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## Influence of charge and metal coordination of *meso*-substituted porphyrins on bacterial photoinactivation



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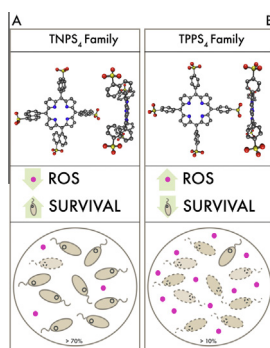
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### HIGHLIGHTS

- Were synthesized and characterized 8 *meso* substituted porphyrins with different substituents.
- Effect of charges and metal coordination in their photochemical characteristics was studied.
- We performed a detailed study of photoinduced antibacterial structure–activity.
- It was obtained a relation among the structural integrity, ROS production and antibacterial activity.

### GRAPHICAL ABSTRACT

**A:** The series of TNPS<sub>4</sub> have higher structural deformity, low production of ROS and reduced antibacterial activity. **B:** The series of TPPS<sub>4</sub> have a low structural deformity, high production of ROS and the greater antibacterial activity.



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### ABSTRACT

The photodynamic effect of *meso*-substituted porphyrins with different charges and metal ions: *meso*-tetraphenylporphyrin tetrasulfonate **1**, its nickel **2** and zinc complexes **3**; *meso*-tetranaphthylporphyrin tetrasulfonate **4**, and its zinc complex Zn **5**; and tetra pyridyl ethylacetate porphyrins **6** and their nickel **7** and zinc **8** complexes, were synthesized and studied their antimicrobial activity against *Escherichia coli*. Fluorescence quantum yields ( $\Phi_F$ ) were measured in water using reference TPPS<sub>4</sub>, obtaining higher values for complexes **3** and **4**. The singlet oxygen  $\Phi_A$  were measured using histidine as trapping singlet oxygen and Rose Bengal as a reference standard. Complexes **1**, **2** and **6** have the highest quantum yields of singlet oxygen formation, showing no relation with the peripheral charges and efficiency as Type II photosensitizers. Meanwhile complexes **3**, **8** and **4** were the most efficient in producing radical species, determined with their reaction with NADH. The photoinduced antibacterial activity of complex was investigated at different concentrations of the photosensitizers with an irradiation time of 30 min. The higher antibacterial activities were obtained for the complexes **1–3** that are those with greater production of ROS and minor structural deformations. Complexes **7** and **8** had moderate activity, while **4–6** a low activity. Thus, in this work demonstrates that the production of ROS and structural deformations due to peripheral substituents and metal coordination, influence the activity of the complexes studied. Therefore, is important to perform comprehensive study physics and structurally when predicting or explain such activity.

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## Introduction

Due to the wide variety of pathogens and how fast their evolutionary changes to become resistant, the field of antimicrobial chemotherapy is an area of study in constant development. Photodynamic therapy (PDT) has been successfully applied in treating cancer as well as in non-cancerous condition [1,2]. Furthermore, there has also been an increasing interest in the application of PDT in the treatment of infectious diseases [3–5]. PDT involves the use of non-toxic dyes, which act as photoactive drugs called photosensitizers (PS) with the visible light of the proper wavelength to excite the PS. The light raises the PS to an excited state, which then interacts with oxygen, leading to the formation of reactive oxygen species (ROS) such as singlet oxygen, resulting in cytotoxicity and direct cell kill [6].

New developments in PDT applications are several approaches, which innovate in the design of PS is very wide. The chemical structure of a photosensitizer, charge and hydrophobicity influences their efficiency of ROS productions and determines how it interacts with itself and with its environment. Theoretically, photosensitizers can be properly designed a function of its chemical structure, but more realistically their effectiveness is determined as a function of the targets to be confronted [7].

The design of porphyrins as PS with microbial activity PDT has been extensively studied mainly focused on develop cationic complexes, due to possibility of interaction with the cell membrane [8,9]. The development of neutral or anionic porphyrins, regardless of their efficiency to produce reactive oxygen species, does not receive great attention because of its low interaction on the membrane [10,11]. Many of those studies report that the latter porphyrins have negligible activity against Gram-negative bacteria such as *Escherichia coli* [12]. It is important to note that the experimental method, bacterial washes are performed system before irradiation, thereby eliminating the PS not attached to the bacteria [13,14]. Taking into account that in large-scale applications, such as in wastewater treatment, these washings not performed, the efficiency of ROS production in the entire system plays an important role in bacterial photoinactivation. Thus, evaluating new photosensitizers on bacterial cultures without pre-irradiation washes, would allow obtaining information about the system under real operating conditions. In this sense, in this work the synthesis and photochemical characterization 8 hydrophilic porphyrins were performed. The aim of this paper is to examine their efficiency as PS in cultures of *E. coli* (no previous washes), and the impact of structural changes in this efficiency.

In this paper the synthesis of the following water-soluble porphyrins is presented: *meso*-tetraphenylporphyrin (TPP) tetrasulfonate ( $S_4$ ): (TPPS<sub>4</sub>, **1**), its nickel (TPPNiS<sub>4</sub>, **2**) and zinc complexes (TPPZnS<sub>4</sub>, **3**) as well as the *meso*-tetranaphthylporphyrin tetrasulfonate (TNPS<sub>4</sub>, **4**) its Zn complexes (TNPZnS<sub>4</sub>, **5**) and tetra piridyl ethylacetate porphyrins (TPyEtAcP, **6**) and their Ni and Zn complexes (TPyEtAcPNi, **7**; TPyEtAcPZn, **8**). Studied photophysical properties of absorption, fluorescence emission and the photochemical characteristic of singlet oxygen generation and oxygen free radicals by synthesized porphyrins are compared in dependence on the chemical structure. Finally, a comparison of the photochemical properties of the synthesized compounds and its efficiency in the photoinduced antibacterial activity is performed.

