BIOORTHOGONALLY ACTIVATABLE CHLORIN E6 PHOTOSENSITIZER FOR TARGETED PHOTODYNAMIC THERAPY: PHOTOPHYSICAL AND BIOLOGICAL PROPERTIES IN VITRO

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INTRODUCTION

Photodynamic therapy uses photosensitizers (PS), light, and intracellular oxygen to produce cytotoxic reactive oxygen species. It's effectiveness is constrained by poor tumor selectivity and the limitations of biomarker-targeted strategies. Bioorthogonal chemistry addresses these challenges by enabling a two-step click reaction strategy that enhances tumor-specific targeting and spatiotemporal control of photosensitizer activation.

Breast cancer, a leading global malignancy with current treatments limited by systemic side effects, may be targeted via photodynamic therapy using compounds that exploits the overexpression of the sodium-dependent multivitamin transporter (SMVT), the biotin uptake receptor upregulated in breast cancer cells compared to normal epithelial cells.

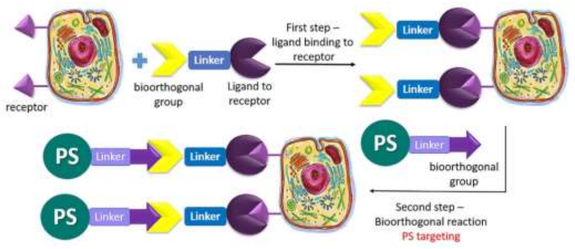

Fig. 1. Bioorthogonal delivery of a photosensitizer to a tumor cell

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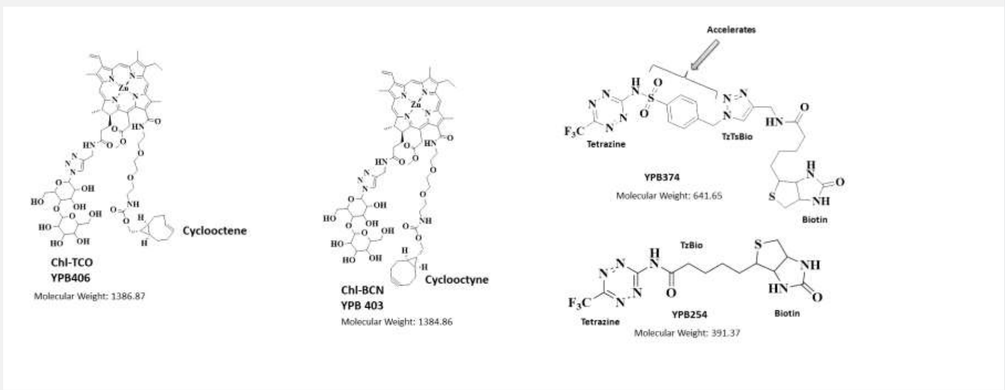
PROJECT AIM

To employ bioorthogonal chemistry to enhance the selectivity and tumor-cell accumulation of different photosensitizers and bioorthogonal pairs. Evaluating and comparing their photophysical properties and accumulation in tumor cells to identify the better bioorthogonal pair.

MATERIALS AND METHODS



Test compounds, Chl-TCO and Chl-BCN, are derivatives of chlorin e6 with a central zinc atom and carbohydrate fragment. Cyclooctene/cyclooctyne is also introduced into the chlorin structure to ensure a bioorthogonal reaction. YPB374 and YPB254 are targeting molecules that are highly specific to the biotin receptor. On the other hand, they ensure the binding of Chl-TCO and Chl-BCN through inverse electron demand Diels-Alder reaction (IEDDA)

- The photophysical properties (absorption and flourescence, quantum yield of ROS, photostability) of the compounds were recorded using a Synergy Mx plate spectrophotometer-spectrofluorimeter.
- *In vitro* studies were performed on cultures of SK-BR-3 cell line. SK-BR-3 is a human breast cancer with high expression of SLC5A6 encoding the target receptor, SMVT.
- Assessment of photosensitizer accumulation in Sk-BR-3 cells using confocal microscopy

RESULT

Determination of the spectral properties

The test compounds exhibited characteristic absorption of chlorin derivatives, and upon excitation at 410 nm, Chl-BCN showed a fluorescence quantum yield more than twice as high, indicating it is a more effective photosensitizer.

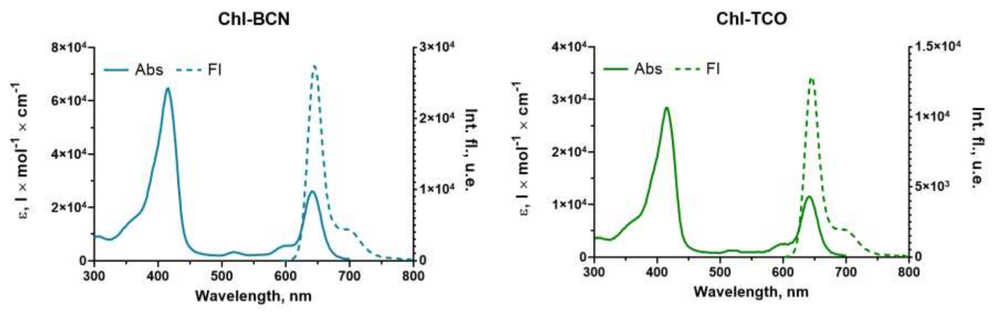


Fig.2. Absorption and fluorescence (λex = 410 nm) spectra of Chl-TCO and Chl-BCN in water (at 5 μM)

