Photodynamic inactivation of bioluminescent *Escherichia coli* by cationic pyrrolidine-fused chlorins and isobacteriochlorins

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Introduction

Photodynamic inactivation (PDI) represents a potential alternative methodology to inactivate microbial cells. This approach is based on the photodynamic therapy concept that comprises the action of three components: a photosensitizer (PS), a light source and oxygen (1). Porphyrins, chlorins and isobacteriochlorins can be used as PSs. In this communication we will report the results obtained in the PDI of bioluminescent *E. coli*, Gram (-) bacteria, in the presence of cationic pyrrolidine fused chlorins and isobacteriochlorins obtained from 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin by 1,3-dipolar cycloadditions and after their immobilization on solid supports.

Results e Discussion

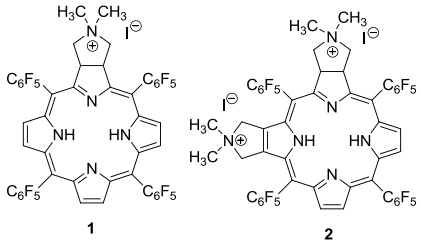

Figure 1. Structures of cationic chlorin **1** and isobacteriochlorin **2** derivatives used in this study.

The fluorescence and singlet oxygen quantum yields of **1** and **2** obtained in dimethylformamide (DMF) are listed in Table 1.

Table 1. Photophysical properties of **1** and **2** in DMF

Compound	λ_{max} /nm	Fluorescence quantum yield(Φ_{fl}) ±0.05	$^1O_2$ quantum yield (Φ_Δ) ±0.05
1	402 (5.23), 500 (4.19), 525 (3.64), 594 (3.69), 647 (4.64)	0.13	0.71
2	408 (5.08), 503 (3.88), 546 (3.97), 599 (4.19), 650 (3.65)	0.08	0.62

The photodynamic inactivation potential of compounds **1** and **2** and also of **1** on a solid support was investigated using the recombinant bioluminescent *E. coli.*

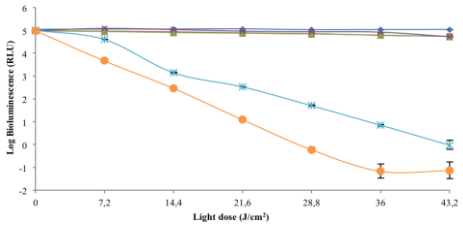

Figure 2. Bioluminescence monitoring of *E. coli* treated with derivatives **1** and **2** after being exposed to different light doses of white light (380–700 nm) at an irradiance of 4.0 mW cm^{-2}; ◆Light control; ■ chlorin **1** (20 μM) dark control; ▲chlorin **1** (20 μM); × isobacteriochlorin **2** (20 μM) dark control; ✳ isobacteriochlorin **2** (5.0 μM); ● isobacteriochlorin **2** (20 μM); Error bars indicate the standard deviation. Lines just combine the points. Small bars are overlapped by the symbols.

In this study the cationic isobacteriochlorin 2 was an effective PS against the bioluminescent *E. coli*, reaching the limit of detection (~6.1 log reduction) after a light dose of 36 J cm-2 for the highest concentration tested (20 μM). Photodynamic inactivation of *E. coli* was reached when derivative **1** was immobilized on a solid support.

Conclusions

The results obtained in this work confirm the high potential of these compounds to be used for potodynamic inactivation of Gram (-) bacteria.

Acknowledgments

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[1] Mesquita, M. Q.; Menezes, J. C. J. M. D. S.; Neves, M. G. P. M. S.; Tomé, A. C.; Cavaleiro, J. A. S.; Cunha, Â.; Almeida, A.; Hackbarth, S.; Röder, B. and Faustino, M. A. F. *Bioorg. Med. Chem. Lett., in press.*
[2] Silva, A. M. G.; Tomé, A. C.; Neves, M. G. P. M. S.; Silva, A. M. S.; Cavaleiro J. A. S. *Chem. Commun.* **1999**, *17*, 1767.