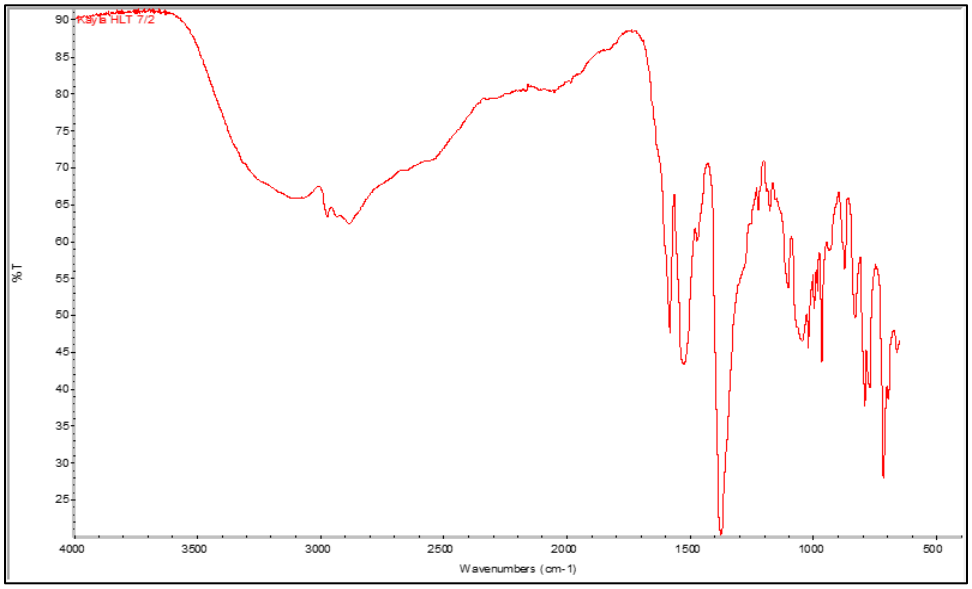

Figure 15. IR Spectrum for H_2TPP-Thr

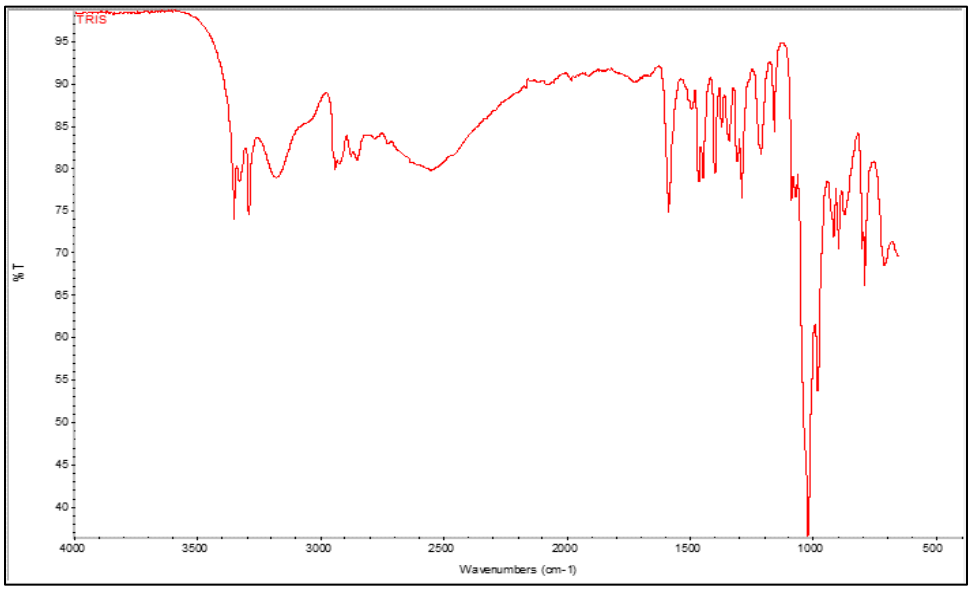

Figure 16. IR Spectrum for tris(hydroxymethyl)aminomethane

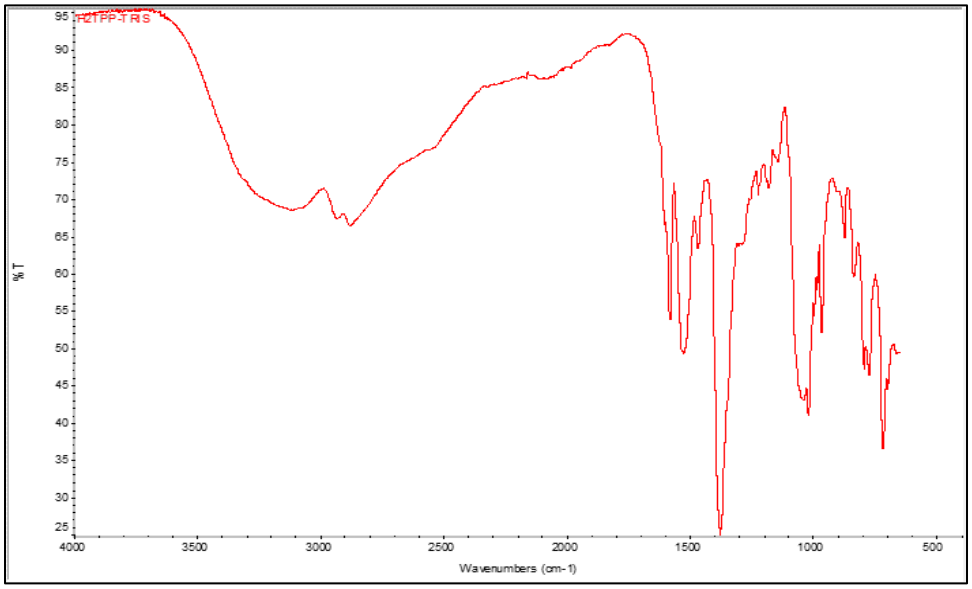

Figure 17. IR Spectrum for H_2TPP-TRIS

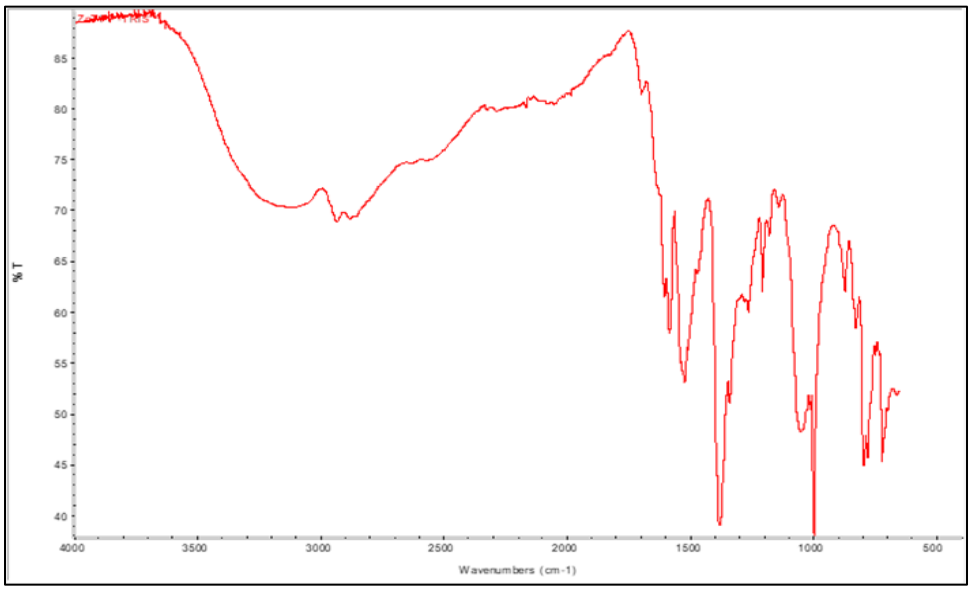

Figure 18. IR Spectrum for ZnTPP-TRIS

UV-Vis Spectroscopy

Background

Conjugated π bonds are characteristic of porphyrin structures. In ultraviolet-visible (UV-Vis) spectroscopy, π electrons are excited to higher energy levels when irradiated with UV-Vis light.[17] Light from the spectrophotometer passes through a cuvette containing the porphyrin sample dissolved in the water solvent. The intensity of the light that passes through at varying wavelengths are compared and a spectrum is generated giving the absorbance at each wavelength for the porphyrin compound. Beer's Law is then utilized to calculate the molar absorptivity, ε, of the porphyrin compound. UV-Vis allows for the characterization of compounds due to individual compounds having unique molar absorptivity. For the scope of this research, a Soret band characteristic of porphyrin compounds was desired at *ca.* 415 nm for the non-metallated porphyrins and *ca.* 423 nm for the metallated zinc porphyrins. Additionally, Q bands were expected to be seen in the final product spectra due to the inherent degenerate transitions.[18]

Results

The following spectra **(Figures 19-21)** confirmed the presence of a highly conjugated system. Additionally, it confirmed structural elements such as the presence of zinc in ZnTPP-TRIS shown through the shifting of the wavelength of absorbance for the Soret band. The Soret bands are shown below in grey, while Q bands are in white. The Soret band for the non-metallated compounds, H_2TPP-Thr and H_2TPP-TRIS **(Figure 19-20)** appeared at a 415 nm wavelength, while the Soret band for the ZnTPP-TRIS **(Figure 21)** was at a wavelength of 421 nm.

H_2TPP-Thr	
λ, (nm)	Molar absorptivity coefficient ($mM^{-1}cm^{-1}$)
415	281
518	11.9
555	6.9
580	5.3
636	3.3

