



Evaluation of *meso*-substituted cationic corroles as potential antibacterial agents

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ABSTRACT

Cationic derivatives of 5,10,15-tris[4-(pyridin-4-ylsulphonyl)-2,3,5,6-tetrafluorophenyl]-corrolate gallium(III) pyridine and 5,10,15-tris[4-(pyridin-2-ylsulphonyl)-2,3,5,6-tetrafluorophenyl]-corrolate gallium(III) pyridine were synthesized and their photosensitizing properties against the naturally bioluminescent Gram-negative bacterium *Allivibrio fischeri* were evaluated. The cationic corrole derivatives exhibited antibacterial activity at micromolar concentrations against this Gram-negative bacterium strain.

Key words: *Allivibrio fischeri*, corroles, photosensitizer, PDI, bioluminescence.

INTRODUCTION

Antimicrobial photodynamic inactivation (PDI) has emerged as an alternative pathway to antibiotics in combating bacteria invasion (Jori and Brown 2004, Almeida et al. 2011, Yin et al. 2015). The goal is to use photoactive drugs (photosensitizers, PS) to trigger a series of processes leading to cells' death, after being light activated in the presence of oxygen. The basic principles are well understood. In essence,

the interaction between light and the PS, generates reactive oxygen species (ROS) through either electron transfer (type I) or energy transfer (type II) reactions. These ROS will react with many cellular components that will induce oxidative processes leading to cell death (Bonnett 2000, Maisch 2009, Ergaieg et al. 2008, Tavares et al. 2011, Costa et al. 2013, Almeida et al. 2015, Wainwright et al. 2017). The great advantage of this methodology is the unlikelihood of the microorganisms to develop resistance mechanisms since there is not a particular target in the cell and also the PS don't have to accumulate in its interior to be efficient (Dahl et al. 1989, Tavares et al. 2010, Costa et al. 2011a, Preuß et al. 2013, Alves et al. 2014a).

PS with a variety of chemical structures have been used to inactivate microbial cells.

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The results obtained with macrocycles such as porphyrins, (Alves et al. 2015), phthalocyanines (Dei et al. 2006, Pereira et al. 2012, Ryskova et al. 2013, Lourenço et al. 2015, Marciel et al. 2017) or porphycenes (Xavier et al. 2010, Ruiz-González et al. 2015, Masiera et al. 2017) are particularly relevant. The discovery that positively charged PS at physiological pH values promote the photoinactivation of microbial cells, namely of antibiotic resistant Gram-negative bacteria has stimulated the development of new useful cationic PS for PDI. (Alves et al. 2015, Mesquita et al. 2014a, Simões et al. 2016).

Corroles are tetrapyrrolic macrocycles belonging to the porphyrinoid family, which have been calling the attention of researchers in this field, since corroles possess interesting and unique properties which confer them significant applicabilities (Aviv-Harel and Gross 2009, Teo et al. 2017). Nowadays corroles can be functionalized by several approaches (Barata et al. 2014, 2017) allowing great flexibility in the synthesis of molecules with unique physical and chemical properties. Although corroles have been investigated in several areas, their therapeutic potential has only recently been disclosed. (Lim et al. 2012, Hwang et al. 2012, Agadjanian et al. 2009, Cardote et al. 2012, Iglesias et al. 2015, Barata et al. 2013, 2015, 2016, Preuß et al. 2014, Pohl et al. 2014). According to that and following our interest in the development of tetrapyrrolic macrocycles with antibacterial activity, (Alves et al. 2009, 2014b, Carvalho et al. 2009, 2010, Costa et al. 2012, Pereira et al. 2012, Gomes et al. 2013, Mesquita et al. 2014a, b, Simões et al. 2016, Lourenço et al. 2015, Rocha et al. 2015, Batalha et al. 2015, Moura et al. 2016, Castro et al. 2017) we decided to synthesize the cationic corrole derivatives **2b** and **3b** and to study, for the first time, their efficiency in the PDI of Gram-negative bacteria. Herein, the synthesis, structural characterisation, photophysical properties (absorbance and singlet

oxygen generation) and biological activity of the new cationic corroles against the naturally bioluminescent bacterium *Allivibrio fischeri* (*A. fischeri*) will be reported.

MATERIALS AND METHODS

Commercial reagents were used without previous purifications according to their purity (with exception of pyridine, which was previously distilled). DMSO and DMF were dried under standard procedures.

