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Developing ZnTPP-4AB as a Potential Photodynamic Therapy Agent

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A Senior Thesis by Kennedy Johnson

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Abstract

Photodynamic therapy is a fairly new technique used in cancer treatment involving the use of a photosensitizer and light exposure to kill malignant cells. In this research, the use of ZnTPP-4AB as a photosensitizer and red light as a source of radiant light energy was explored in terms of cytotoxicity in the A549 lung cancer cell line. ZnTPP-4AB was synthesized by replacing the carbonyl functional groups on the ZnTPPC porphyrin with 4-amino-1-butanol. Following synthesis, the new porphyrin was purified through the use of Sephadex LH-20 and G-50 in column chromatography in order to remove impurities. To confirm the identity and structure of the product, ZnTPP-4AB was analyzed through nuclear magnetic resonance, infrared, and ultraviolet-visible spectroscopies. Purity of the final compound was determined using HPLC. Lastly, the efficacy of ZnTPP-4AB as a photosensitizer was examined by analyzing its cytotoxicity when paired with red light using MTT assay on the A549 lung cancer cell line. An additional experiment was also conducted in which the A549 cells were put into a hypoxic environment (mimicking a tumor) prior to treatment with the porphyrin, and these were compared with the first MTT assay.

Background

Lung cancer is the number one cause of cancer-related death in the world. In America, one out of every seventeen people will be diagnosed with lung cancer in their lifetime. The current therapy methods used for lung cancer can vary depending on the type and stage, but typically include either surgery, chemotherapy, radiation therapy, targeted therapy, or a combination. Although each of these methods have proven to be successful in their own ways, they all have one thing in common: causing intense physical tolls on the human body. Removing a lung tumor through physical extraction, high energy radiation, or through chemotherapy drugs all come with negative consequences with the biggest being the destruction of healthy cells. Lung cancer patients

desperately need a technology that can target cancer cells specifically without damaging other cells in the body.[3]

Introduction

Photodynamic Therapy

Photodynamic therapy (PDT) is a fairly new, yet promising approach to lung cancer treatment (**Figure 1**). The US Food and Drug Administration approved the first photosensitizing drug for use in cancer treatment in 1995; since then, PDT has continued to improve and expand into other cancer types. When undergoing this therapy, patients are injected with a photosensitizing drug that preferentially localizes in cancerous cells (**Figure 1, a**). In this research, the novel photosensitizer that was synthesized and tested on lung cancer cells was ZnTPP-4AB. After injection, the drug incubates in the body for 0.5 to 4 hours, allowing the malignant cells to absorb it depending on the size of the targeted tumor (**Figure 1, b**).[1]

In order to cause cell death, the photosensitizer must be exposed to light. In the presence of light, the drug is transformed into an excited electronic state that can react with oxygen to produce singlet oxygen. Singlet oxygen is an extremely reactive molecule that induces oxidative damage to a cell, thus disrupting its normal function and causing it to die. The ideal wavelength of light that can permeate into tissues to prompt this reaction has been found to be between 600 and 850 nm, with shorter wavelengths having less tissue penetration and longer ones reaching deeper.[1] In this experiment, the effects of white light and red light were each explored as sources of photosensitizer activation. The white light uses a variety of colors on the spectrum, while red light has a wavelength of 632 nm. During PDT, light is only shown directly at the targeted tissues for short amounts of time (**Figure 1, c**). Following light exposure, the cancerous cells die (**Figure 1, d**).[1]

In comparison to chemotherapy and radiotherapy, photodynamic therapy has the advantage of being selectively toxic. By targeting the tumor, the remainder of the body is conserved during treatment allowing patients fewer widespread effects of toxicity. Another important factor of PDT is its ability to be repeated multiple times in the same

location. Other therapy types that utilize radiation cannot be used more than once, thus leaving malignant cells in the body to continue growing. In contrast, PDT can be applied to a tumor as many times as needed in order to remove all cancerous cells. Lastly, photodynamic therapy typically costs less than other therapy types that may be less accurate.[1]

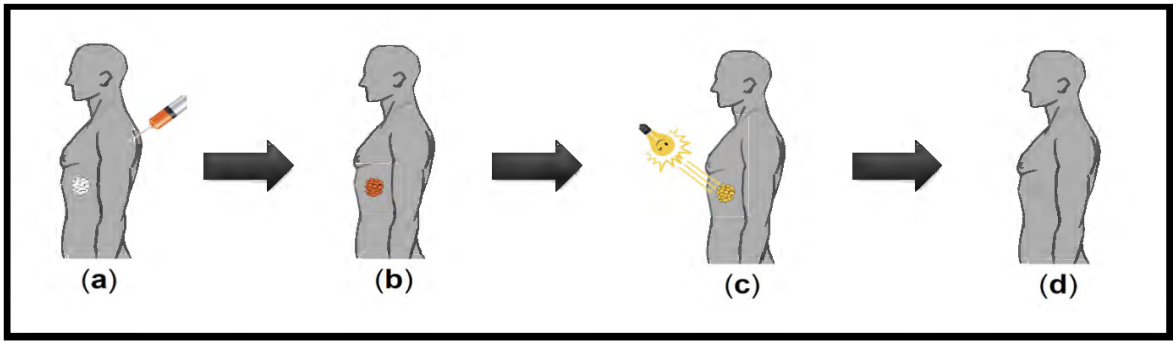


Figure 1: Photodynamic Therapy Diagram

Porphyrins

Porphyrins are organic compounds that are essential to many biological processes in the human body. They have key roles in electron transport, gene regulation, hormone synthesis, and more. Although there are many different kinds of porphyrins, they all share a common structure.[7] Each contains four pyrrole rings connected to each other by methine groups (**Figure 2**). This highly conjugated molecular structure makes them successful photosensitizers in PDT due to their ability to absorb light.[10] Ambiguity between the structures of different porphyrins can be found both in the center of the porphyrin and in the functional groups on the outside of the porphyrin.

This research focused on the use of a porphyrin as the photosensitizer in photodynamic therapy to kill malignant cells and various concentrations. The porphyrin that was synthesized and tested was called ZnTPP-4AB. It was metallated, meaning that the porphyrin ring contained zinc, and its functional groups were 4-amino-1-butanol (**Figure 3**).