Table 1. UV-VIS Molar absorptivity coefficients for H_2TPP-Thr spectral peaks

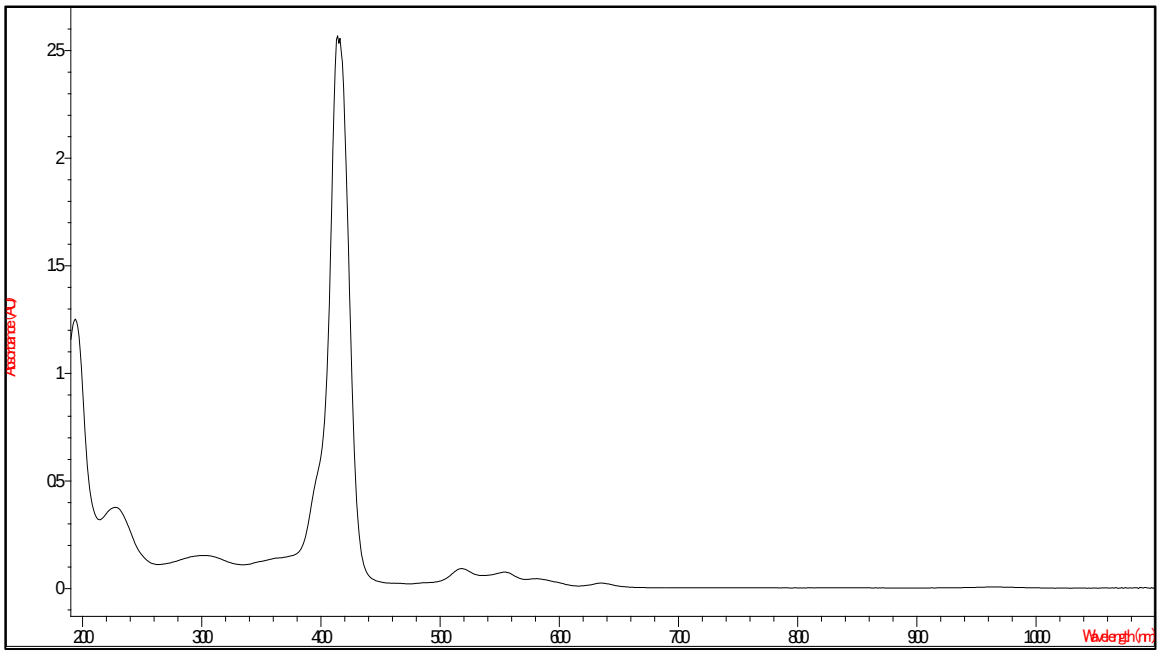

Figure 19. UV-VIS Spectrum for H_2TPP-Thr

H_2TPP-TRIS	
λ, (nm)	Molar absorptivity coefficient ($mM^{-1}cm^{-1}$)
415	330
518	11
555	6.7
581	4.8
636	3.2

Table 2. UV-VIS Molar absorptivity coefficients for H_2TPP-TRIS spectral peaks

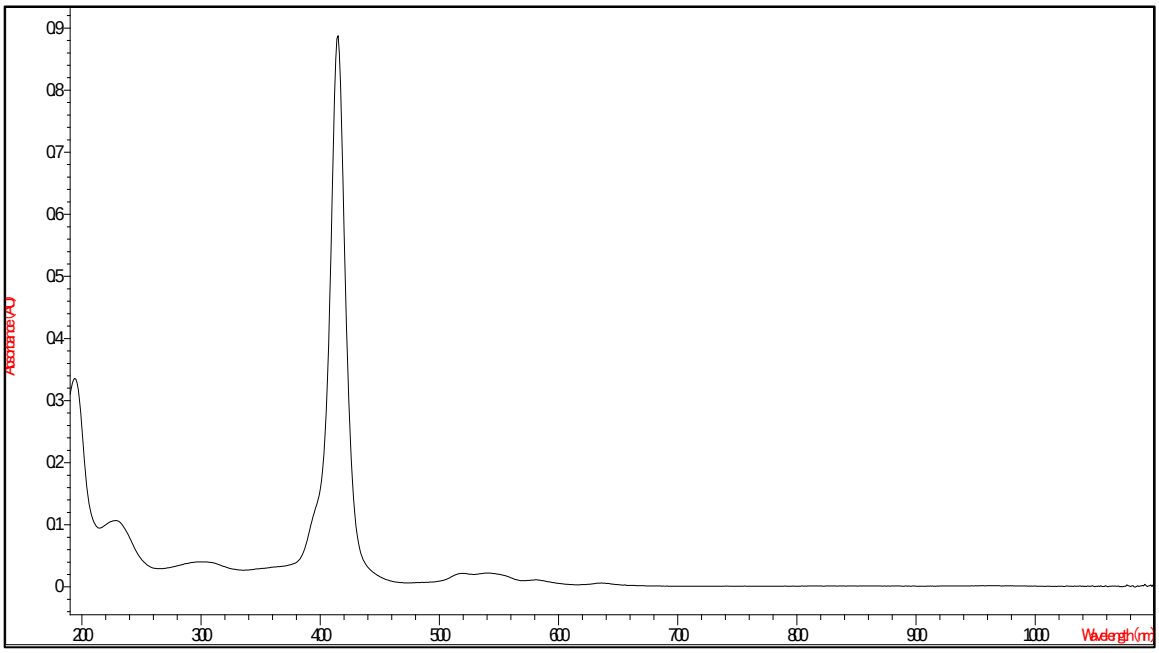

Figure 20. UV-VIS Spectrum for H_2TPP-TRIS

ZnTPP-TRIS	
λ, (nm)	Molar absorptivity coefficient ($mM^{-1}cm^{-1}$)
421	330
558	16.1
598	8.6

