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The Photodynamic Therapy Potential of a Novel Water Soluble Gallium Porphyrin

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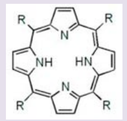
Abstract

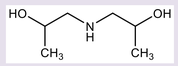
Photodynamic therapy (PDT) has potential use in the treatment of cancer and other health disorders. PDT utilizes light and a photosensitive agent that once activated by light generates singlet oxygen that affects surrounding cells. Metalloporphyrins have been shown to accumulate in tumors as the result of preferential binding to low-density lipoproteins. Additionally, gallium(III) porphyrins have been shown to be effective potential photosensitizers for PDT. The goal of this research was to synthesize and characterize the novel photosensitive agent, GaTPP-DIPA, as a PDT agent. The GaTPP-DIPA was purified using column chromatography and characterized using IR, UV-Vis, and NMR spectroscopies, purity was determined using HPLC. Cytotoxicity testing of the GaTPP-DIPA using U87 glioblastoma cells in both light and dark conditions determined that novel material has potential as a next generation PDT agent.

Figure 1: Standard Porphyrin Core Structure (Unsubstituted)



A standard poryphrin ring structure, unsubstituted.

Figure 2: R-Group attached to Porphyrin Core



Diisopropanolamine

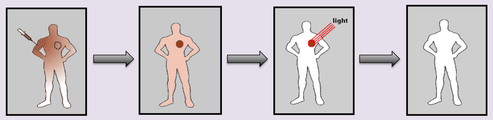
Introduction

U87 Cell Line

- Glioblastoma Cancer Cells found in the brain
- Typically results in death 15 months after diagnosis
- Each year, 20,000 Americans are diagnosed
- Spreads quickly
- 5 year survival rate is 10%

Photodynamic Therapy

- A patient diagnosed with a tumor is injected with a photosensitizer.
- Due to the nature of the photosensitizer, it collects in the tumor over time.
- The tumor is exposed to light for a given amount of time, activating the photosensitizer.
- The tumor is selectively destroyed.



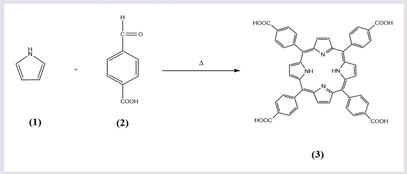
Porphyrins

- A porphyrins' usefulness is largely due to its conjugated structure, light absorbing quality, and efficacy as photosensitizer.
- Various uses of porphyrins include gene regulation, drug and iron metabolism, hormone synthesis, electron transfer medium (conducting polymers), oxygen transport medium (hemoglobin), solar cell (convert light or chemical energy), metal binder (ligand).
- This research specifically utilizes and tests the light sensitivity of the porphyrins and how they react to cells.

Synthesis

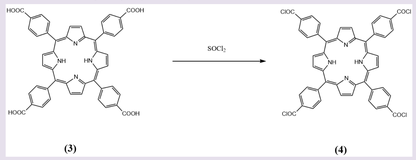
Reaction 1

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



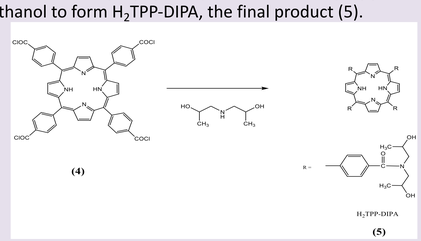
Reaction 2

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



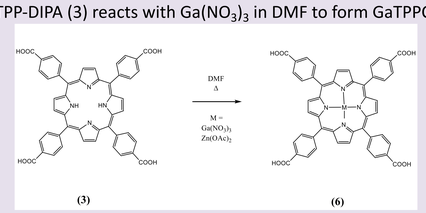
Reaction 3

- The acid chloride porphyrin (4) reacts with diisopropylamine in methanol to form H_2TPP-DIPA, the final product (5).



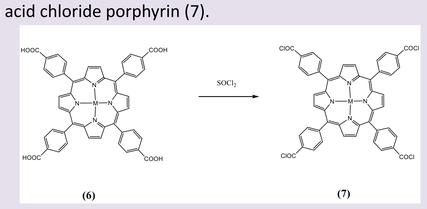
Reaction 4

- H_2TPP-DIPA (3) reacts with $Ga(NO_3)_3$ in DMF to form GaTPPC (6).



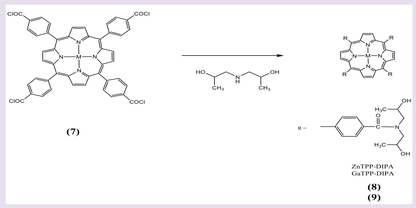
Reaction 5

- GaTPPC (6) reacts with thionyl chloride in dimethylformamide, forming an acid chloride porphyrin (7).