Thin layer chromatography (TLC) was used to monitor the reactions using plastified sheets coated with Silica Gel 60 0.2 mm (Merck). Purifications through preparative chromatography were performed in glass plates (20 x 20cm) previously coated with Silica Gel 60 0.5 mm and activated at 100 °C during 12 h. Column chromatography purifications were performed with glass columns filled with Silica Gel 60 with granulometry 32-63 or 63-200 mesh.

¹H and ¹⁹F NMR spectra were recorded on a Bruker Avance-300 spectrometer at 300.13 and 282.38 MHz, respectively. The chemical shifts are expressed in δ (ppm) and the coupling constants (*J*) in Hertz (Hz). TMS was used as internal reference for ¹H NMR spectra and C₆F₆ was used as reference for the ¹⁹F NMR spectra. CDCl₃ and C₅D₅N were used as solvents for the neutral compounds, while for the cationic compounds NMR spectra were recorded with CD₃OD and C₅D₅N; unequivocally attribution of NMR signals was attained with 2D-NMR experiments COSY and NOESY.

UV-Vis spectra were recorded in DMSO solutions on a UV-2501-PC Shimadzu spectrophotometer using quartz cells with 1 cm of optical length. MALDI-MS spectra were acquired from a MALDI/TOF/TOF 4800 Applied Biosystems MDS Sciex using CH₂Cl₂ or CH₃OH, with and without matrix. ESI mass spectra were acquired with a Micromass Q-ToF 2 (Micromass,

Manchester, UK), operating in the positive ion mode, equipped with a Z-spray source, an electrospray probe and a syringe pump. ESI⁺ HR-MS were recorded on a VG Autospec M in University of Vigo, Spain.

SYNTHESIS OF THE CORROLE MACROCYCLES

Synthesis of 5,10,15-tris[4-(pyridin-4-ylsulphanyl)-2,3,5,6-tetrafluorophenyl]-corrolategallium(III)pyridine (2a): In a sealed reaction vessel 10.0 mg of **1** (10.6 μ mol) were added to 12 mg of 4-mercaptopyridine (10 eq., 0.11 mmol) in the presence of K₂CO₃ in excess (60 mg), using 0.3 mL of dried DMSO as solvent. The reaction was carried out at room temperature under stirring and N₂ atmosphere for 1h30min. The reactional mixture was neutralized with aqueous citric acid solution followed by extraction of the organic phase with CH₂Cl₂, and then dried with anhydrous sodium sulphate. The crude mixture was then separated by column flash chromatography using as eluent a mixture of hexane:ethyl acetate:pyridine (150:50:1). Compound **2a** was obtained in 27% yield (3.0 mg).

¹H NMR (CDCl₃ and C₅D₅N; 300,13 MHz): δ 9.31 (d, 2H, *J* 4.1 Hz, H-2,18); 8.98 (d, 2H, *J* 4.6 Hz, H-7,13); 8.91 (d, 2H, *J* 4.1 Hz, H-3,17); 8.77 (d, 2H, *J* 4.6 Hz, H-8,12); 8.60 – 8.64 (m, 6H, H-Ar); 7.37 – 7.33 (m, 6H, H-Ar) ¹⁹F NMR (CDCl₃ and C₅D₅N; 282.38 MHz): δ -155.18 to -155.50 (m, 6F, F_{ortho}); -159.03 to -159.27 (m, 6F, F_{meta}) UV-Vis in DMSO λ_{max} (log ϵ): 436 (5.22), 575 (4.08) 607 (4.39). HR-MS ESI⁺ *m/z*: calcd. for C₅₂H₂₁F₁₂GaN₇S₃ 1136.0079 [M-py+H]⁺, found 1136.0079.

Synthesis of 5,10,15-tris[4-(pyridin-2-ylsulfanyl)-2,3,5,6-tetrafluorophenyl]-correlategallium(III)pyridine (3a): In a sealed reaction vessel 10.0 mg of **1** (10.6 μ mol) were added to 12 mg of 2-mercaptopyridine (10 eq., 0.11 mmol) in the presence of K₂CO₃ in excess (60 mg), using

0.3 mL of dried DMSO as solvent. The reaction was carried out at room temperature under stirring and N₂ atmosphere for 3h30min. The reactional mixture was neutralized with aqueous citric acid solution followed by extraction of the organic phase with CH₂Cl₂, and then dried with anhydrous sodium sulphate. The crude mixture was then separated by column flash chromatography using as eluent a mixture of hexane:ethyl acetate:pyridine (150:50:1). Compound **3a** was obtained in 43% yield (4.8 mg).