Table 3. UV-VIS Molar absorptivity coefficients for ZnTPP-TRIS spectral peaks

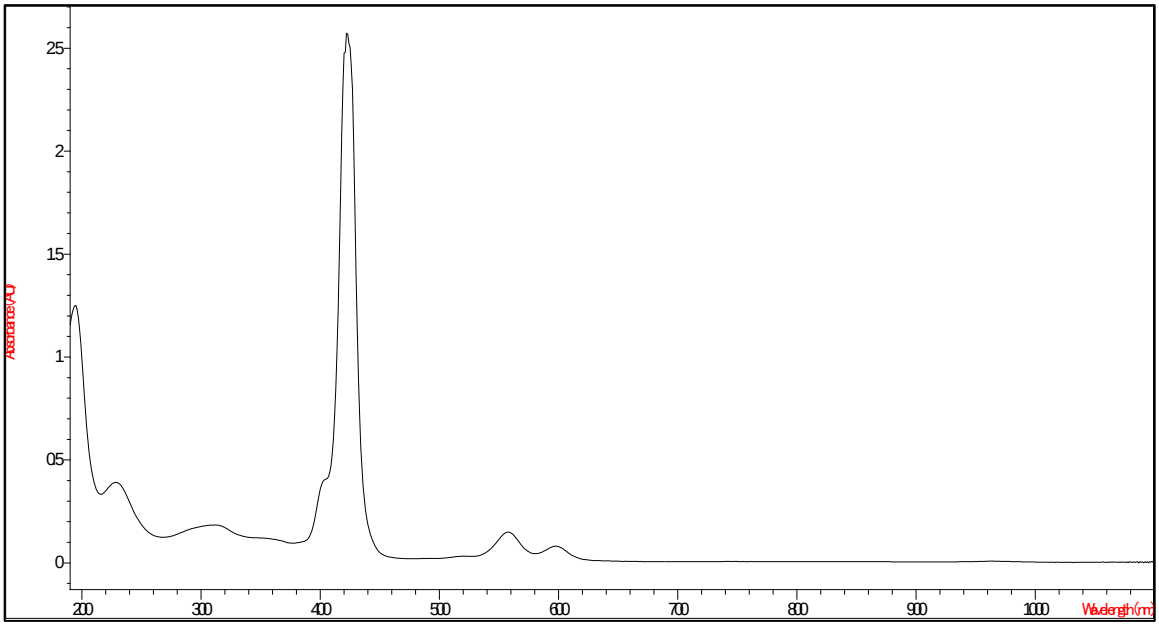

Figure 21. UV-VIS Spectrum for ZnTPP-TRIS

Nuclear Magnetic Resonance Spectroscopy (NMR)

Background

Nuclear Magnetic Resonance Spectroscopy (NMR) is a type of magnetic spectroscopy that allows for the characterization of compounds on the basis of their nuclei's spin and magnetic dipole. Different nuclei within a compound have differing chemical environments leading to different total magnetic fields. As a result, nuclei absorb varying amounts of magnetic field frequency and show different chemical shifts.[17]

Results

NMR spectra were obtained on the final porphyrin products and each of the free amines shown in **Figures 22-26**. Water eliminated fourier transform (WEFT) was utilized to suppress the water signal and allow for better spectral visualization for each of the final porphyrin products. The final porphyrin products showed chemical shifts characteristic of both the porphyrin ring's aromatic hydrogens *ca.* 8 ppm as well as the aliphatic hydrogens characteristic of the free amine.

