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ZnTPPEA as a Potential Photosensitizer in Photodynamic Therapy

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Abstract

Photodynamic therapy (PDT) is an emerging treatment that can be utilized against certain types of cancer and other various diseases. It functions using a photosensitizer in the presence of light that contributes to cell death in the desired tissues. This research centered on the development of a novel water-soluble porphyrin that could be utilized as one of these photosensitizers. This was achieved by adding the hydroxyamine, ethanolamine, to the outer portion of the ZnTPPC core. The resulting compound, ZnTPPEA, was created using two different synthetic strategies. Purification was carried out using column chromatography with Sephadex G-50 followed by Sephadex LH-20. The desired ZnTPPEA was then characterized through ultraviolet-visible spectroscopy (UV-vis), nuclear magnetic resonance (NMR), and infrared spectroscopy (IR), as well as, the use of high performance liquid chromatography (HPLC) to determine the purity of the final product. The ZnTPPEA was tested on the A549 lung cancer cell line using an MTT assay under light and dark conditions to assess the compound's effectiveness as a photosensitizer for PDT.

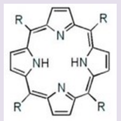


Figure 1: Standard Porphyrin Core Structure (Unsubstituted)

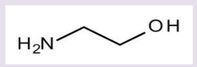


Figure 2: R-Group attached to Porphyrin Core: Ethanolamine

Introduction

A549 Cell Line

- Non-small cell lung cancer
- Most common type of lung cancer
- Represents about 80-85% of lung cancer diagnoses
- Its 5 year survival rate is about 25%

Photodynamic Therapy

The patient is injected with a photosensitizer after being diagnosed with a tumor

Over time, the photosensitizer collects in the tumor

The photosensitizer is activated by exposing the tumor to light for a designated time period

The tumor is selectively destroyed by the photodynamic therapy treatment

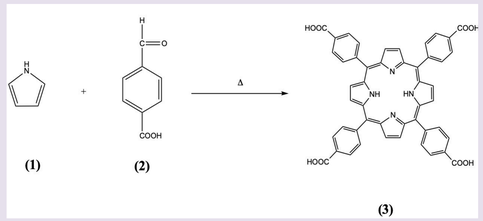
Porphyrins

- Porphyrin compounds have many uses due to their conjugated structure, light absorbing qualities, and ability to act as photosensitizers.
- Porphyrins are able to perform various functions including gene regulation, hormone synthesis, oxygen transport medium (hemoglobin), solar cell (convert light or chemical energy), as well as potentially treating conditions such as different forms of cancer, atherosclerosis, rheumatoid arthritis, macular degeneration, and various autoimmune diseases.
- This research specifically investigates the light sensitivity of the porphyrins and their ability as a photosensitizer.

Synthesis

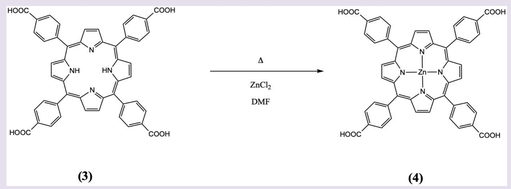
Reaction 1

- 4-formylbenzoic acid (2) reacted with pyrrole (1) in a propionic acid solution to form H_2TPPC (3).



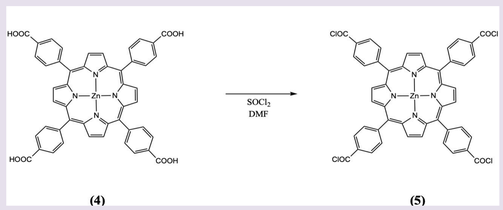
Reaction 2

- H_2TPPC (3) reacts with zinc chloride in dimethylformamide, forming an acid chloride porphyrin- ZnTPPC (4).



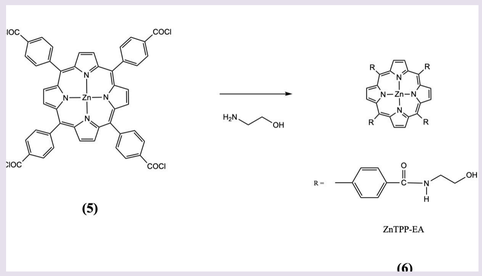
Reaction 3

- The acid chloride porphyrin (4) reacts with thionyl chloride in dimethylformamide to form the acid chloride intermediate (5).