¹H NMR (CDCl₃ and C₅D₅N; 300,13 MHz): δ 9.27 (d, 2H, *J* 4.1 Hz, H-2, 18); 9.01 (d, 2H, *J* 4.6 Hz, H-7, 13 or H-8, 12); 8.92 (d, 2H, *J* 4.1 Hz, H-3, 17); 8.79 (d, 2H, *J* 4.6 Hz, H-7,13 or H-8, 12); 8.59-8.56 (m, 3H, H-Ar); 8.55-8.51 (m, 3H, H-Py); 7.75-7.69 (m, 3H, H-Ar); 7.65-7.62 (m, 2H, H-Py); 7.45 (m, 3H, H-Ar); 7.27-7.19 (m, 3H, H-Ar). ¹⁹F NMR (CDCl₃ and C₅D₅N, 282.38 MHz): δ -154.5 (dd, 4F, *J* 25.4 Hz, F_{ortho}); δ -154.6 (dd, 2F, *J* 25.4 Hz, F_{ortho}); δ -159.3 to -159.5 (m, 6F, F_{meta}). UV-Vis in DMSO λ_{max} (log ϵ): 428 (5.30) 596 (4.34). MS MALDI-TOF *m/z* 1135 [M-py]⁺, *m/z* 1116 [(M-py)-HF+H]⁺, *m/z* 1096 [(M-py)-2HF+H]⁺, *m/z* 1076 [(M-py)-3HF+H]⁺.

General procedure for the cationization of the neutral corrole macrocycles: In a reaction sealed vial, it was added to the neutral corrole derivatives dissolved in dried DMF, CH₃I in large excess; then, the resulting mixture was maintained at 40 °C overnight and under stirring. After this period the reaction vial was cooled down to 0 °C (using ice) and it was added diethyl ether in order to promote the precipitation of the alkylated compound. The resulting precipitate was filtered and washed several times with cool diethyl ether. The solid was dissolved in a methanol:water solution and then precipitated in a metanol:diethyl ether solution.

Synthesis of 5,10,15-tris[4-(1-methylpyridin-4-ylsulfanyl)-2,3,5,6-tetrafluoro-phenyl]correlategallium(III)(pyridine) tri-iodide (2b):In

a reaction vial 18.2 mg (0.016 mmol) of corrole **2a** and 2.0 mL (3.2 mmol) of CH_3I were added to 3.0 mL of dried DMF. Following the conditions described in the general procedure compound **2b** was obtained quantitatively.

^1H NMR (CD_3OD and $\text{C}_5\text{D}_5\text{N}$; 300.13 MHz): δ 9.37 (d, 2H, J 4.0 Hz, H-2,18); 9.20 (d, 2H, J 4.4 Hz, H-7,13); 9.07 (d, 2H, J 4.0 Hz, H-3,17); 9.00 (d, 2H, J 4.4 Hz, H-8,12); 8.85 (d, 6H, J 6.6 Hz, H-Ar); 8.23 (d, 6H, J 6.6 Hz, H-Ar); 4.42 (s, 9H, 3 x CH_3 , H-5',5'',5'''). **^{19}F NMR** (CD_3OD and $\text{C}_5\text{D}_5\text{N}$; 282.38 MHz): δ -157.59 to -157.85 (m, 6F, F_{ortho}); -160.46 to -160.79 (m, 6F, F_{meta}). **UV-Vis** in DMSO λ_{max} (log ϵ): 430 (5.10), 577 (4.67), 596 (4.51). **HR-MS** ESI⁺ m/z: calcd. for $[\text{C}_{55}\text{H}_{29}\text{F}_{12}\text{GaN}_7\text{S}_3]^{3+}$ ·589.53108 [M-py-H]²⁺, found 589.5311.

Synthesis of 5,10,15-tris[4-(1-methylpyridin-2-ylsulfanyl)-2,3,5,6-tetrafluoro-phenyl]corrolategallium(III)(pyridine) tri-iodide (3b): In a reaction vessel 18.2 mg (0.016 mmol) of corrole **3a** and 2.0 mL (3.2 mmol) of CH_3I were added to 3.0 mL of dried DMF. Following the conditions described in the general procedure compound **3b** was obtained quantitatively.