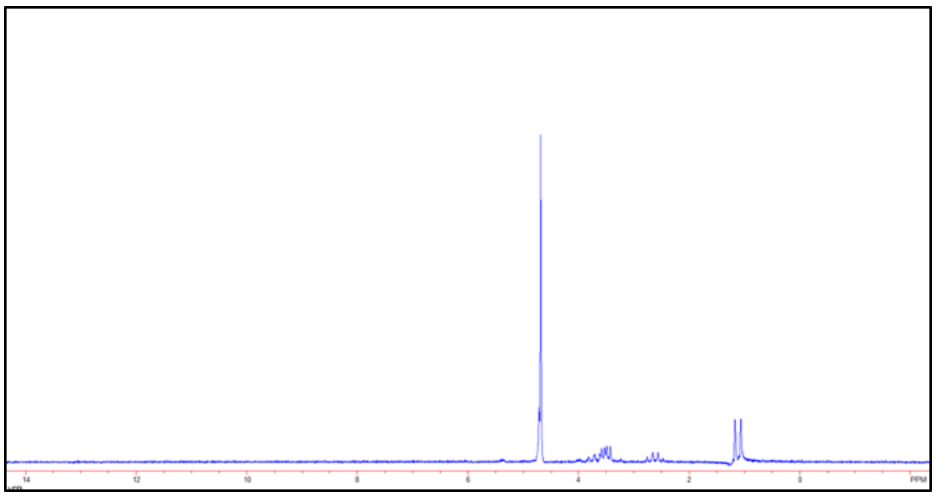

Figure 22. NMR spectrum for L-threoninol

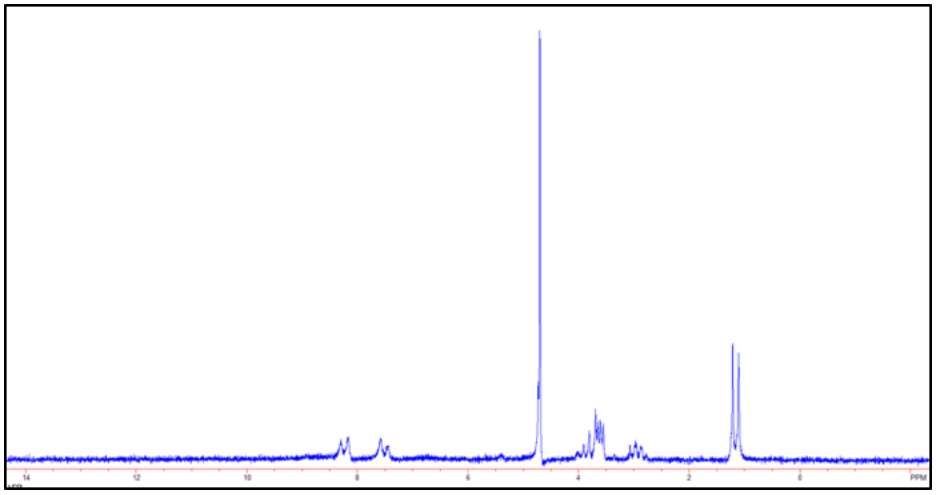

Figure 23. NMR spectrum for WEFT H_2TPP-Thr

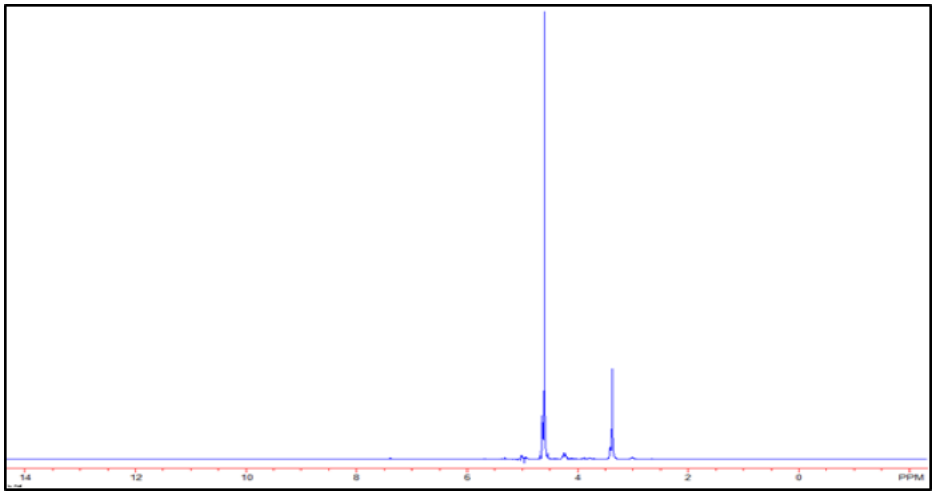

Figure 24. NMR spectrum for tris (hydroxymethyl)aminomethane

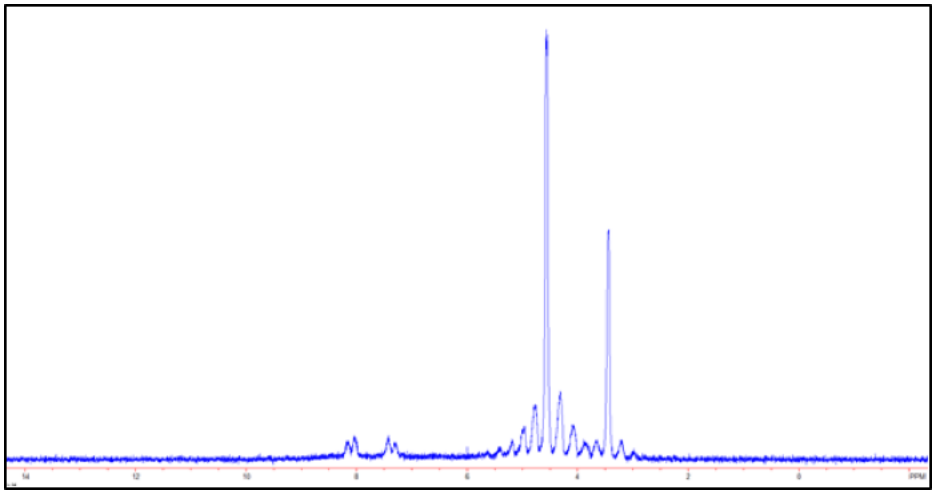

Figure 25. NMR spectrum for WEFT H_2TPP-TRIS

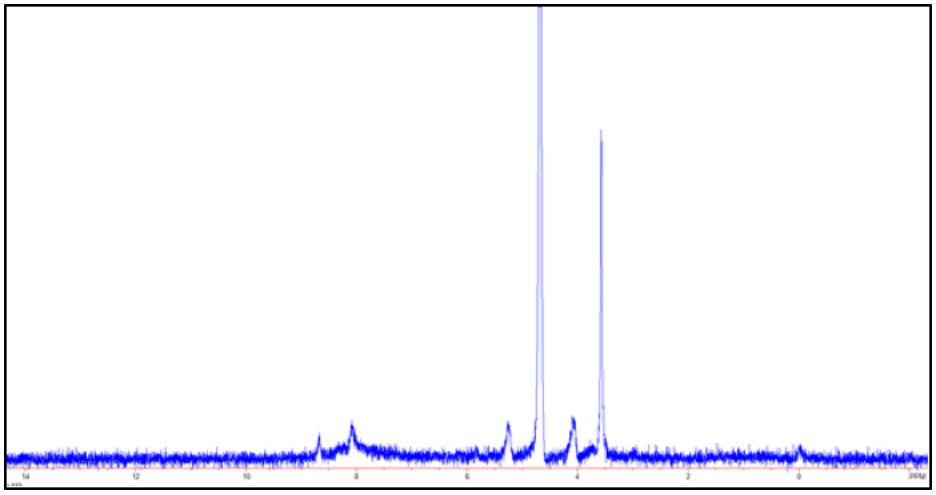

Figure 26. NMR spectrum ZnTPP-TRIS

High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is utilized to separate, identify, and quantify soluble compounds. In HPLC, high pressure is used to create a flow for liquid chromatography to take place in a packed column. HPLC allows for the determination of the purity of a sample.[19]

The purity of each porphyrin product was determined by dissolving the product in Milli-Q H_2O and using a Hamilton PRP-1 5µm 4.1 x 150 mm column. A 100% acetonitrile solvent was used at a flow rate of 1.00 mL/min. The H_2TPP-Thr had a purity of 98.9, the H_2TPP-TRIS had a purity of 98.6%, and the ZnTPP-TRIS had a purity of 97.8%.

