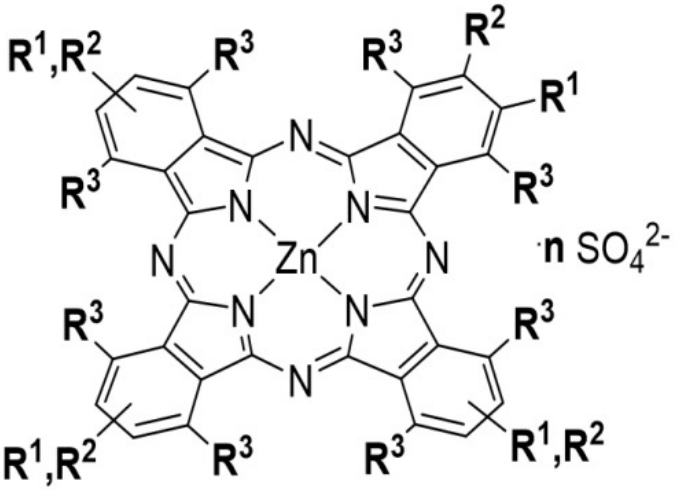
Killing microbes with red light

Photosensitization is a process in which a chemical compound (photosensitizer) that absorbs energy from light is able to transfer that energy to oxygen molecules. As a consequence, reactive oxygen species (ROS), highly toxic to living cells, are produced. This strategy - photodynamic inactivation (PDI) - is currently envisaged as a promising alternative to antibiotics because several bacterial structures are simultaneously attacked, limiting the capacity of cells to recover from damage or to develop mechanism of resistance.

Phthalocyanines (Pc) represent a family of light-absorbing blue-green compounds that have been tested for PDI of microorganisms. These molecules are particularly interesting because they efficiently transfer the energy to oxygen, producing large amounts of ROS, in particular singlet oxygen, which is extremely lethal to microbial cells. Another interesting aspect is that PC efficiently absorb light in the red region of the visible spectrum, which allows a better penetration in living tissues when envisaging clinical applications. As a major drawback, Pc may not easily dissolve in water and, in biological conditions, it is difficult to obtain their optimal therapeutic action/biological activity. The introduction of adequate chemical groups on the periphery of the Pc has been attempted to make these molecules more water soluble and improve the affinity to the outer structures of the bacterial cell, making them suitable to target a wider range of bacteria.



Pc	R^1	R^2	R^3	n
1a	F	$\oplus$ $N(CH_3)_3$	H	2
2a	$\oplus$ $N(CH_3)_3$	$\oplus$ $N(CH_3)_3$	F	4
3a	Cl	$\oplus$ $N(CH_3)_3$	H	2

Fig. 1. Chemical structures of Zn-Pc 1a, 2a and 3a.

With these aims, a new family of zinc(II)-containing phthalocyanines (Zn-Pc), differing in the nature, number and position of the chemical groups attached to the core-molecule (Fig. 1), was synthesized and characterized in terms of photochemical (capacity to produce ROS such as singlet oxygen, photostability when exposed to light), photophysical (behaviour towards light) and

photobiological performance (affinity towards bacterial cells and efficiency of its destruction). A genetically transformed bioluminescent strain of the Gram negative bacterium *Escherichia coli* was used as a model organism for the real time monitoring of the photodynamic inactivation.

The new Zn-Pc synthesized were generally water-soluble and photostable. However, they revealed very different capacities for singlet oxygen production which was the highest with Pc **3a** and almost undetectable with Pc **1a**. The molecules were also very different in terms of affinity towards *E. coli* cells. In fact, Zn-Pc **2a** was more efficiently bound to bacterial cells than any of the other two molecules.

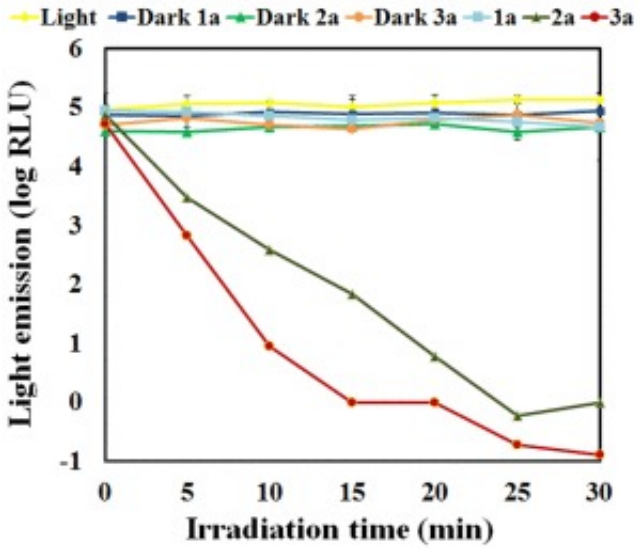


Fig. 2. Photodynamic inactivation (reduction of light emission) of bioluminescent Escherichia coli with Zn-Pc 1a, 2a and 3a upon irradiation with red light.

When *E. coli* cells suspended in an aqueous medium were irradiated 30 min with white light (400–800 nm), in the presence of these three compounds, only Zn-Pc **3a** caused a significant reduction of bioluminescence (aprox. 2 logarithmic units). However, under red light (620–750 nm), a much more efficient inactivation (aprox. 5 logarithmic units) was attained with Zn-Pc **2a** and **3a** (Fig. 2). None of the Zn-Pcs caused any inactivation in the absence of light. Considering the overall results, according to the PDI efficiency, the new Zn-Pc were ordered as **1a**?**2a** 3a.

The introduced modifications in the Pc core had a significant impact in terms of singlet oxygen generation capacity and affinity towards bacterial cells, which are major determinants of the inactivation efficiency. Zn-Pcs **2a** and **3a** showed the most promising results and further modifications of this Pc are under consideration to fine-tune their properties and optimize their potential as PDI agents.

Publication

Photodynamic inactivation of Escherichia coli with cationic ammonium Zn(ii) phthalocyanines.
Rocha DM1, Venkatramaiah N, Gomes MC, Almeida A, Faustino MA, Almeida Paz FA, Cunha Â, Tomé JP
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