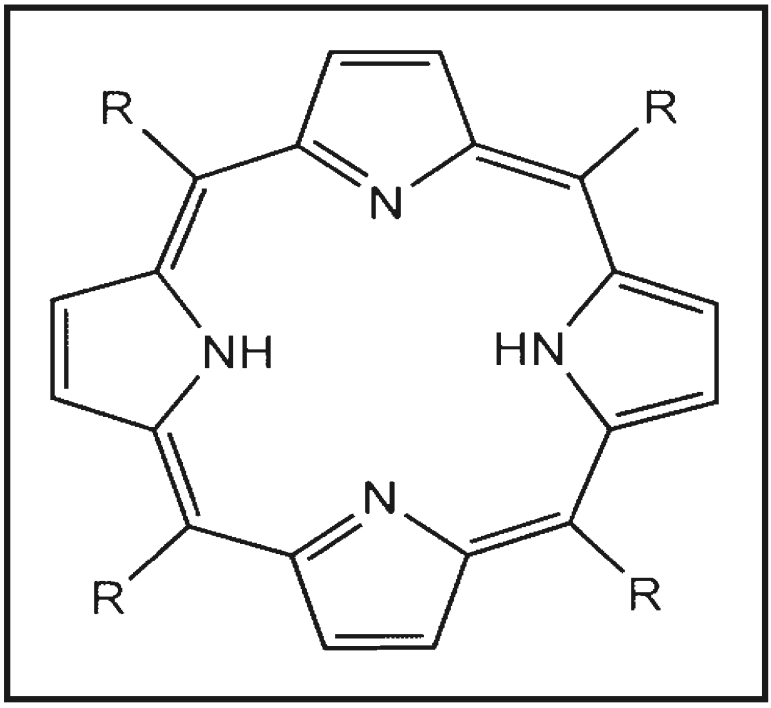


Figure 2: Generic Porphyrin Structure

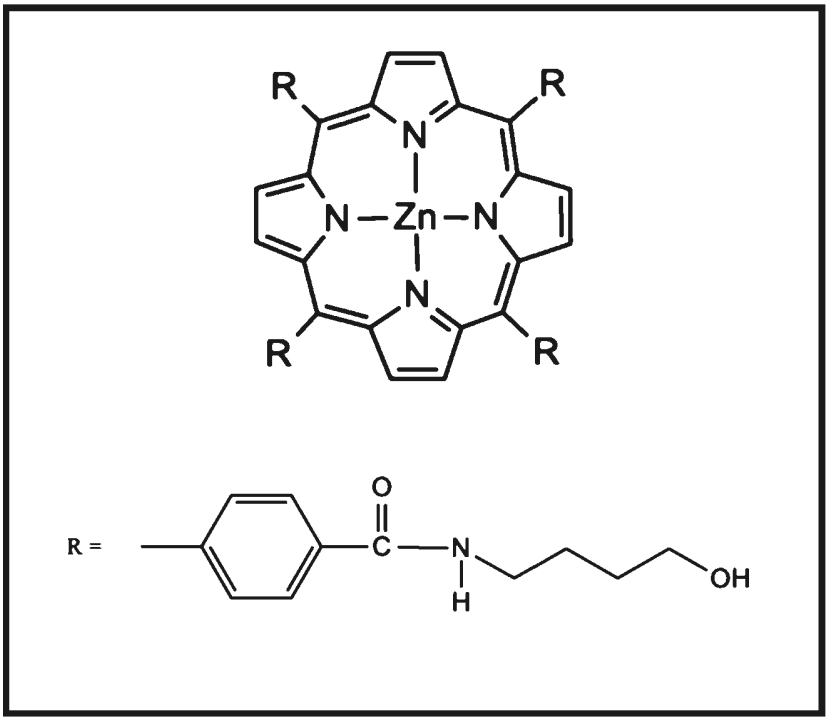


Figure 3: ZnTPP-4AB Porphyrin Structure

Methods

Synthesis

Reaction 1: Synthesis of H_2TPPC

The acronym H_2TPPC **(3)** can be broken down as follows: H_2 representing the two hydrogens in the center of the molecule, TPP standing for tetraphenyl porphyrin, and the C indicating the carboxylic acid functional groups on each of the four benzene rings of the molecule. To synthesize this product, a 500 mL round bottom flask was filled with 3.0 g of 4-carboxybenzaldehyde **(2)**, 150 mL of propionic acid, 1.5 mL of pyrrole **(1)** , and a stir bar. The flask was then wrapped with aluminum foil to minimize exposure to light and refluxed for 1 hour **(Figure 4)**. Following the reaction, the cooled flask was stoppered and stored at -20ºC overnight.

The product was then vacuum filtered using a medium sintered glass fritted filter. In order to remove impurities, dichloromethane was used to wash the product three times. The final product, H_2TPPC **(3)**, was allowed to air dry overnight before being added to a vial for storage. The reaction yielded 1.530 g of product (9.68% yield). Although this yield may seem very low, a yield of around 10-12% is typical for this reaction.

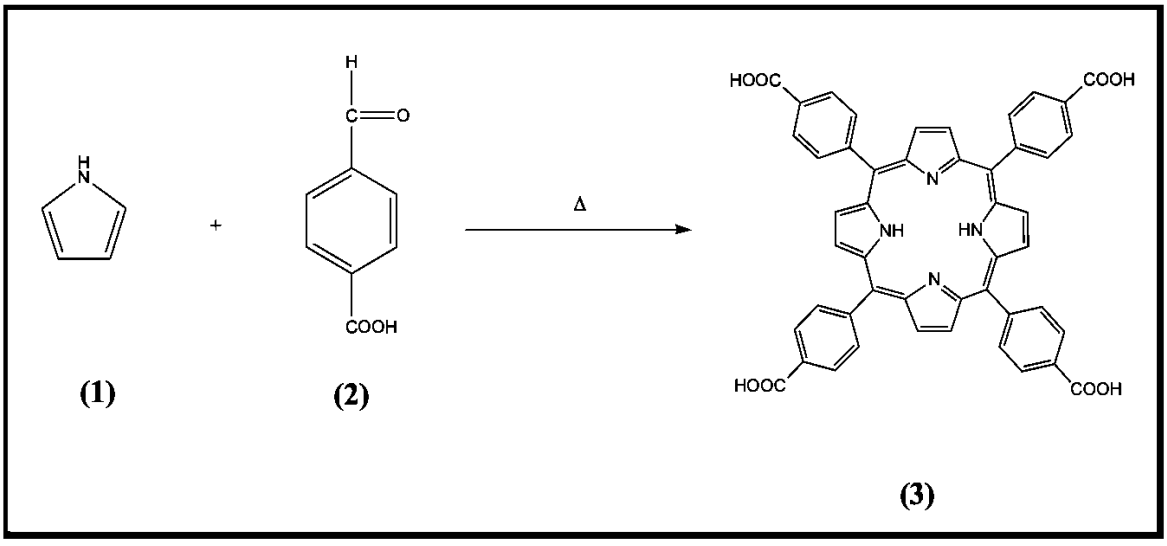


Figure 4: Synthesis of H_2TPPC

Reaction 2: Synthesis of ZnTPPC

In the reaction below (**Figure 5**), H_2TPPC **(3)** is being metallated with zinc to create ZnTPPC **(4)**. The two hydrogens at the center of the molecule are being replaced with Zn. Zinc porphyrins have been shown in previous research to be more efficient at killing A549 lung cancer cells than other metalloporphyrins and non-metallated porphyrins.[18]

A 125 mL round bottom flask was filled halfway with dimethylformamide (DMF), 1.0 g of H_2TPPC **(3)**, and 0.365 g of $ZnCl_2$. The flask was then heated and refluxed for 3 hours. To remove the DMF, the product was placed under vacuum. The crude ZnTPPC was next dissolved in methanol and filtered. Methanol was then rotovapped off, leaving only ZnTPPC. The purple product was allowed to cool before drying in an oven for 30 minutes and being placed in a vial for storage.