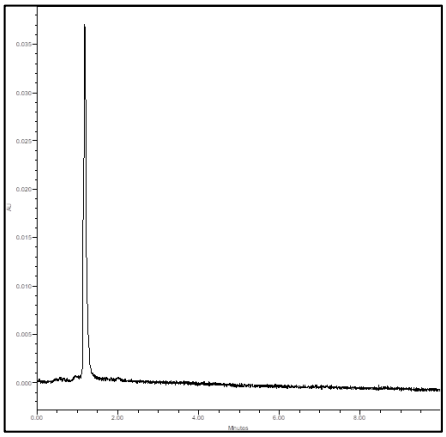

Figure 27. HPLC Trace for ZnTPP-TRIS

Compound	Purity
H_2TPP-Thr	98.9%
H_2TPP-TRIS	98.6%
ZnTPP-TRIS	97.8%

Table 4. HPLC Purities for final porphyrin products

Cytotoxicity Testing

Background

An MTT assay is a colorimetric assay utilized for measuring the metabolic activity of cells, and therefore their viability. An MTT assay allows for the determination of the efficacy of the porphyrin compound by measuring the interruption of cell metabolism and therefore the killing of cells. In an MTT assay, metabolically active cells reduce yellow tetrazolium salt to purple formazan crystals. These crystals are then dissolved, and the absorbance can be measured with a spectrophotometer. The darker purple corresponds to the more metabolically active cells. Thus, cells exposed to light with no porphyrin compound or lesser concentration should have greater metabolic activity or more living cells. Porphyrin compounds with greater efficacy should have decreased metabolic activity when exposed to light, preferably at small concentrations. Cells containing the porphyrin compound that are not exposed to light, should have minimal cytotoxicity, as the porphyrin compound should only be activated when exposed to light.[20]

MTT Assay Methodology

A549 cells from the American Type Culture Collection (ATCC) were plated on two 96-well plates and cultured with MEM growth medium. Varying concentrations of the porphyrin compound being tested were added after cells were ~25% confluent, with each concentration being replicated in eight of the wells. The plates were wrapped in aluminum foil to prevent light contamination and were placed back in the incubator. After approximately 22 hours, one of the plates was exposed to white light (0.5 J/cm^2) for

20 minutes while the other plate was kept in the dark. The plates were then incubated again. After 72 hours, MTT reagent was placed on the cells for 3 hours to allow time for the purple formazan product to form. The formazan crystal product was then dissolved in DMSO. A spectrophotometer was used read at wavelengths of 570 nm and 630 nm. The difference between the two wavelengths was used to quantify and compare the amount of purple coloring, indicating the cell's viability, and thus porphyrin compound toxicity.

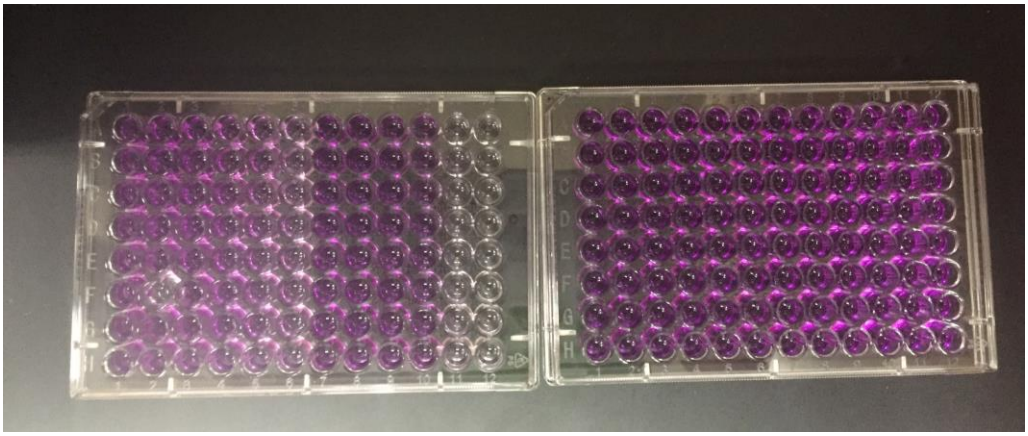

Figure 28. MTT Assay for ZnTPP-TRIS **(8)** in last 6 columns with purple indicating greater cell metabolism

MTT Assay Results

Six MTT assay trials were done to test the cytotoxicity of H_2TPP-Thr. A preliminary trial used concentrations of 0, 1, 3, 10, and 30 µM. The second and third trials used concentrations of 0, 50, 75, 100, 125, and 150 µM, while the fourth through sixth trials utilized concentrations of 0, 100, 150, 200, and 250 µM. It was determined that H_2TPP-Thr had a lethal dose, the concentration where 50% of the cells are killed, LD_{50}, *ca.* 200 micromolar **(Figure 29)**.