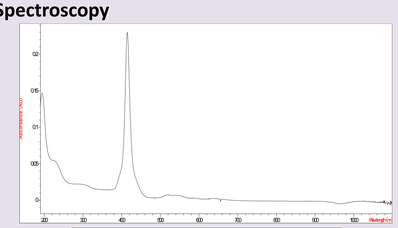
Reaction 6

- The acid chloride porphyrin (7) reacts with diisopropylamine in methanol to form GaTPP-DIPA, the final product (8).



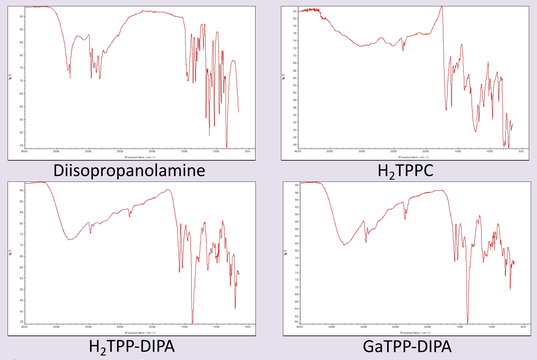
Characterization

UV-vis Spectroscopy

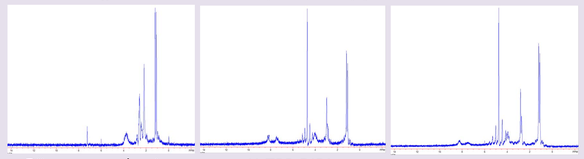


Peaks (nm)	Molar Absorptivity Coefficient, e ($cm^{-1}mM^{-1}$)
415	321
518	11.9
555	6.80
581	5.20
635	3.60

Infrared Spectroscopy

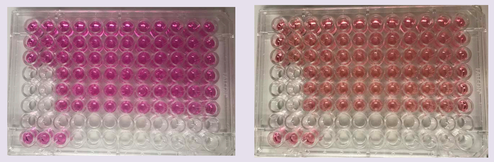


Nuclear Magnetic Resonance Spectroscopy



In-Vitro Cytotoxicity

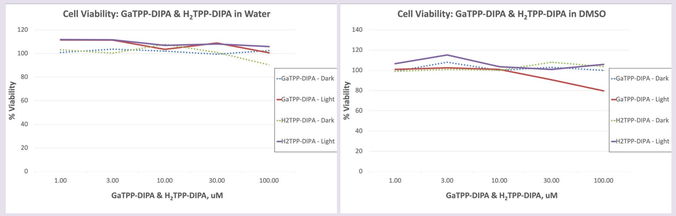
- Cells were exposed to white light (0.5 J/cm^2) for approximately 20 minutes after 24 hours of porphyrin treatment. A parallel plate was kept in the dark.
- The cells were treated with Alamar Blue.



In-vitro Cytotoxicity of GaTPP-DIPA and H2TPP-DIPA

- GaTPP-DIPA is in wells A3-A7, B3-B7, C3-C7 diluted in DMSO
- H_2TPP-DIPA is in wells A8-A12,B3-B7, C3-C7 diluted in DMSO
- GaTPP-DIPA is in wells D3-D7, E3-E7, F3-F7 diluted in H_2O
- H_2TPP-DIPA is in wells D8-D12, E8-E12, F3-F7 diluted in H_2O
- The plate on the left was kept in the dark
- The plate on the right was exposed to light and shows moderate cell death at concentration 100uM of porphyrin

Results



- The *In-Vitro* Cytotoxicity indicates cell viability is greater in cells that were kept in the dark compared to those that were exposed to light.
- The toxicity in the light and in the dark for each porphyrin derivative is concentration dependent.
- This experiment shows that at concentrations around 100 uM, the cells exposed to light showed significantly lower cell viability than those kept in the dark.

Conclusions

- A novel water-soluble porphyrin was successfully synthesized.
- The compound was characterized by UV-vis, IR, and NMR spectroscopies.
- The spectrums indicate that the correct compound has indeed been formed.
- *In-Vitro* Cytotoxicity revealed cell death at a porphyrin concentration range of 30uM to 100uM when exposed to light. *In-Vitro* Cytotoxicity also showed normal cell growth under dark conditions, making the novel porphyrin a viable PDT agent.
- The metalloporphyrins caused more cell death than the unmetalloporphyrins.
- The results indicate that GaTPP-DIPA is effective against U87 Glioblastoma cancer cells in relatively small concentrations.

Future Direction

- Examine the cytotoxicity of GaTPP-DIPA among normal cells and other cancer cell lines.
- Synthesize additional novel water-soluble metallic porphyrin derivatives, such as ZnTPP-DIPA, to see if other derivatives are more desirable in killing U87 cancer cells and are, therefore, candidates for PDT.
- Run HPLC of H_2TPP-DIPA and GaTPP-DIPA

Acknowledgements

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