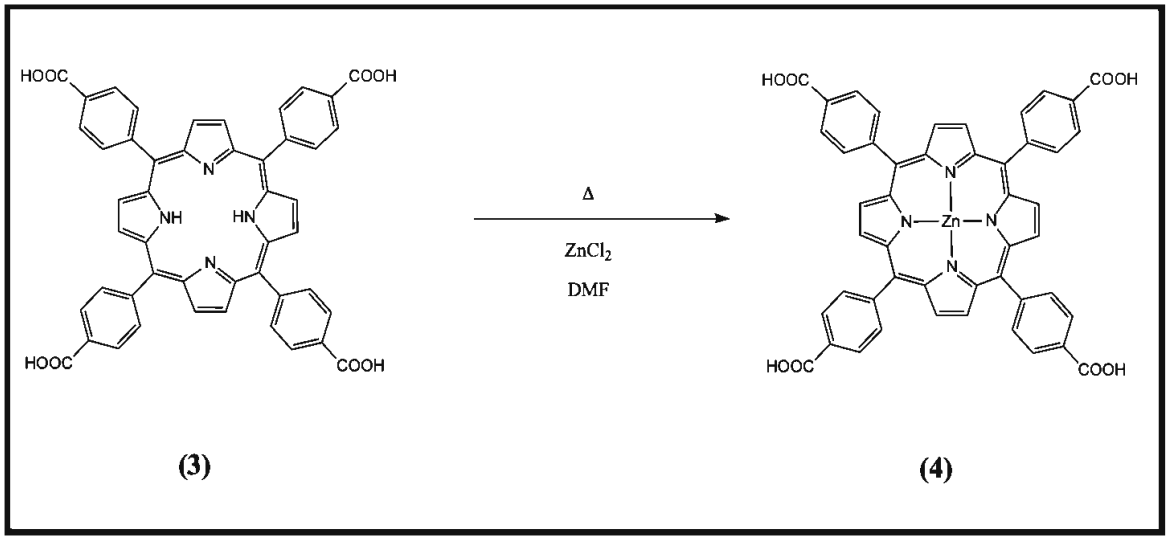


Figure 5: Synthesis of ZnTPPC

Reaction 3: Synthesis of Acid Chloride ZnTPP

In order to create the acid chloride ZnTPP **(5)** (**Figure 6**), the four carboxylic acid groups (COOH) on each of the ZnTPPC **(4)** porphyrin's corners were reacted with thionyl chloride to form the acid chloride (COCl). The purpose of this reaction is to switch the OH group with Cl, since chloride will be easier to remove and substitute with our desired R group in later reactions.

For this reaction to be carried out correctly, water must not react with the product at any point in time. In order to eliminate water contact through air moisture, a 125 mL round bottom flask was dried in an oven and immediately stoppered. During the duration of this reaction, the round bottom flask was kept under a nitrogen atmosphere. A 0.15 g portion of ZnTPPC **(4)** was added to the flask with enough dried DMF to fill the flask half way. Using a syringe, 0.15 mL of thionyl chloride was added to the flask and allowed to stir for 1 hour under nitrogen. After reaction completion, the DMF was rotovapped off and the product was kept under vacuum overnight.

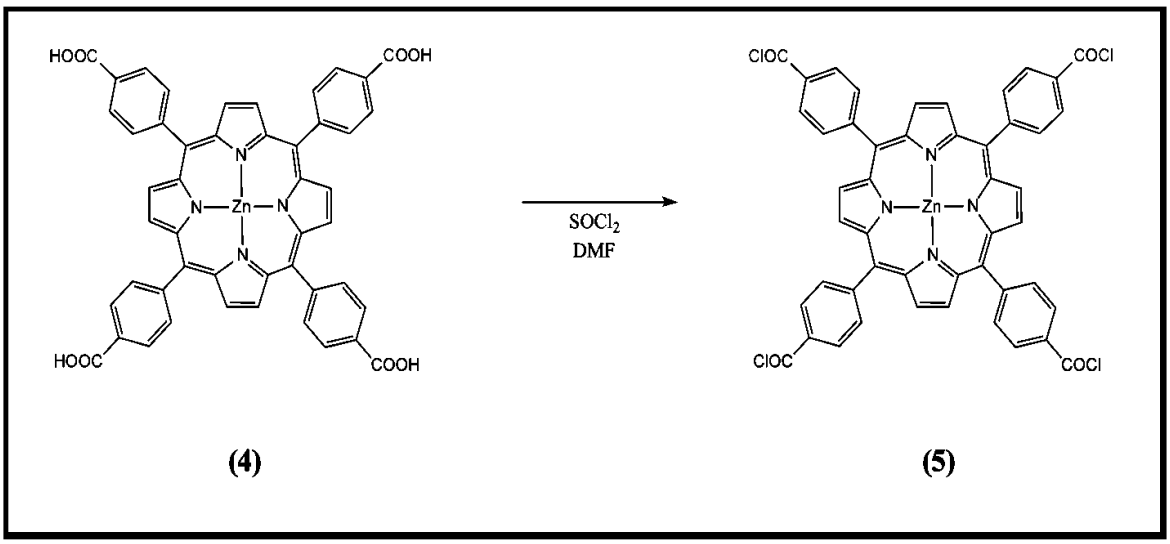


Figure 6: Synthesis of Acid Chloride ZnTPPC

Reaction 4: Synthesis of ZnTPP-4A

In the reaction below (**Figure 7**), the acid chloride of ZnTPPC **(5)** was reacted with 4-amino-1-butanol (4AB) in order to form ZnTPP-4AB **(6)**. The 4-amino-1-butanol group is what makes this porphyrin unique to this research. Previous researchers have used different R groups (primarily amines) and tested their efficacy in photodynamic therapy. In order for this porphyrin to have clinical relevance, it is important to ensure that it is soluble in water. Its hydrophilicity will allow cells in the human body to absorb it during the photodynamic therapy process.

To begin Reaction 4 (**Figure 7**), the round bottom flask from Reaction 3 was quickly removed from vacuum and placed again under a nitrogen atmosphere. Next, a vial containing 0.310 g of 4-amino-1-propanol and 40 mL of methanol was quickly added

to the round bottom flask. The mixture was allowed to stir for one hour under the N_2 atmosphere. The methanol was then removed using the rotovap. Following completion, a small amount of the final product ZnTPP-4AB **(6)** was mixed with Milli-Q H_2O to test its solubility. After solubility was ensured, the product was stored in a vial and the synthesis portion of this project was repeated four times.