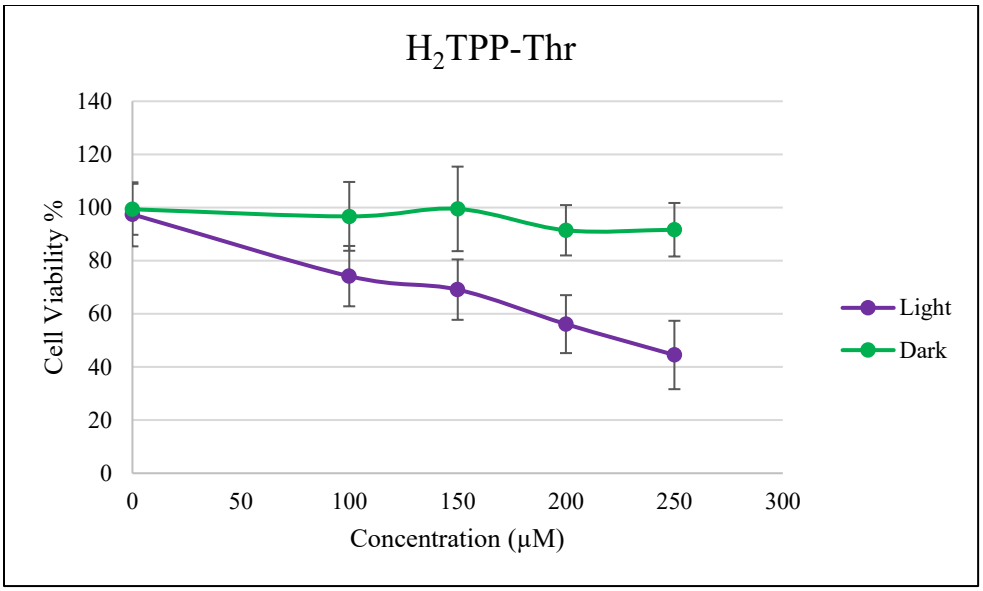

Figure 29. Spectrophotometric MTT assay results for H_2TPP-Thr **(5)**

Five trials were done to test the cytotoxicity of H_2TPP-TRIS. The first trial utilized concentrations of 0, 1, 3, 10, 30, and 100 µM concentrations. The second through fifth trials utilized 0, 25, 50, 75, 100, and 125 µM. As seen in Figure 30, H_2TPP-TRIS has an LD_{50} of approximately 100 µM.

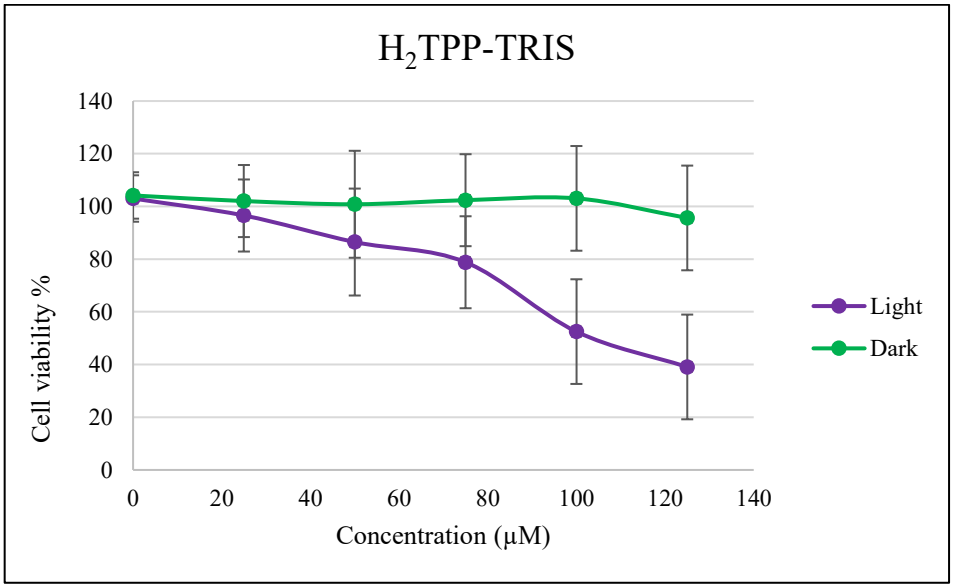

Figure 30. Spectrophotometric MTT assay results for H_2TPP-TRIS **(6)**

A preliminary trial of ZnTPP-TRIS was done utilizing 0, 1, 3, 10, 30, 100, and 150 µM. The LD_{50} was around 50 micromolar. This compound was tested further during the summer of 2021 using 0, 13, 16, 20, 23, and 26 µM concentrations. This second trial showed the LD_{50} may be as low as 16 µM. Further testing is needed to confirm this lower LD_{50}. In both trials, the LD_{50} was lower than that of the non-metallated porphyrin, H_2TPP-TRIS.

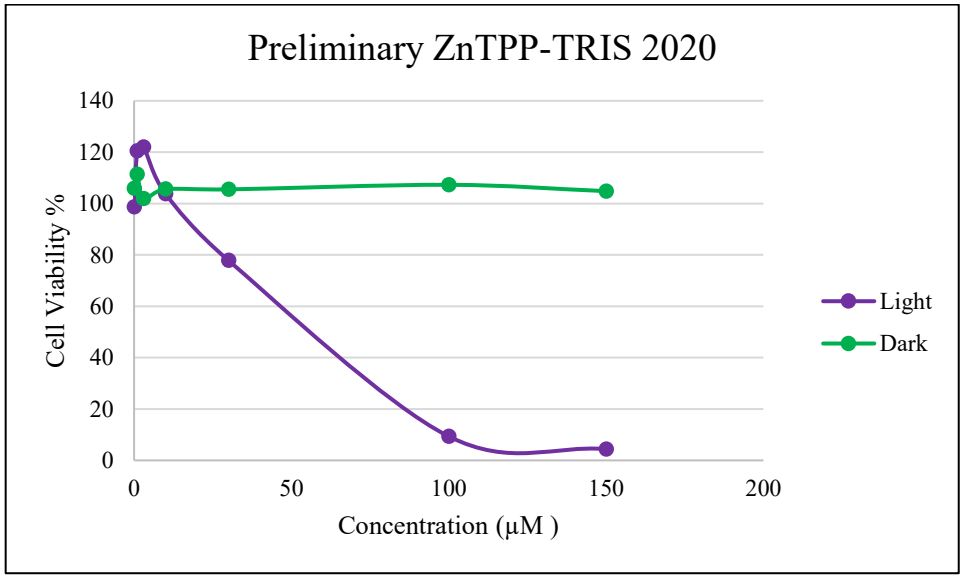

Figure 31. Spectrophotometric MTT assay results for preliminary ZnTPP-TRIS trial **(8)**