^1H NMR (CD_3OD and $\text{C}_5\text{D}_5\text{N}$; 300.13 MHz): δ 9.39 (d, J 4.0 Hz, H- β); δ 9.25 (d, J 4.5 Hz, H- β); 9.14 – 9.11 (m, H- β); 9.05 (d, J 4.3 Hz, H-Ar); 8.95 (d, J 6.0 Hz, H-Ar); 8.66 – 8.61 (m, H-Ar); 8.55 (sl, H-Py); 8.50 – 8.47 (m, H-Ar); 8.31 (d, J 8.3 Hz, H-Ar); 8.06 – 8.01 (m, H-Ar); 7.87 (sl, H-Py); 7.70 (d, J 8.9 Hz, H-Ar); 7.55 – 7.5 (m, H-Ar); 7.46 (sl, H-Py); 4.69 (s, 6H, CH_3); 4.08 (s, 3H, CH_3). **^{19}F NMR** (CD_3OD and $\text{C}_5\text{D}_5\text{N}$; 282.38 MHz) δ -157.1 to -157.4 (m, 6F, F_{ortho}); -160.1 to -160.3 (m, 6F, F_{meta}). **UV-Vis** em DMSO λ_{max} (log ϵ): 433 (5.24) 605 (4.47) **MS** ESI⁺ m/z: 393.4 [M-py]³⁺.

Determination of the Singlet Oxygen Generation

Stock solutions of each PS at 0.1 mM in DMF and a stock solution of 1,3-diphenylisobenzofuran

(DPBF) at 10 mM in DMF were prepared. A mixture of 50 μM of DPBF and 0.5 μM of the PS derivative in DMF in glass cells (2 mL) was irradiated with a LED array at an irradiance of 9.0 mW cm^{-2} . A LED square array (5 x 5 LEDs light sources) with an emission peak centered at 640 nm and a bandwidth at half maximum of ± 20 nm was constructed and used as light source. Irradiation was conducted at room temperature under stirring. The breakdown of DPBF, indicative of singlet oxygen production, was monitored by measuring the decreasing of the absorbance at 415 nm at irradiation intervals of 1 min for 10 min.

Biological assays

Bacteria cells were conditioned at -80 °C in 10% glycerol. Fresh cultures were maintained in solid BOSS at 4% (1% peptone, 0.3% meat extract, 0.1% glycerol, 3% NaCl, 1.5% agar, pH 7.3). For each assay, in asepsis, an isolated colony was inoculated in 30 mL of liquid BOSS medium for one day at 25 °C under stirring (120 rpm). An aliquot of this culture was subcultivated (240 μL) in 30 μL of liquid BOSS medium and grown overnight at 25°C under stirring (120 rpm) until an optical density of 1.0 was attained at 620 nm ($\text{DO}_{620} \approx 1.0$), meaning around 10^8 cells/mL.

Bacterial strain and growth conditions

The bacterial model used in this work was the marine bioluminescent bacterium *Allivibrio fischeri* (*A. fischeri*) ATCC 49387 (USA). Cells were stored at -80 °C in 10% glycerol. Fresh plate cultures of *A. fischeri* were maintained in solid BOSS medium at 4 °C (BOSS medium: 1% peptone, 0.3% beef extract, 0.1% glycerol, 3% NaCl, 1.5% agar, pH 7.3). A concentration of 20–40 g L^{-1} of NaCl is necessary to maintain the osmotic pressure of cells required to natural light emission to occur. Before each assay, one isolated colony was aseptically inoculated in 30 mL of liquid BOSS medium and

it was allowed to grow for one day at 25 °C under stirring (120 rpm). An aliquot of this culture (240 mL) was subcultured in 30 mL of BOSS medium and grew overnight at 25 °C under stirring (120 rpm) to reach an optical density (OD_{620}) of ~ 1.0 , corresponding to $<10^8$ cells mL^{-1} .

Bioluminescence versus Colony forming units of an overnight culture

The correlation between the colony-forming units (CFU) and the bioluminescence signal of *A. fischeri* was evaluated and a high correlation ($R=0.99$) between viable counts and the bioluminescence signal of overnight cultures was observed. It was used a luminometer (0.5 mL) (TD-20/20 Luminometer, Turner Designs, Inc., Madison, WI, USA) to determine the bioluminescence signal. Thus, the bioluminescence results reflect the viable bacterial abundance, allowing to obtain results in real time in a cost-effective way.