Table 1. Absorbance and flourescence of PS

Photosensitizers	Abs, nm (log Ɛ)		Fl, nm	fluorescence quantum yield, %
	Soret	Q-band		
Chl-TCO	414 (4,45)	640 (4,06)	646	1,47 ± 0,28
Chl-BCN	416 (4,8)	640 (4,4)	644	3,2 ± 0,97

Determination of the photostability

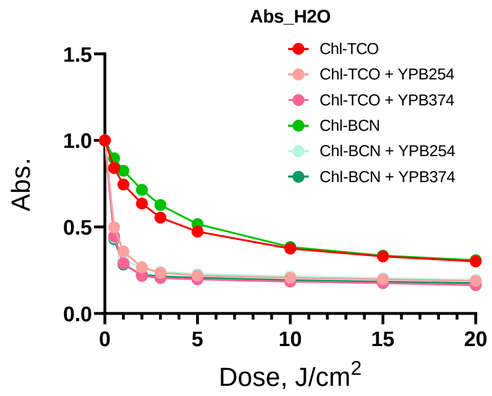


Fig. 4. Result of photostability study of the PS at increasing radiation dose

Chl-TCO and Chl-BCN exhibited low photostability, with absorptivity decreasing by approximately 50% upon irradiation at 5 J/cm²; however, Chl-BCN showed better photostability than Chl-TCO. Furthermore, association with YPB374 and YPB254 (bioorthogonal pair) resulted in an approximately ten-fold decrease in photostability.

Assessment of reactive oxygen species (ROS) generation quantum yield (ΔΦ)

Chl-BCN generated nearly twice the ROS compared to Chl-TCO, indicating its greater potential to induce oxidative damage in tumor cells.

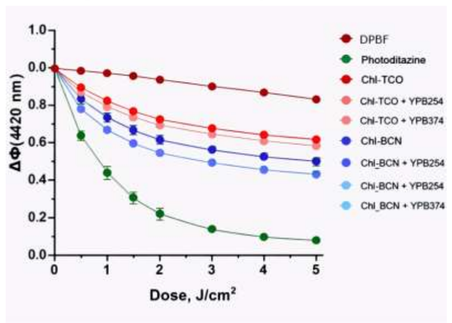
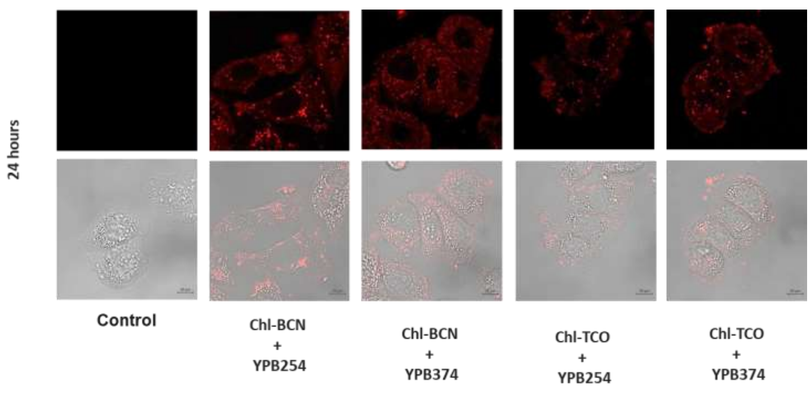


Fig. 5. ROS quantum yield of PS in DMSO

Assessment of Photosensitizer Accumulation in Tumor Cells



Chl-BCN demonstrated better tumor cells accumulation than Chl-TCO. The vector moieties enhanced tumor cells accumulation of both photosensitizers.

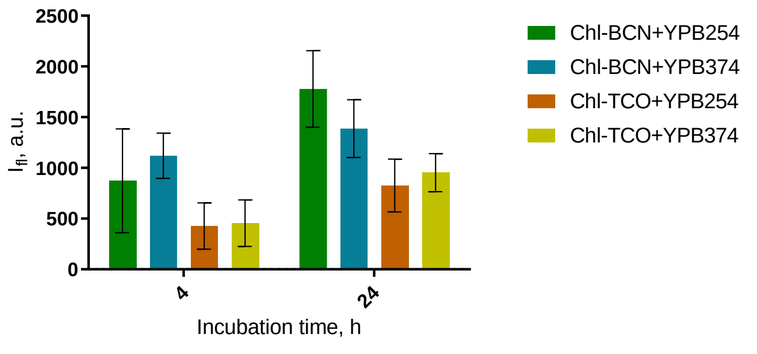


Fig. 3. Tumor cell accumulation of bioorthogonal pair with final concentration of 5 μM at 24 h incubation

CONCLUSION

The study leveraged bioorthogonal chemistry to enhance tumor-specific targeting of photosensitizers. Both Chl-BCN and Chl-TCO exhibited favorable spectral properties, strong ROS generation, and low photostability, which further decreased upon conjugation with the targeting molecules YPB254 and YPB374. Overall, Chl-BCN demonstrated superior performance, showing higher ROS production, better photostability, and greater tumor cell accumulation after 24 hours incubation.

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