Design and Evaluation of PpIX–Capsaicin Conjugates for Antimicrobial Photodynamic Applications

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ABSTRACT

Antimicrobial Photodynamic Therapy (aPDT) is a promising strategy for combating multidrug-resistant bacteria, bypassing the limitations of conventional antimicrobials due to the lack of acquired resistance. In this study, two derivatives of Protoporphyrin IX (PpIX), functionalized with one or two fragments of capsaicin, were synthesized and evaluated. The derivatives showed an increase in singlet oxygen quantum yield and greater photodegradation, with a significant increase in lipophilicity in the compound with two fragments. Biological tests revealed high efficacy against *Staphylococcus aureus* (reduction 1.75 times greater than PpIX), while *Escherichia coli* showed lower sensitivity, possibly due to the lipopolysaccharide outer membrane. The results highlight the PpIX-capsaicin 1 derivative as a promising candidate for aPDT applications.

Keywords: Antimicrobial Photodynamic Therapy, antibiotic resistance, porphyrin.

Introduction

Antimicrobial resistance is a critical global health challenge, with increasing rates of treatment failure due to the spread of multidrug-resistant pathogens (1). Conventional antibiotics are losing effectiveness, and resistance to last-line drugs continues to rise (1). In this context, Antimicrobial Photodynamic Therapy (aPDT) emerges as a promising alternative. aPDT combines a photosensitizer (PS), visible light, and molecular oxygen to generate reactive oxygen species (ROS), leading to oxidative damage and microbial death (2). Unlike antibiotics, aPDT acts on multiple cellular targets, reducing the likelihood of resistance development (3).

Porphyrins are among the most studied PSs due to their strong light absorption and high ROS yield (4). However, their efficacy against Gram-negative bacteria remains limited by structural barriers such as the outer membrane (5, 6). In this study, two novel derivatives of Protoporphyrin IX (PpIX) were synthesized by conjugating one or two capsaicin fragments. These modifications aimed to improve photophysical behavior and biological activity.

Experimental

Synthesis and characterization

PpIX-capsaicin 1 and 2 were synthesized via Steglich esterification using 1 or 2 equivalents of capsaicin, respectively, with DMAP and EDC in DMF. Both derivatives were characterized by NMR and HRMS.

Photophysical parameters

PSs were dissolved in ethanol (Abs < 0.3). Fluorescence and singlet oxygen phosphorescence (λ ≈ 1270 nm) were recorded to calculate quantum yields, using PpIX as the standard.

Aggregation and LogP

PSs solutions were prepared in DMSO/water (1–6% DMSO) and analyzed by UV-Vis absorption to assess self-aggregation. LogP was determined via shake-flask method.

Photobleaching

PSs solutions were irradiated using green LED (0–5 J/cm²) or white LED (0–70 J/cm²). Absorbance spectra were recorded to monitor Soret band decrease as a measure of photodegradation.

Photodynamic Inactivation (PDI)

PSs were added (78–1.2 μM) to bacterial suspensions (10^8 CFU/mL), and incubated. Samples were kept in the dark or irradiated with white (30 J/cm²) or green LED (2.5 J/cm²). After irradiation, cell suspensions were diluted (10^{-1}–10^{-7}), plated on agar for CFU counting.

Results and Discussion

Synthesis and characterization

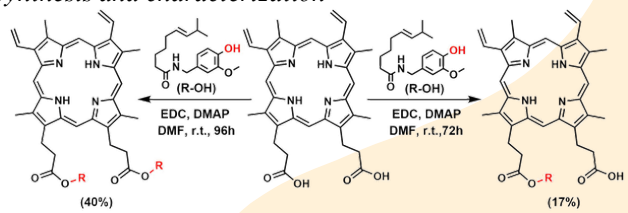

Figure 1. Synthesis of PpIX-capsaicin 1 and 2.