Photosensitizer stock solutions

Stock solutions of the photosensitizers used in the biological studies were prepared in dimethylsulfoxide (DMSO) at a concentration of $500 \mu mol L^{-1}$, and diluted with phosphate buffered saline (PBS) to reach the final concentrations used in the photoinactivation experiments.

Light source

All the photoinactivation experiments were performed under white light from a compatible fibre optic probe (400–800 nm) attached to a 250 W quartz/halogen lamp (LumaCare®, USA, model LC122) with an irradiance of $100 mW cm^{-2}$. All the irradiances were measured with a Power Meter Coherent FieldMaxII-Top combined with a Coherent PowerSens PS19Q energy sensor

PDI experimental setup

The assays were carried out by exposing the *A. fischeri* suspension to each PS added from the stock solution to achieve the final concentrations (10 and 20 μM). After the addition of the PS, all beakers were protected from accidental light exposure with an aluminium foil and pre-incubated for 15 min in the dark, under 100 rpm. After this period, the irradiation was conducted under white light. During the irradiation, the inactivation kinetics was assessed by periodically collecting aliquots of the cell suspensions for bioluminescence signal determinations in the luminometer during the irradiation period (0 and 10, 20, 30, 45, 60, 75, 90 and 120 min irradiation). For each PS and concentration, three independent experiments in duplicate were conducted and the final results were expressed as the overall average. The survival curves were constructed by plotting bioluminescence against irradiation time (min).

RESULTS AND DISCUSSION

The access to the cationic derivatives involved, in a first step, the synthesis of the corresponding neutral corroles **2a** and **3a** via nucleophilic substitution of the *para*-fluorine atoms in the pentafluorophenyl groups of the gallium(III) complex of 5,10,15-tris(pentafluorophenyl)corrole **1** (Gross et al. 1999) using the adequate pyridine derivatives, 4-mercaptopyridine and 2-mercaptopyridine (Figure 1).

This approach, although largely explored to functionalize the analogue 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin, (Costa et al. 2011b, Castro et al. 2017) has also been envisaged for the functionalization of corrole **1** (Cardote et al. 2012, Hori and Osuka 2010, Barata et al. 2013). After a systematic evaluation of the experimental conditions, namely solvent type, temperature, base, number of equivalents of the nucleophile, it was verified that the best yields of the tris-substituted

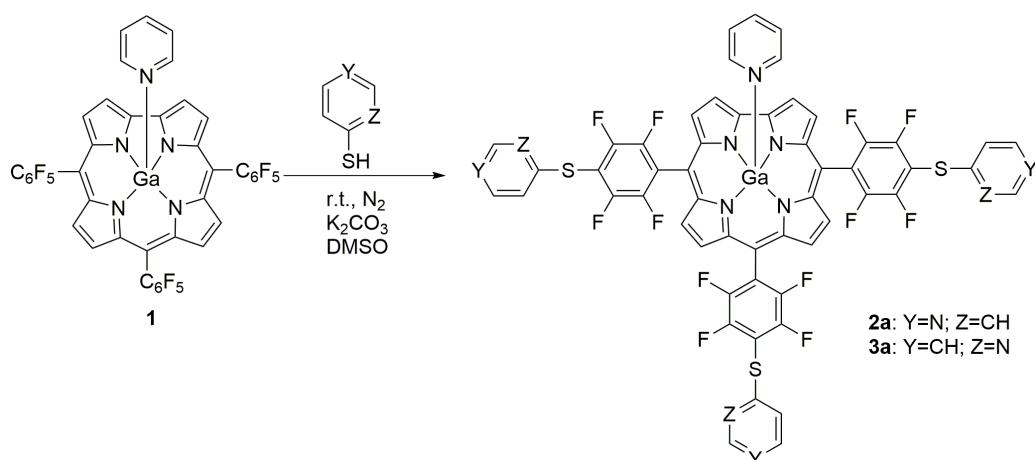


Figure 1 - Reaction of gallium(III) complex of 5,10,15-tris(pentafluorophenyl)corrole **1** with adequate pyridine derivatives.