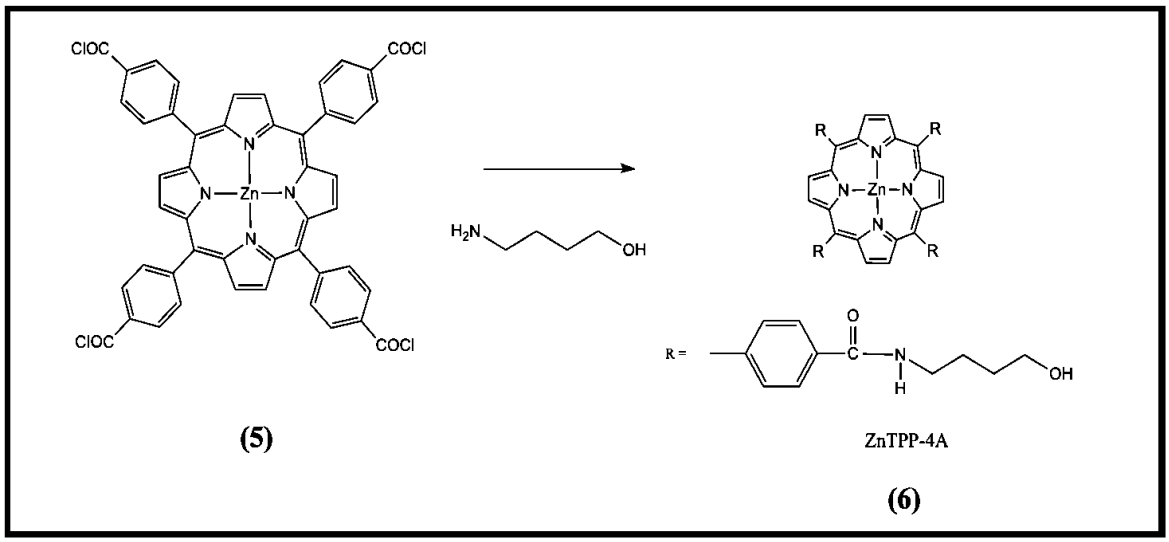


Figure 7: Synthesis of ZnTPP-4AB

Purification

Column Chromatography: Sephadex LH-20

Sephadex is a cross-linked dextran gel that is used for gel filtration, a technique used for the filtration of small organic molecules.[2] The Sephadex LH-20 powder was dissolved in a 50/50 mixture of methanol (MeOH) and Milli-Q H_2O before being added into a column apparatus. The column was assembled where a 50 mL erlenmeyer flask could be placed under the drip guard to catch any liquid that passed through the Sephadex column (**Figure 8**). ZnTPP-4AB was diluted with 50/50 MeOH and H_2O before being filtered through a syringe filter. With air flowing through the top of the column to speed up the process, the filtered ZnTPP-4AB was added to the column.

Almost immediately, the product began moving down the column in two separate color components: brown and purple. The brown fraction, indicating impurities, moved quicker through the Sephadex LH-20 and was collected into an Erlenmeyer flask. As

soon as the purple product began dripping into the flask, the Erlenmeyer flasks were exchanged to ensure that only pure product was collected. Once all of the ZnTPP-4AB was filtered through the column, the pure purple product was placed on the rotovap to remove the MeOH/H_2O solvent. The collected product was a purified form of ZnTPP-4AB.

Figure 8: Column Chromatography: Sephadex LH 20

Column Chromatography: Sephadex G50

Following chromatography using Sephadex LH-20, the process was repeated using a separate column filled with Sephadex G50 that used Milli-Q H_2O instead of the MeOH/H_2O mixture as the eluent. Sephadex G50 separates based on molecular size, whereas Sephadex LH-20 separates based on lipophilicity and polarity.[12] When the product was run through the column, there were several different colored impurities that were separated from the desired product **(Figure 9)**. As before, only the desired purple product was kept and was placed onto the rotovap to remove the Milli-Q water. The final

purified product, ZnTPP-4AB, was collected.

Figure 9: Column Chromatography Impurities

Characterization

Infrared Spectroscopy

To ensure that the desired product, ZnTPP-4AB, was synthesized, Infrared Spectroscopy (IR) was performed. Infrared Spectroscopy is a characterization technique that sends infrared radiation through a molecule, causing its bonds to vibrate. During this interaction, different bonds will absorb the radiation at different wavenumbers. The different absorption data for each bond provides information on functional groups within the molecule.[4] The IR spectrum shown in **Figure 11** for ZnTPP-4AB indicated the desired compound was successfully synthesized. For comparison, IR was also performed on the free amine, 4-amino-1-butanol (**Figure 10**). When comparing the two spectra, appearances of a broad alcohol peak and three amide bands for ZnTPP-4AB confirm the formation of the final product. See **Table 1** for information about which functional groups correspond to each peak.

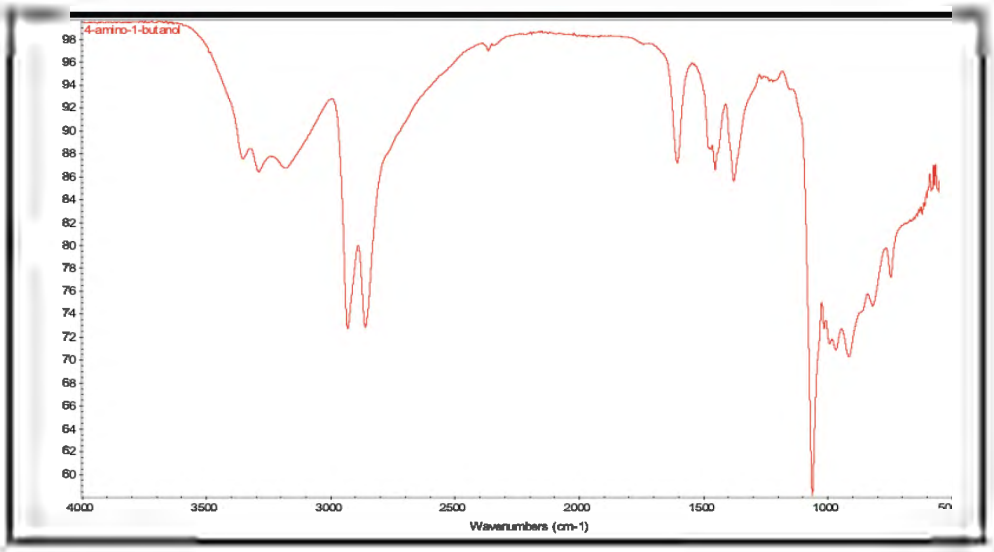


Figure 10: IR Spectroscopy: 4AB

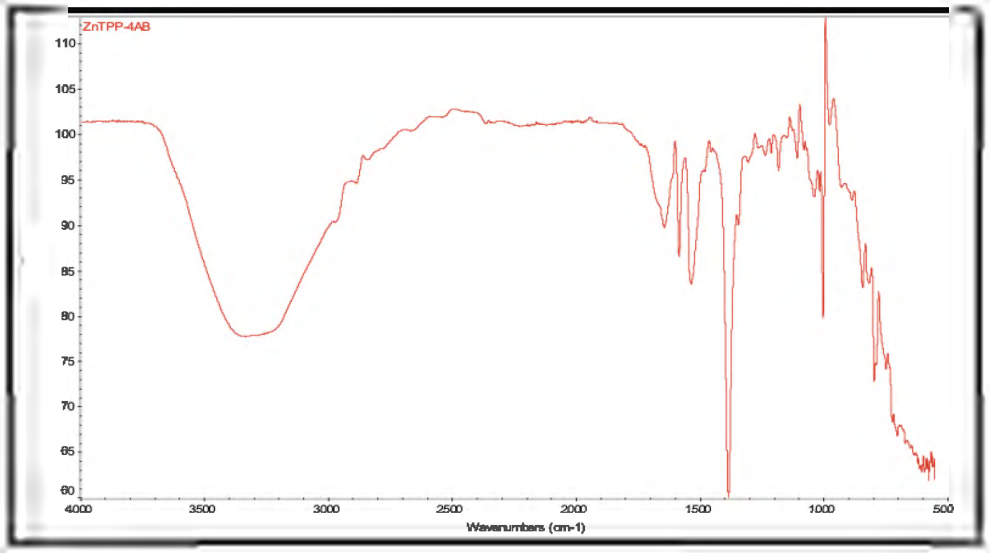


Figure 11: IR Spectroscopy: ZnTPP-4AB

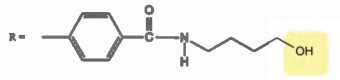
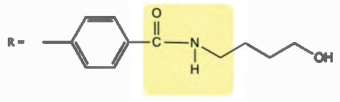
Bond type	Peak location	Location on Porphyrin
Alcohol	3300 cm-1	
2º Amide	1470-1570 cm-1	