The new compounds showed enhanced singlet oxygen quantum yields (Table 1), increased photodegradation, and, in the case of the bis-substituted derivative, higher lipophilicity (Table 1). Antimicrobial assays demonstrated greater efficacy for PpIX-capsaicin 1 against *S. aureus* (Figure 4), while *E. coli* showed lower susceptibility (Figure 5). In contrast, PpIX-capsaicin 2 showed no photoactivity against the tested bacterial strains. These results highlight PpIX-capsaicin 1 as a promising candidate for aPDT applications.

Photophysical parameters and LogP

Table 1. Photophysical and lipophilicity parameters determined for PpIX, PpIX-capsaicin 1, and PpIX-capsaicin 2.

PSs	Φ_f [a]	Φ_Δ [b]	LogP
PpIX	0.070	0.92	0.63 ± 0.07
1	0.076	0.99	0.75 ± 0.01
2	0.081	1.01	1.24 ± 0.04

[a] Measured in ethanol (λ_{exc} = 402 nm).
[b] Measured in ethanol (λ_{exc} = 400 nm).

Aggregation

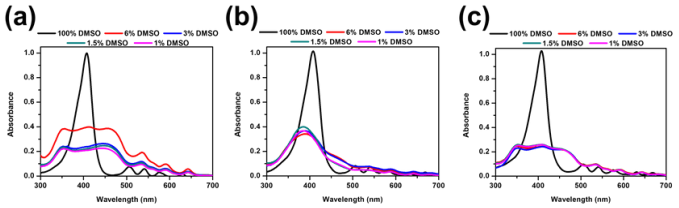

Figure 2. UV–visible absorption spectra of (a) PpIX (b) PpIX-capsaicin 1, and (c) PpIX-capsaicin 2, varying proportions of water and DMSO.

Photobleaching

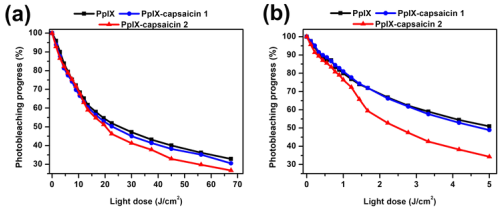

Figure 3. Photodegradation progress of each PS, PpIX (black line), PpIX-capsaicin 1 (blue line) and PpIX-capsaicin 2 (red line) under different irradiation setups: (a) White LED (0–70 J/cm^2) and (b) Green LED (0–5 J/cm^2).

Photodynamic Inactivation (PDI)

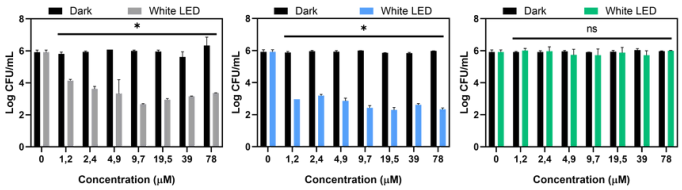

Figure 4. PDI of *S. aureus* (ATCC 29213) using PpIX, PpIX-capsaicin 1, and PpIX-capsaicin 2 (left to right).

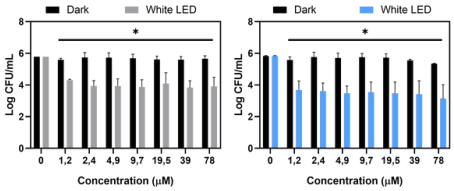

Figure 5. PDI of *E. coli* (ATCC 25922) using PpIX and PpIX-capsaicin 1 (left to right).

Conclusions

Two derivatives of PpIX structurally modified with capsaicin fragments were synthesized and biologically evaluated. Photophysical studies revealed improved results, along with a higher photodegradation rate. Self-aggregation and lipophilicity analysis revealed a significant increase only in the derivative with two capsaicin fragments. PDI assays demonstrated higher efficacy against *S. aureus* resulting in a 2.9-log reduction. *E. coli* exhibited lower susceptibility, due to structural challenges associated with these strains.

Acknowledgments

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