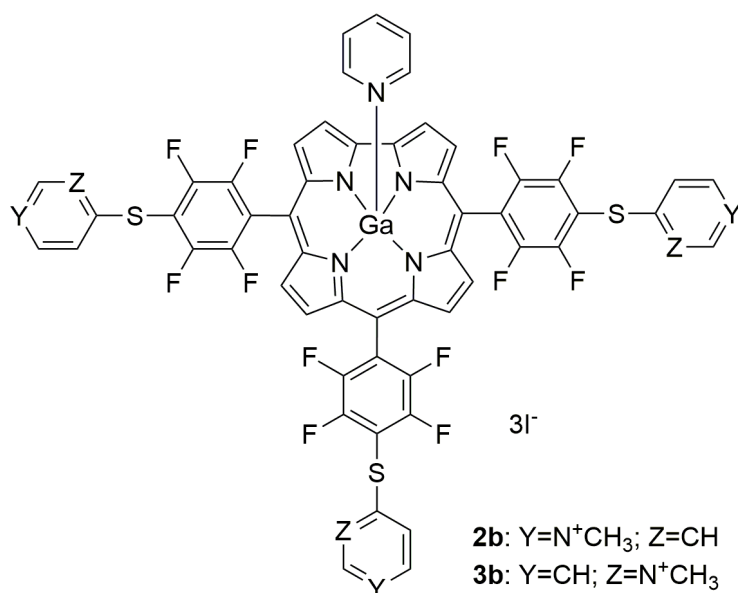


Figure 2 - Structures of the 5,10,15-tris[2,3,5,6-tetrafluoro-4-(1-methylpyridinium-4-sulfanyl)phenyl]corrolatogallium(III)(pyridine) tri-iodide **2b** and 5,10,15-tris[2,3,5,6-tetrafluoro-4-(1-methylpyridinium-2-sulfanyl)phenyl]corrolatogallium(III)(pyridine) tri-iodide **3b**.

derivatives were obtained when the reactions were performed at room temperature under nitrogen, in DMSO and using K₂CO₃ as the base. The coupling reactions of 2- or 4-mercaptopyridines were performed at room temperature in the presence of ten equivalents of the nucleophile. In both cases, in the range 1h30min - 3h30min, the TLC analysis revealed the formation of an important product that was identified, after workup and chromatographic

purification of the crude material, as being the expected tris-substituted derivatives **2a** (27%) and **3a** (43%).

The quaternization of the pyridyl units in corroles **2a** and **3a** was performed in DMF, with methyl iodide at 40 °C overnight, affording the expected tricationic derivatives **2b** and **3b** in quantitative yields after precipitation with ethyl ether (Figure 2).

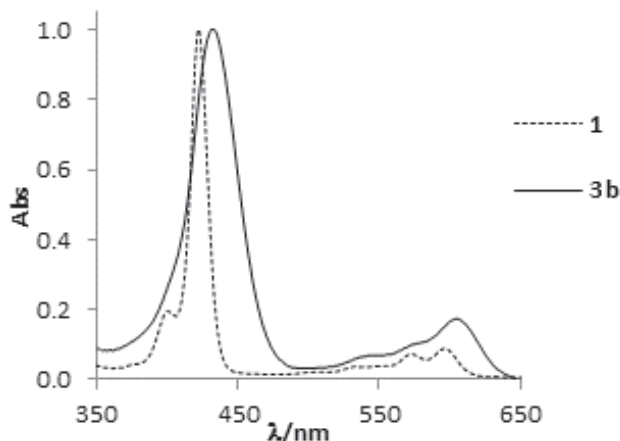


Figure 3 - Normalized UV-Vis spectra of compounds **1** and **3b** in DMSO.

All compounds were characterized by ^1H and ^{19}F NMR spectroscopy and mass spectrometry. The spectroscopic data of all new derivatives were in accordance with the proposed structures. The tri-substituted derivatives were easily identified by the presence at ^{19}F NMR spectra of only two sets of signals due to the *ortho* and *meta* fluorine atoms with the complete disappearance of the signals due to the resonance of the *para*-fluorine atoms. The cationization of the pyridyl groups was confirmed by the appearance of the characteristic singlets due to the resonance of the methyl groups in the ^1H spectra of compounds **2b**, **3b**. All attempts to reach the corresponding tri-substituted free-bases from 5,10,15-tris(pentafluorophenyl)corrole afforded such derivatives in very low yields ($< 10\%$). The absorption spectra of all compounds in DMSO are dominated by an intense absorption band around 430 nm (Soret band) and the less intense Q-bands between 540-610 nm. As an example, in Fig. 3 shows the UV-Vis spectrum of the derivative **3b** and the one due to the starting corrole **1**.