Table 1: ZnTPP-4AB IR Spectroscopy Corresponding Peaks

Nuclear Magnetic Spectroscopy

Nuclear Magnetic Spectroscopy (NMR) is another characterization technique that was used to further ensure the synthesis of the desired product (ZnTPP-4AB). However, instead of using infrared light to interact with the molecule, ^{1}H NMR focuses on the magnetic properties of the protons in the nuclei of the molecule.[5] The location of peaks, measured in parts per million (ppm), is based on the location and characteristics of different hydrogen atoms on ZnTPP-4AB. An analysis of the peaks on the ^{1}H NMR spectrums of both the free amine **(Figure 12)** and the porphyrin **(Figure 13)** confirmed the identity of the sample to be ZnTPP-4AB. The final porphyrin contained peaks *ca.* 8 ppm that were characteristic of the hydrogen atoms on the pyrrole and aromatic rings as well as aliphatic protons *ca.* 1.5-4.0 ppm.

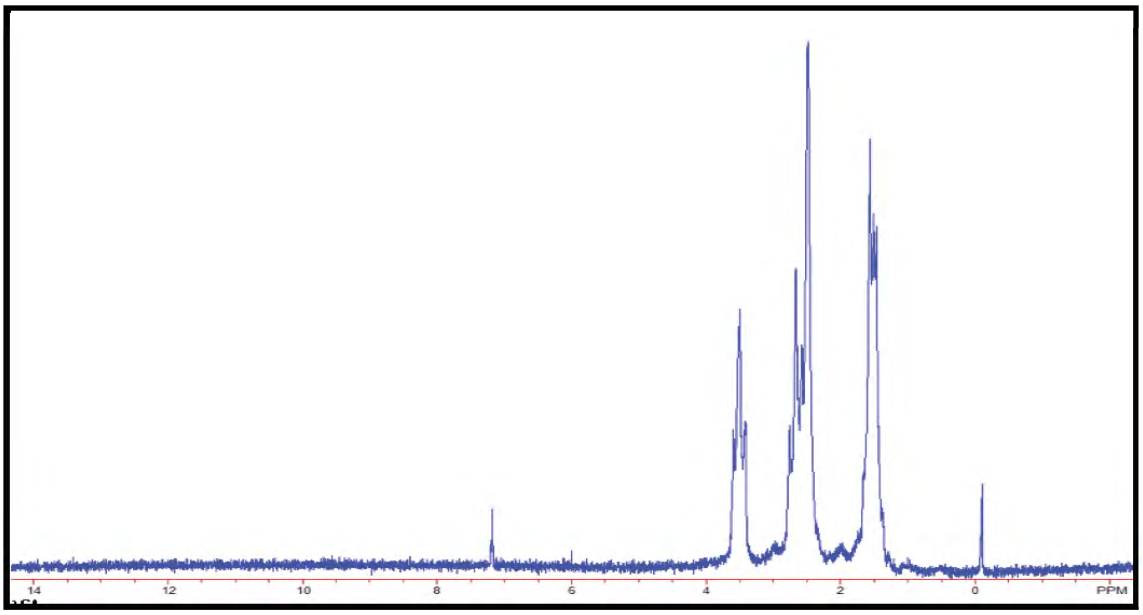


Figure 12: H NMR Spectroscopy: 4-amino-1-butanol in $CDCl_3$

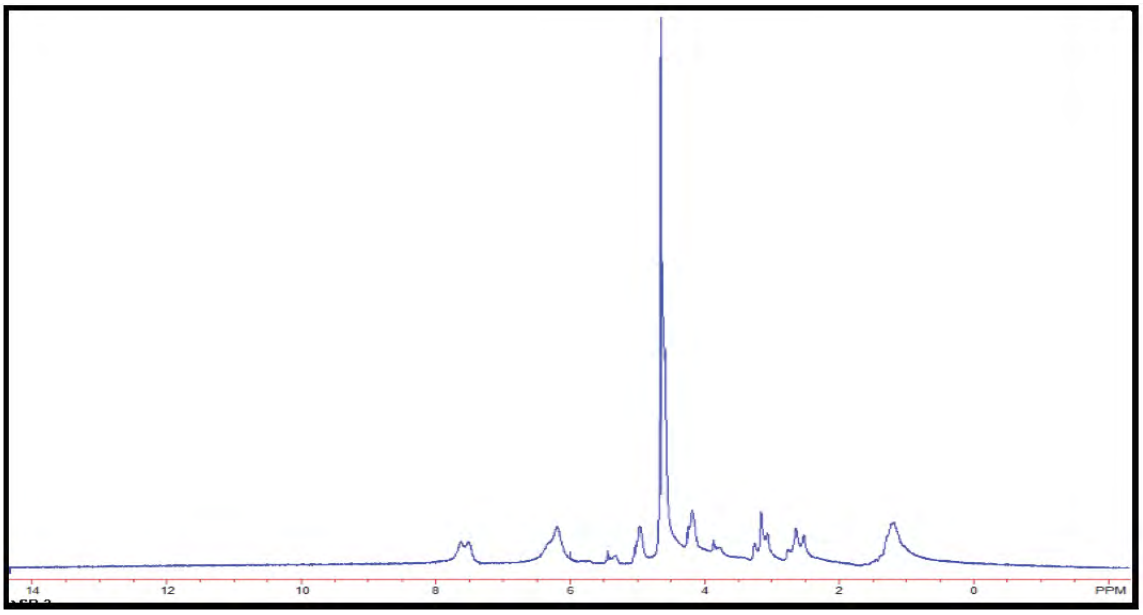


Figure 13: H NMR Spectroscopy: ZnTPP-4AB in D_2O

Ultraviolet - Visible Spectroscopy

The last spectroscopic technique that was performed to confirm the identity of the sample was Ultraviolet-Visible Spectroscopy (UV-Vis). UV-Vis the technique measures the wavelengths of ultraviolet and visible light that are absorbed by the sample. While passing through the sample, the light excites electrons to higher energy states which can be detected by the spectrophotometer to identify the unique structure of the molecule.[6] The varying wavelengths for the porphyrin compound are each associated with an absorbance value that can be used in Beer's Law to calculate the molar absorptivity (ε) of the molecule.[15] The molar absorptivity coefficient can give information about light absorption capabilities of a molecule, with larger values indicating stronger absorption.[16] Looking at **Figure 14**, a Soret band peak is located at 423 nm with an ε value of 497 $mm^{-1}cm^{-1}$. A Soret band at 423 nm indicates that a porphyrin is metallated with Zn, while a Soret band at 415 nm would indicate an unmetallated porphyrin. Because ZnTPP-4AB is a porphyrin that is metallated with zinc, this data indicated that the metallation step of synthesis was completed successfully. Two additional peaks are located at 558 nm and 597 nm. The molar extinction coefficient, ε, for each of the three peaks were calculated and are shown in **Table 2**.

