Synthesis of a Novel Water-Soluble Porphyrin Derivative for use as a Potential Phototherapeutic Cancer Treatment

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Abstract

Photodynamic therapy (PDT) agents are light-activated materials used for localized disease treatment. Porphyrins are classic representations of photosensitive agents. Identified by their large, planar molecular structure, porphyrins are centrally composed of four pyrrole rings. Through this project, the starting material, H₂TPPC, a tetra-carboxylic acid porphyrin, was first synthesized and later modified through the addition of the amine, morpholin-2-ylmethanol. This multi-step synthesis resulted in the highly water-soluble porphyrin product H₂TPP-MorphMeOH. Following this synthesis, the compound was purified using liquid chromatography, and then structurally identified and classified using infrared (IR), nuclear magnetic resonance (NMR), and UV-Vis spectroscopies. Lastly, to determine its utility as a potential PDT agent, the cytotoxicity of H₂TPP-MorphMeOH was determined using an MTT assay on MDA-MB-231 triple-negative breast cancer cells comparing exposure and absence of white light.

Background

- Triple-negative breast cancer (TNBC) accounts for 10 to 15% of breast cancer cases. These patients have an increased likelihood of recurrence, as well as a higher risk of death within five years of diagnosis. Standard cancer treatments, such as chemotherapy, have only limited effectiveness against TNBC.
- Photodynamic therapy (PDT) was devised to circumvent many of the side effects of traditional chemotherapy, as well as for treating cancers that do not respond well to other methods. The PDT agents are administered systemically and are non-toxic until exposed to light, which causes production of reactive oxygen species.
- Several distinct photosensitizers are used in PDT, including chlorophylls, dyes, and porphyrins.
- Porphyrins are naturally occurring heterocyclic macrocycles. The best known porphyrin is Heme.
- Several porphyrin derivatives have already been approved by the FDA as PDT agents for treatment of several types of cancer.

Porphyrins

- The multi-ring structure and light absorbing qualities of porphyrins give them unique properties.
- Some natural and artificial uses of porphyrins include the following:
 - Metal binders (ligands)
 - Solar cells (convert light or chemical energy)
 - Oxygen transportation medium (hemoglobin)
 - Electron transfer medium (conducting polymers)
 - Gene regulation
 - Hormone synthesis
- This research utilizes the light absorbing property of porphyrins to allow the molecule to be “activated” by light, resulting in a desired reaction with the targeted cells.

Porphyrin Synthesis

The porphyrin used in this research project was synthesized through three reactions.

First, 4-formylbenzoic acid (2) was reacted with pyrrole (1) in propionic acid, forming H₂TPPC (3).

Next, H₂TPPC (3) was reacted with thionyl chloride, SOCl₂, in dimethylformamide to form an acid chloride porphyrin (4).

Finally, the acid chloride (4) was reacted with morphyl-2-ylmethanol in methanol to form the final product, H₂TPP-MorphMeOH (5).

Characterization

UV-Vis Spectroscopy

H₂TPP-MorphMeOH

Peaks (nm)	Molar Absorptivity Coefficient, ε (cm ⁻¹ mM ⁻¹)
224	17.4
302	7.45
413	120
518	5.35
555	2.93
581	2.45
636	1.95

Infrared Spectroscopy

morpholin-2-ylmethanol

H₂TPP-MorphMeOH

Nuclear Magnetic Resonance Spectroscopy

morpholin-2-ylmethanol

H₂TPP-MorphMeOH

Characterization Cont.

HPLC

H₂TPP-MorphMeOH

- The HPLC analysis performed on a sample revealed that the compound was 98% pure. The eluent used in this analysis was acetonitrile.

MTT Assay

Cells - MDA-MB-231 triple-negative breast cancer cells were cultured in the appropriate. H₂TPP-MorphMeOH porphyrin was added in multiple concentrations approximately 72 hrs. after plating. Each treatment was repeated on 8 replicate wells.

Exposure to light - Cells were exposed to white light (0.5 J/cm²) for approximately 17 minutes after 18-24 hours of porphyrin treatment. A parallel plate was kept in controlled darkness.

Phototoxicity - three days after light treatment, the cytotoxicity of the modified porphyrin was examined using an MTT assay. Active mitochondria reduce MTT to an insoluble purple formazan, which was quantitated spectrophotometrically.

Results

- The MTT assay indicates cell viability is greater in cells that were kept in the dark compared to those that were exposed to light, and that the toxicity in the light and in the dark for the porphyrin derivative is concentration dependent.
- This experiment shows that only at concentrations nearing 100 uM does cell viability begin to noticeably decrease, whereas lower concentrations show negligible increase in cytotoxicity.

Results Cont.

- A second MTT assay was performed with a series of higher concentrations, ranging from 50 uM to 250 uM. These concentrations were selected using the cytotoxicity trend present in the initial assay.
- Although some error is present in the plotting of the graph, the trend of increasing toxicity versus concentration is still present in the second assay. This experiment shows that once concentrations increase beyond 200 uM, the derivative does have a dramatic effect on cell viability.

Conclusions

- The research was successful in synthesizing a novel, water soluble porphyrin with confirmed structure and high purity.
- However, the porphyrin did not appear to consistently kill MDA-MB-231 cancer cells when exposed to light except when applied in high concentrations. Consequently, relatively normal growth was seen under dark conditions.
- The results indicate that H₂TPP-MorphMeOH is not very effective against the MDA-MB-231 triple negative breast cancer cells in relatively small concentrations; however, more trials, using both the same and larger concentrations, must be completed to acquire more conclusive data.

Future Work

- Since the novel porphyrin doesn't currently appear to have the potential to be a PDT agent in low concentrations, more cell testing and MTT assays will be conducted in an attempt to further investigate the application of this porphyrin, in increasing concentrations.
- In addition, several different water-soluble porphyrins will be tested to see if other derivatives are more desirable in killing MDA-MB-231 cancer cells and becoming candidates for PDT.

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