Considering the potential application of the new corroles as photosensitizers it was evaluated their ability to generate singlet oxygen. This was qualitatively evaluated by monitoring the photodegradation of 1,3-diphenylisobenzofuran (DPBF) and the data obtained are displayed in Fig.

4. The results show that the DPBF photodegradation was, in general, enhanced in the presence of the corrole. All the corrole derivatives (**2a**, **3a**, **2b** and **3b**) although with different efficiency, proved to be able to produce singlet oxygen. The DPBF decomposition caused by the neutral derivatives **2a** and **3a** is higher than that caused by the cationic derivatives **2b** and **3b**. The results show also that the *para*-substituted corrole **2a** has a higher efficiency to generate singlet oxygen than the corresponding *ortho*-substituted corrole **3a**.

Antibacterial Activity

To assess the antibacterial effect of the cationic corroles **2b** and **3b**, it was used a light emitting Gram-negative bacterium *Allivibrio fischeri* (*A. fischeri*) under irradiation with white light (400-800 nm), at an irradiance of 100 mW cm^{-2} for 120 min (total light dose of 0.72 kJ cm^{-2}). The light output from these bioluminescent bacteria is a highly sensitive marker of their metabolic activity and it can be easily read in a luminometer with a strong correlation between bioluminescence (measured in relative light units, RLU) and viable cell counts. (Tavares et al. 2010, Alves et al. 2011a, b, Mesquita et al. 2014b). The efficiency of the

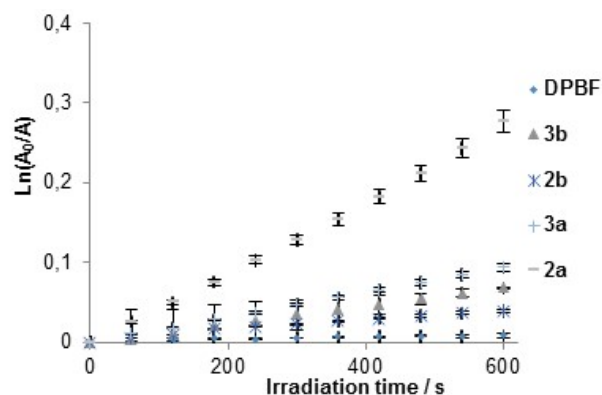


Figure 4 - Time dependent photodecomposition of DPBF (50 mM) photosensitized by corroles derivatives **2a,b** and **3a,b** in DMF upon irradiation with a LED array with an emission peak centred at 640 nm and a bandwidth at half maximum of $\pm 20 \text{ nm}$ (9.0 mW cm^{-2}) with or without PS ($0.5 \mu\text{M}$).

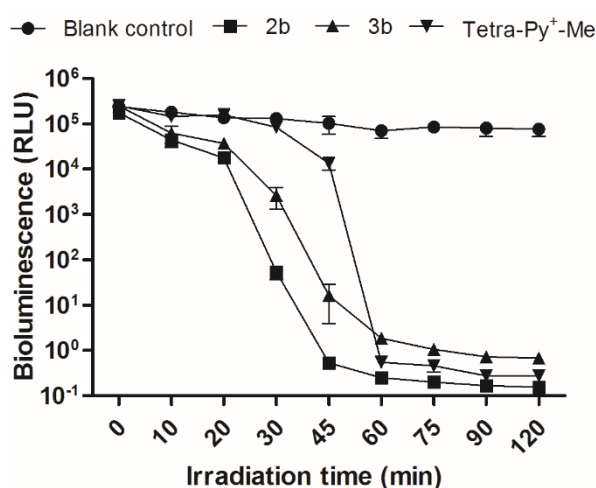


Figure 5 - Bioluminescence monitoring of *A. fischeri* treated with 10 mM of cationic PSs **2b** and **3b** and **Tetra-Py⁺-Me** after irradiation with white light (400-800 nm) at an irradiance of 100 mW cm⁻² during 120 min. Each value represents the mean \pm standard deviation of three independent experiments, with two replicates each. Error bars indicate the standard deviation.

cationic corroles **2b** and **3b** was compared with that of 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin tetra-iodide (**Tetra-Py⁺-Me**) which has been extensively studied in bacterial PDI studies (Almeida et al. 2011, Pereira et al. 2014). The kinetics of photoinactivation of *A. fischeri* was evaluated at two different corrole concentrations (20 and 10 μ M). The significance of the difference in bacterial inactivation among the different compounds was assessed by the Kruskal-Wallis test along with post-hoc tests, using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp. Armonk, NY). A value of $p < 0.05$ was considered significant.