Figure 14: UV-Vis Spectroscopy Full ZnTPP-4AB Spectra

Wavelength (nm)	Molar Extinction Coefficient ($mM^{-1}cm^{-1}$)
423	497
558	15.4
597	9.05

Table 2: NMR Molar Extinction Coefficients

High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a technique commonly used to assess the purity of a sample. A solution of the sample is injected through a packed column under high pressures, forcing the separation of the solution into constituent parts.[11] A Hamilton PRP 1-5µm 4 x 150 mm column was used with 100% acetonitrile as the solvent at a flow rate of 1.00 mL per minute. HPLC was performed on the ZnTPP-4AB sample (**Figure 15**) and yielded only one peak. The purity was found to be >99% pure. This validated that the sample did not contain significant amounts of impurities, as other peaks would have been present if there were other impurities.

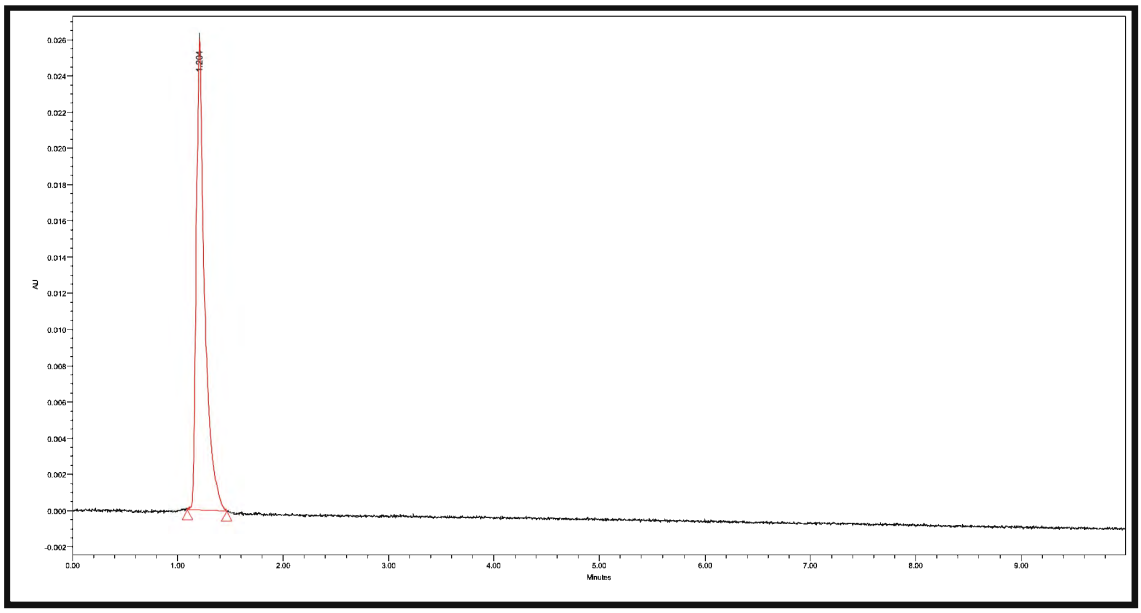


Figure 15: HPLC: ZnTPP-4AB

Cytotoxic Testing

Preparation

In order to determine the efficacy of the porphyrin ZnTPP-4AB as a PDT agent, it was tested on adenocarcinomic human alveolar basal epithelial cells that came from cancerous lung tissue. The A549 cell line used was obtained from the American Type Culture Collection. To keep the cells alive for the duration of the research, cell culture techniques were performed. MEM growth medium was routinely exchanged in each cell flask to maintain a healthy living environment for the cells. To prevent overcrowding and subsequent cell death, cells were "split" into two separate flasks once they reached 80% confluency. When it was time to begin cytotoxic testing on the cells, they were separated into three 96-well plates so that roughly 1,000 cells were in each well. Only rows 8-12 on each of the three plates were filled.

The growth medium in each well was replaced with differing concentrations of the ZnTPP-4AB porphyrin three days after initial plating . Each successive row of wells had an increasing concentration of porphyrin, following the order of 1µM, 3µM, 10µM, 30µM, 100µM. Refer to **Table 3** for specific components of each well. 100 µL of each

dilution was added to its appropriate row and immediately wrapped with foil to exclude excess light. Overnight, the cells were kept in the incubator to absorb the porphyrin.

Row #	1	2	8	9	10	11	12
Concentration	**Cntrl**	**DMSO**	**1 µM**	**3 µM**	**10 µM**	**30 µM**	**100 µM**
Stock Type	N/A	DMSO	Porph. 1 mM	Porph. 1 mM	Porph. 10 mM	Porph. 10 mM	Porph. 46 mM
Stock Amount	N/A	9 µL	3 µL	9 µL	3 µL	9µL	6.52 µL
Media Amount	3 mL	3 mL	3 mL	3 mL	3 mL	3 mL	3 mL

Table 3: Components of the 96-well Plates

Light Exposure

In past research, white light was used exclusively to activate the porphyrin for use in photodynamic therapy. White light uses a variety of colors on the spectrum while red light has a larger wavelength of 632 nm. In theory, red light could be used to penetrate deeper into a patient's body to reach a tumor. This research project focused on the effects of both red and white light in combination with using the ZnTPP-4AB porphyrin.

Once the porphyrin-containing cells reached *ca.* 50% confluency, each of the three 96-well plates were removed from the incubator. One of the plates was assigned to the "dark" treatment, serving as a control by not receiving any light exposure. The dark plate stayed in the incubator, wrapped in foil. A second well plate was assigned to the "white light" treatment. This plate was exposed to white light for 26.5 minutes to receive a total of 0.5 Joules/cm^2. The third plate received the "red light" treatment. It was exposed to red light for 56 minutes, totaling 0.5 Joules/cm^2. Following the treatments, all three plates were kept in the incubator for 3 days. Foil was lightly placed on top of each plate to eliminate any excess light exposure.

Hypoxia

During the cytotoxic testing portion of the project, the possibility of running the same experiment on cells in a hypoxic (without oxygen) environment was introduced.

This idea originated from the fact that the fast-growing nature of cancerous cells creates overcrowded areas (tumors) that result in reduced amounts of oxygen and nutrients.[8] Putting the cells in a hypoxic environment before treating them with the porphyrin may better simulate how cancer cells respond in the human body. In order to add this new component to the project, three additional 96-well plates were filled with roughly 1,000 cells in each well. Then, the plates were placed inside a nitrogen gas chamber. After running nitrogen gas through the chamber for four minutes, the chamber was sealed and placed in the incubator for four hours. Following incubation, the ZnTPP-4AB porphyrin was added to rows 8-12 in differing concentrations. In order to identify a specific concentration of porphyrin for a "lethal dose", concentrations of the porphyrin occupied a smaller range than those used on the non-hypoxic cells. These concentrations were 1µM, 2.5 µM, 5 µM, 7.5 µM, and 10 µM. Refer to **Table 4** for specific components to each well. 100 µL of each dilution was added and the plate was immediately wrapped with foil to exclude excess light. For three days, the cells were kept in the incubator to absorb the porphyrin.