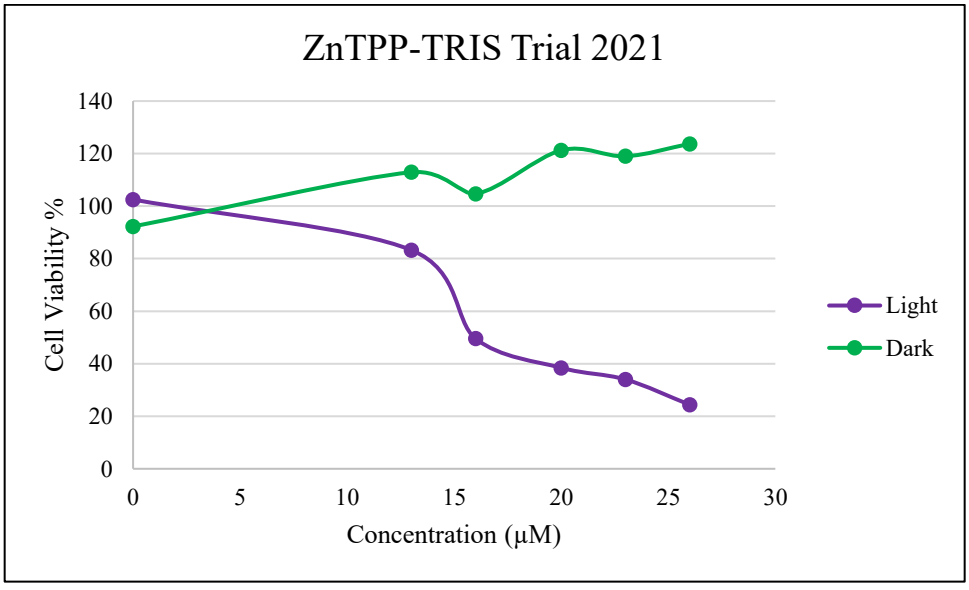

Figure 32. Spectrophotometric MTT assay results for additional ZnTPP-TRIS trial **(8)**

CONCLUSIONS

Three novel water-soluble porphyrin compounds were developed for potential use as photosensitive agents in photodynamic therapy. Two free amines, thought to be biologically friendly were successfully attached to the porphyrin core. All three compounds showed minimal to no cytotoxicity in the dark. This result is promising because the porphyrin products should only be killing cells when exposed to light. H_2TPP-Thr had an LD_{50} of *ca.* 200 µM and H_2TPP-TRIS showed an LD_{50} of *ca.* 100 µM when exposed to light. H_2TPP-TRIS showed higher cytotoxicity at lower concentrations than H_2TPP-Thr. This could potentially be due to greater water solubility due to an additional -OH group. Finally, preliminary results show the LD_{50} of ZnTPP-TRIS to be between 15 and 50 µM when exposed to light. Further testing is necessary to confirm these preliminary results. ZnTPP-TRIS showed greater toxicity at lower concentrations than H_2TPP-TRIS, showing promise that metallized porphyrin compounds such as ZnTPP-TRIS may have greater efficacy as PDT agents. New, second generation PDT agents such as these synthesized, may help to eliminate health disparities in the treatment of lung cancer. The further implementation of PDT in the treatment of lung cancer could limit surgical procedures and side effects. It could also minimize treatment costs, which is particularly important for patients of low socio-economic status. It could also lead to better survival outcomes which could help to close the health disparity gap for minority groups like African Americans, Asian Americans, Native Americans and underrepresented groups like women.

FUTURE WORK

In the future, further testing is needed to confirm the true LD_{50} value for ZnTPP-TRIS. The synthesis and comparison of the cytotoxicity in the presence and absence of light of additional metallated and non-metallated porphyrins using the same amine coupled to the periphery of the porphyrin core. This is needed to generalize if metallated porphyrins have greater efficacy than non-metallated porphyrins for a particular cancer cell line. These compounds should also be tested on other cancer cell lines. Additionally, these porphyrin compounds should have further cytotoxicity testing using red light **(Figure 32),** rather than the white light previously used. Red light is classically used *in vivo* for PDT treatment, as it has a greater wavelength, and thus increased penetration depth of tumor tissue.[21] If the novel water-soluble porphyrin compounds show similar or greater efficacy using red light, they can be tested *in vivo* with mice in collaboration with Dr. Theresa Busch at the University of Pennsylvania School of Medicine.

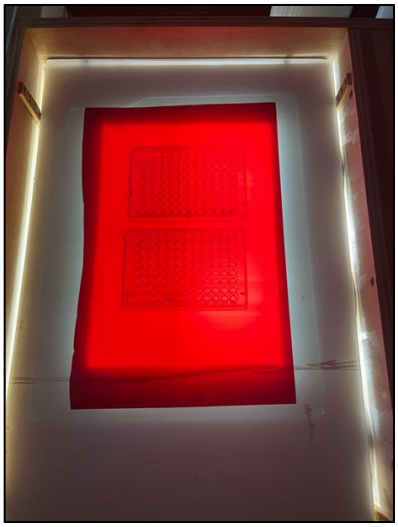

Figure 33. Red light filter.

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