At 20 μ M, corroles **2b** and **3b** were able to reduce *A. fischeri* bioluminescence of $> 6 \log_{10}$ RLU after 30 min of irradiation (0.18 kJ cm⁻²) and the results were not significantly different from the pattern of **Tetra-Py⁺-Me** (data not shown).

At the concentration of 10 mM (Fig.5), the limit of detection ($> 6 \log_{10}$ RLU reduction) was reached for corrole **2b** after 45 min (0.27 kJ cm⁻²) of irradiation. At this irradiation time, PDI with

Tetra-Py⁺-Me was significantly different from the corroles **2b** and **3b** ($p = 0.046$) (figure 5). However the same reduction on *A. fischeri* was attained with corrole **3b** and **Tetra-Py⁺-Me** after 60 min of irradiation ($p > 0.05$). It is worth to refer that all the studied compounds did not exhibit toxicity against the bacterial strain in the absence of light (dark controls) at the tested concentrations or by direct exposure to light in the absence of PS (blank control).

Since corroles usually show single properties when compared with porphyrins, we decided to evaluate if the neutral precursors **2a** and **3a** were able to photoinactivate *A. fischeri* (Fig. 6). The non-cationic corroles **2a**, **3a** and 5,10,15,20-tetra(4-pyridyl)porphyrin were not able to efficiently affect the Gram-negative strain by irradiation which is in accordance with literature (Carvalho et al. 2007, Mesquita et al. 2014a). It is well established that the efficient photoinactivation of Gram-negative bacteria requires the presence of positively charged PS at physiological pH or the presence of

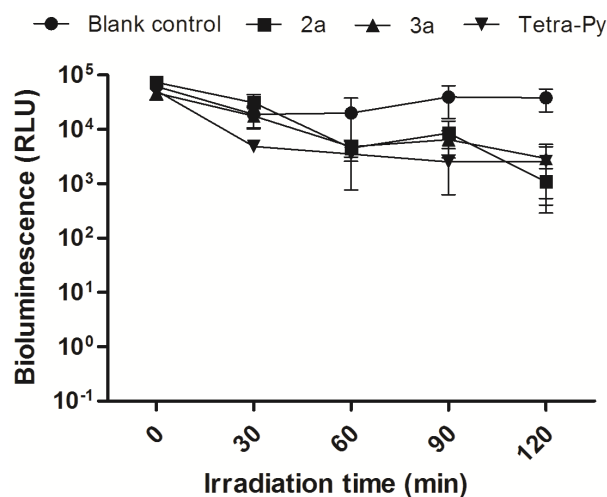


Figure 6 - Bioluminescence monitoring of *A. fischeri* treated with 10 mM of neutral corroles **2a** and **3a** and 5,10,15,20-tetra(4-pyridyl)porphyrin (**Tetra-Py**) after irradiation with white light (400-800 nm) at an irradiance of 100 mW cm⁻² during 120 min. Each value represents the mean \pm standard deviation of three independent experiments, with two replicates each. Error bars indicate the standard deviation.

membrane disruptors such as EDTA or polymyxin B nonapeptide (Costa et al. 2011a, Malik et al. 1992, Preuß et al. 2012).

CONCLUSIONS

In this study, it is described the access to positively charged metallocorroles and their potential application as antibacterial agents. Contrary to the neutral derivatives, both cationic corrole derivatives exhibit ability to photoinactivate the Gram-negative bacterium *A. fischeri* under white light irradiation (100 mW cm⁻²) at all tested concentrations without demonstrating any dark toxicity. Corrole **2b** demonstrated at the lower tested dose, notable efficiency when compared with the well-known **TetraPy⁺-Me**, achieving total photoinactivation (> 6 log₁₀ RLU reduction) after 45 min of irradiation. This preliminary study highlights the properties of cationic corroles for being used in antimicrobial photoinactivation.

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