Row #	**1**	**2**	**8**	**9**	**10**	**11**	**12**
Concentration	**Cntrl**	**DMSO**	**1 µM**	**2.5 µM**	**5µM**	**7.5µM**	**10µM**
Stock Type	N/A	DMSO	Porph. 1 mM	Porph. 1 mM	Porph. 1 mM	Porph. 10 mM	Porph. 10 mM
Stock Amount	N/A	20 µL	1 µL	10 µL	20 µL	3µL	4 µL
Media Amount	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL

Table 4: Components of the Hypoxic 96-well Plates

MTT Assay

An MTT Assay is a widely used technique to measure cell toxicity in relation to drugs. MTT is a material (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) that is converted into formazan crystals by living cells. The purple crystals are identified by the assay as indicators of cell viability, which can in turn expose the cells that are not living.[17] **Figure 16** shows the three (non-hypoxic) plates after MTT was added to the

cells. It is possible to see the differences in cell viability between different light treatments and different porphyrin concentrations. The MTT assay for this research was performed using a TECAN microplate reader with Magellan 6 software.

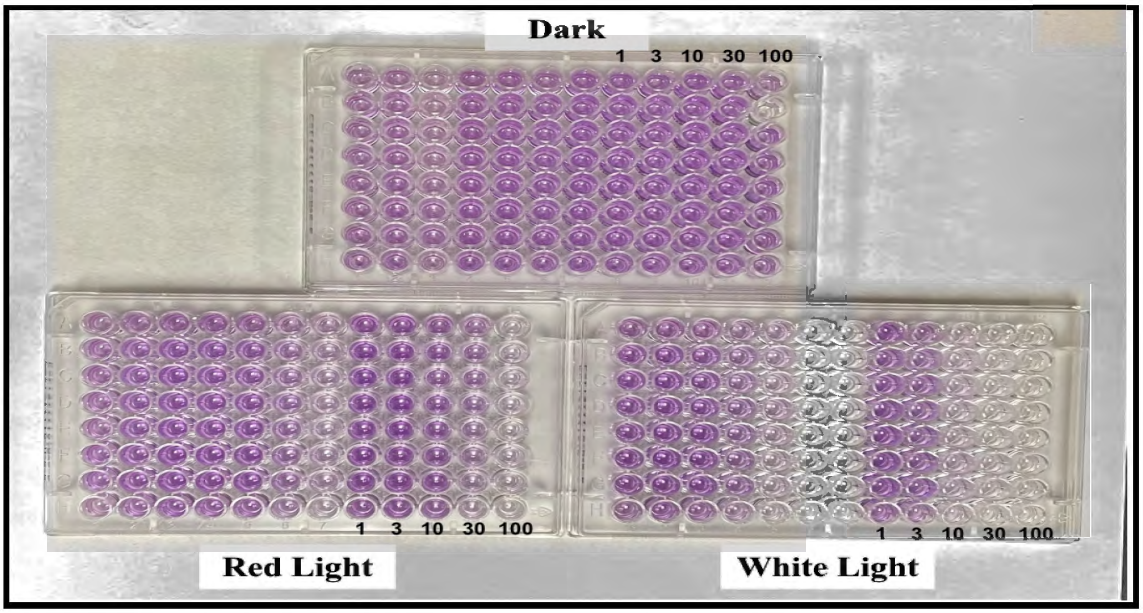


Figure 16: Visualization of Cell Viability Using MTT

Results

Based on the data gathered from the MTT assay, cell viability was assessed based on porphyrin concentration and light exposure (**Figure 17**). Cell toxicity was dependent on ZnTPP-4AB concentration under both red and white treatments. For both light treatments, increasing the concentration of porphyrin decreased cell viability. The white light treatment killed more cells at lower porphyrin concentrations than red light. The lethal dose of ZnTPP-4AB that killed 50% of the cells (LD_{50}) was 6 µM under white light and 30 µM under red light.

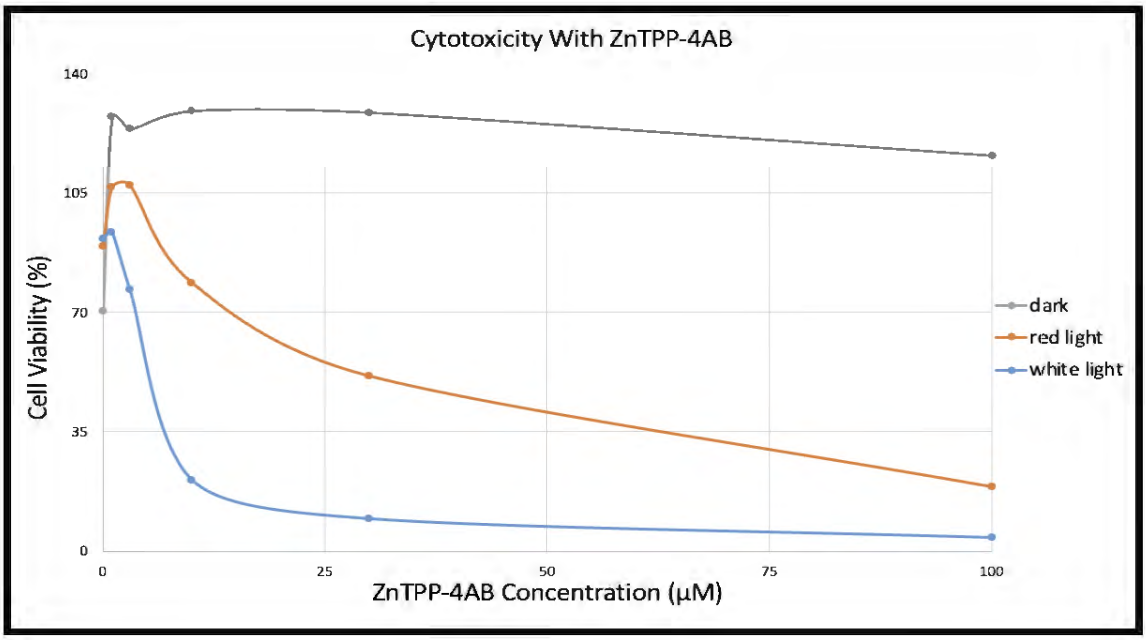


Figure 17: Cell Viability Based on Porphyrin Concentration and Light Exposure

Data summarizing cell viability in hypoxic conditions is found in **Figure 18**. There was little to no difference in cytotoxicity between no light and red light treatment under hypoxia in the concentration range tested (0-10µM ZnTPP-4AB). Both hypoxic and oxygen treated plates under white light had high cytotoxicity, but the hypoxic environment paired with white light had the highest cytotoxicity, with an LD_{50} at 6 µM.