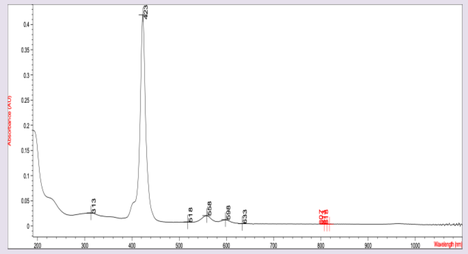
Reaction 4

- The acid chloride intermediate (5) reacts with ethanolamine in to form ZnTPP-EA, the final product. (6).



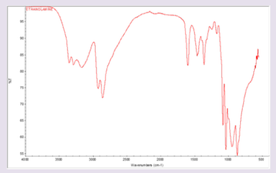
Characterization

UV-vis Spectroscopy

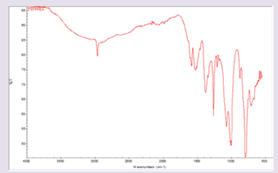


Peaks (nm)	Molar Absorptivity Coefficient, e (cm-1mM-1)
423.0	419.44
313.0	25.69
558.0	20.42
598.0	12.29
518.0	7.96

Infrared Spectroscopy

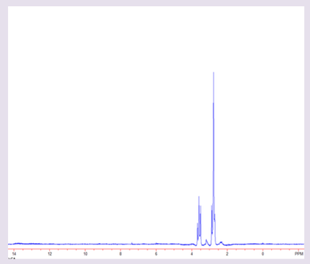


H_2TPPC

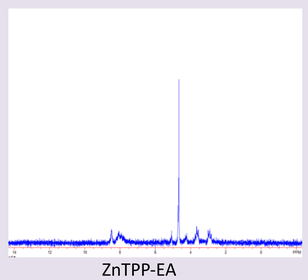


ZnTPPEA

Nuclear Magnetic Resonance Spectroscopy

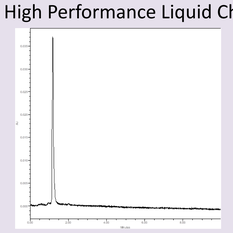


Ethanolamine



ZnTPP-EA

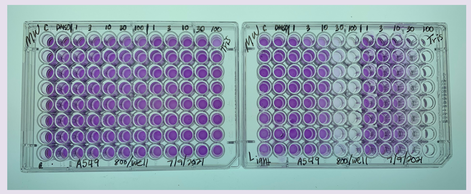
High Performance Liquid Chromatography



- Solvent: 100% acetonitrile
- Flow rate: 1.00 mL per minute
- Column: Hamilton PRP-1
- 5 µm 4 x 150 mm
- Sample dissolved in H_2O
- 99% pure

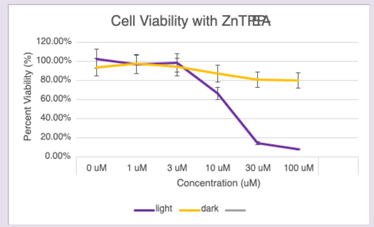
MTT Assay

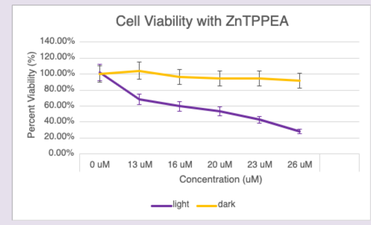
- ZnTPPEA is shown in columns 3-7 on each plate
- Living cells are shown by the purple color
- The plate on the left was kept in the dark
- The plate on the right was exposed to white light for approximately 26 minutes 18-24 hours after treatment with the porphyrin compound



Results

- Exposure to light resulted in much lower cell viability when compared to the cells that were kept in the dark
- Cell viability is also affected by concentration of porphyrin. compound in both light and dark conditions
- The first MTT assay shows that significant cell death began around 10 µM when cells were exposed to light
- Concentrations were narrowed down between 1—30 µM in the second MTT assay to investigate where cell viability was approximately 50%. This value was around a concentration of 20 µM .





Conclusions

- A novel water-soluble porphyrin was successfully synthesized.
- The compound was characterized by UV-vis, IR, and NMR spectroscopies as well as high performance liquid chromatography.
- The spectrums show that the correct compound was formed.
- MTT assays showed cell death from concentrations of 1—100 µM when exposed to light but normal cell growth in dark conditions, making this compound a legitimate photosensitizer.

Future Direction

- Test various versions of porphyrin compound with a certain wavelength of red light that could become useful in further development of treatments
- Begin in vivo testing of porphyrin compounds with live animals
- Synthesize an unmetylated version of this compound and test using MTT assays.

Acknowledgements

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