## Materials and methods

### Chemicals

All analytical or HPLC grade solvents were obtained from Merck (Darmstadt, Germany) and used without further purification.

Benzaldehyde,  $\alpha$ -naftaldehyde, chloro ethyl acetate, pyrrole, propionic acid, tetra-pyridyl porphyrin, chloroform, histidine, Rose Bengal, horseradish peroxidase (HRP), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), and *p*-nitrosodimethyl aniline were purchased from Sigma–Aldrich (St. Louis, MO, USA). 3-Aminophthalhydrazide (Luminol) and hydrogen peroxide, 30 wt.% solution in water, were purchased from Aldrich (Milwaukee, USA). Phosphate buffered saline solution (PBS) pH 7.4 (0.01 mol l<sup>-1</sup> phosphate buffer and 0.135 mol l<sup>-1</sup> NaCl) was prepared daily before each experiment.

<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded with a Bruker Avance 500 and 300 MHz respectively, in chloroform-*d* with tetramethylsilane (Me<sub>4</sub>Si) as an internal standard. Chemical shifts ( $\delta$ ) are given in parts per million. Infrared spectroscopy (IR) spectra were performed using a Nicolet Magna 560 FT-IR spectrometer. ElectroSpray Ionization mass spectra and MS/MS spectra were obtained with a Thermo-Finnigan TSQ Quantum Ultra AM spectrometer coupled to a HPLC ElectroSpray (ESI). Elemental analyses were performed in a Fisons Instrument EA-1108. The samples were prepared by addition of the compound of interest to water. The absorption spectra were recorded on a Perkin Elmer Lambda-35 UV-Vis spectrophotometer. The fluorescence spectra and the quantum yields were registered in a Perkin Elmer LS-45.

### Synthesis of the porphyrins derivatives

#### Tetraphenyl porphyrins (TPP)

The synthesis of TPP (Scheme 1) was carried out following the experimental procedure reported by Alder and coworkers [15] with some modifications: benzaldehyde (0.67 mol) and pyrrole (0.65 mol) were added simultaneously to refluxing propionic acid (200 ml) and the mixture was refluxed for 1 h before being allowed to cool and stand at room temperature overnight. The product was filtered off and washed with water and methanol to give purple crystals. Yield 20% (20.5 g). **F.T-IR** (KBr, thin film)  $\nu_{\max}$  (cm<sup>-1</sup>): 3309 (N–H); 3047 (C=C); 2361 (C=N); 1593, 1466 (aromatic C=C); 698 (C–H). **UV-vis** (1.0 × 10<sup>-5</sup> mol l<sup>-1</sup> in CHCl<sub>3</sub>),  $\lambda_{\max}$  (nm): 419, 515, 550, 590, 674; **<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>)  $\delta$  = ppm: 8.87 (s, 8H, H-pyrrolic), 8.21–8.28 (d, *J* = 5.0 Hz, 8H, *o*-phenyl), 7.72–7.85 (m, 12H, H-phenyl), –2.73 (s, NH). **<sup>13</sup>C NMR** (125 MHz, CHCl<sub>3</sub>)  $\delta$  = ppm: 142.21 (2C-phenyl), 134.6 (CH-*o*-phenyl), 131.15 (CH-pyrrolic), 127.74 (2C-pyrrolic), 126.72 (2CH-phenyl), 120.18 (2C).

#### Tetranaphthylporphyrins (TNP)

The synthesis of TNP (Scheme 2) was carried out following the experimental procedure reported by Regimol and coworkers [16] with some modifications: benzaldehyde (0.10 mol) and pyrrole (0.14 mol) were added simultaneously to refluxing propionic acid (100 ml). The reaction mixture was left at reflux for a ten days, after which it was allowed to cool to room temperature, and then it was kept at 5 °C overnight. The product was filtered off and washed with water and methanol and the solid obtained was purified by preparative chromatography on silica plates, using chloroform as mobile phase, yielding purple crystals. Yield of 19.8% (5.44 g). **F.T-IR** (KBr, thin film)  $\nu_{\max}$  (cm<sup>-1</sup>): 3432 (N–H), 2357 (C=N), 3048 (C=C), 1568, 1499 (aromatic C=C), 1021 (C–H). **UV-vis** (1.0 × 10<sup>-5</sup> mol l<sup>-1</sup> in CHCl<sub>3</sub>),  $\lambda_{\max}$  (nm): 423. **<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>)  $\delta$  = ppm: 8.47 (d, 8H, *J* = 4.5 Hz, H-pyrrolic), 8.28–7.73 (m, 28H, H-naphthalene), –2.76 (s, 2H, NH). **MS** (APCI) *m/z*: 815.22 (M + H)<sup>+</sup>, (cal: M: C<sub>60</sub>H<sub>38</sub>N<sub>4</sub> = 814.97).

#### Tetrapiridyl ethylacetate porphyrins

(TPyEtAcP, **6**): The synthesis of water-soluble porphyrin TPyEtAcP (Scheme 3) was performed according to changes in the methodology reported by Berezin and coworkers [17]: tetrapiridyl

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