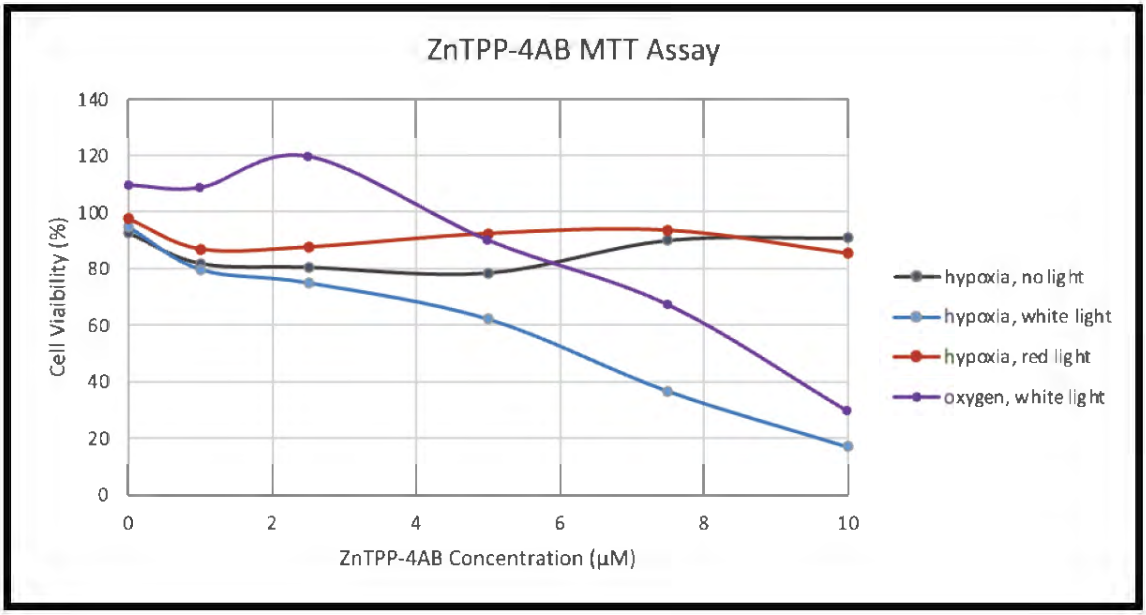


Figure 18: Cell Viability Based on Oxygen Presence, Porphyrin Concentration, and Light Exposure

Conclusions

ZnTPP-4AB, a novel water-soluble porphyrin, was successfully synthesized. Its structure was validated through characterization using UV-vis, IR, and NMR spectroscopies. Without light exposure, the porphyrin had minimal to no effect on cell viability. This result is favorable because PDT agents should only be killing cells after being exposed to light. Light exposure paired with increasing ZnTPP-4AB concentration caused cytotoxicity, with the white light treatment having a lower LD_{50} than red light. A lower LD_{50} is ideal for photodynamic therapy agents in order to keep the body's exposure to external porphyrins as low as possible. White light treatment given in a hypoxic environment caused more cell death than when under normal oxygen conditions. This is promising since tumors are typically in a hypoxic environment in the human body, meaning that potentially more cancerous cells could be killed using PDT.

ZnTPP-4AB is an effective photosensitizing agent for A549 non-small cell lung cancer cells with an LD_{50} of 6 μM. Choosing ZnTPP-4AB over other porphyrin compounds will prevent lung cancer patients from unnecessary toxicity while maximizing the efficacy of photodynamic therapy. Implementing PDT into cancer therapies could decrease the potential physical and financial tolls that are associated with other treatments such as radiation and chemotherapy.

Future Directions

In the future, this research could be expanded to explore hypoxic effects on a larger range of porphyrin concentrations. In doing this, more information can be gained about how a real tumor in the body may react to ZnTPP-4AB paired with light. Once this information is gained, ZnTPP-4AB could be used *in vivo* to evaluate tumor reaction in specimens that have lung cancer. This would be the best way to see how a legitimate lung cancer tumor reacts to the porphyrin in the body. Further, data regarding tumor recurrence after treatment with photodynamic therapy could also be gathered. Additionally,

ZnTPP-4AB could be tested on other cancer cell lines such as HCT116 colorectal carcinoma or A431 epidermal carcinoma in order to examine and compare its cytotoxic effects on these cell lines.

PDT in Dermatology

Photodynamic therapy's use in dermatology has recently become a topic of high interest. Skin cancer is a prevalent issue that often gets overlooked. In fact, some may be surprised to find out that in the United States, skin cancer gets diagnosed more each year than all of the other cancers combined.[13] Before a region of the skin becomes fully cancerous, they typically present as precancerous actinic keratoses (AK's).[13] These spots are "erythematous, flat, scaly papules that can range from a few millimeters to a few centimeters in diameter".[14] Because of its constant exposure to the sun, the scalp and face are two of the most common areas for AK's to appear. It is imperative to catch actinic keratoses before they become fully cancerous due to their high probability of transforming into squamous cell carcinomas.[14] The use of photodynamic therapy as a way to treat precancerous AK's has grown increasingly popular in dermatology. Yale University is one of the leading pioneers of this novel technique. Dr. Christensen, a dermatologist at Yale, claims that PDT "can be an effective way to treat skin issues before they become a problem, and before they even appear."[9]

Because of the external location of skin, PDT can be practiced without the need for needles or endoscopes. Instead, clinicians can apply a topical cream, containing a photosensitizing agent, to the area of concern before using a variety of light sources to activate the material. During application, the photosensitizer is usually spread across a large region of the skin to include multiple precancerous spots, a technique coined "field therapy".[9] Research is still being conducted to determine the most successful photosensitizing agent, but the two front runners that are currently licensed for clinical use are Aminolevulinic acid (ALA) and methyl aminolevulinate (MAL).[14] After administering the topical cream, a period of incubation is required for the skin cells to absorb the material. This period can vary, depending on the size and severity of the target region, but typically falls within the range of 90 to 540 minutes.[9] In order to activate the

photosensitizing agent, light is administered to the target region. Although the efficacy of different light sources is still being evaluated, researchers at Yale University use a U-shaped blue, fluorescent lamp as an activator. During the 16-minute exposure period, patients may experience "mild tingling or a burning sensation" and for up to two weeks after application, the treated area may present with symptoms similar to a sunburn, such as redness and peeling.[9] Because of an increased sensitivity to light after treatment, patients are instructed to cover the affected area and to stay out of direct sunlight or intense indoor lighting for 48 hours.

In comparison to other skin treatment options for precancerous spots such as liquid nitrogen and excision, photodynamic therapy offers a more practical solution that is less painful. However, even with such low stakes surrounding the technique, researchers have continued to search for PDT techniques that are still more convenient. The most recent topic of interest has been using daylight as an activating source of light, an approach called D-PDT. In comparison with conventional PDT (C-PDT), daylight PDT offers higher convenience through lower incubation times and even less adverse skin reactions after treatment.[14] Light exposure can be administered as early as 30 minutes after applying the topical cream rather than a minimum of 1.5 hours. Studies conducted to determine differences in efficacy between C-PDT and D-PDT have concluded that in the long term, no significant differences were found in the complete response rates of subjects who used each technique on half of their face.[14] The research surrounding photodynamic therapy's use in dermatology is far from finished and will continue to seek the most efficient techniques to treat skin cancer.

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