Photochemical & Photobiological Sciences

An international journal

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: F. S. Sagrillo, C. Dias , A. T. P. C. Gomes, M. A. Faustino, A. Almeida, A. G. D. Souza , A. R. P. D. Costa, F. D. C. S. Boechat, M. C. Souza, M. D. G. P. M. S. Neves and J. A. S. Cavaleiro, *Photochem. Photobiol. Sci.*, 2019, DOI: 10.1039/C9PP00102F.

This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

View Article Online
DOI: 10.1039/C9PP00102F

ARTICLE

Synthesis and photodynamic effect of new porphyrin/4-oxoquinoline derivatives in the inactivation of *S. aureus*

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

[c] Fernanda Savacini Sagrillo,[a,b] Cristina Dias,[b] Ana T. P. C. Gomes,*[c] Maria A. F. Faustino,[a] Adelaide Almeida,*[c] Alan Gonçalves de Souza,[b] Amanda Rodrigues Pinto da Costa,[b] Fernanda da Costa Santos Boechat,[b] Maria Cecília Bastos Vieira de Souza,*[b] Maria G. P. M. S. Neves*[a] and José A. S. Cavaleiro*[a]

New porphyrin/4-oxoquinoline conjugates were synthesized from the Heck coupling reaction of a β-brominated porphyrin with 1-allyl-4-oxoquinoline derivatives, followed by demetallation and deprotection affording the promissing photossensitizers **9a-e**. The singlet oxygen studies have demonstrated that all the porphyrin/4-oxoquinoline conjugates **9a-e** were capable to produce that cytotoxic species and revealed to be excellent photossensitizer agents in the inactivation of *S. aureus* after the antimicrobial photodynamic therapy (aPDT) protocol.

Introduction

The emergence of resistance by microbial pathogens to conventional antimicrobial drugs is a serious and growing problem worldwide.[1, 2] Antimicrobial photodynamic therapy (aPDT) emerged as an effective solution against multidrug resistant strains and against biofilm formation and destruction.[3-5] This therapeutic approach involves the use of a photosensitizer molecule (PS) that after being excited by light can react with molecular oxygen producing cytotoxic reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$) and/or hydroxyl radicals, superoxide and hydrogen peroxide. These ROS can react with biological molecules (*e.g.* nucleic acids, proteins and lipids) causing microbial death.[3-6] aPDT presents several advantages when compared with the use of traditional antimicrobials. This approach shows to be efficient, independently of the antimicrobial resistance profile[5] and is able to prevent further development of resistance even after several cycles of treatment.[7-9] In fact, this approach has been efficiently applied to inactivate several microorganisms, such as Gram-negative[10, 11] and Gram-positive bacteria,[12,13] fungi[14, 15,16] and viruses.[9, 17,18-20]

One of the bacteria that deserves special attention due to its antibiotic resistance development is *Staphylococcus aureus*.[21] This Gram-positive bacterium is one of the most important human pathogens both in hospital and community context and has been a privileged target of therapeutic researches. In fact, numerous antimicrobial agents, as well as vaccine attempts, were specifically designed to combat this bacterium.[21,22] However, multi resistant strains, such as methicillin-resistant *S. aureus* (MRSA) infection has become a more common cause of healthcare-associated infections worldwide. MRSA is associated with significant morbidity and mortality and imposes a huge burden on healthcare resources.[22] This strain is frequently found in epidemic hospital infections[22] and was already found in US patients with HIV infection[22, 23] and in patients with diabetic foot infections.[24] Moreover, in this case, from patients undergoing antibiotic therapy, 93% of the antibiotic regimens were inadequate based on the antibiotic susceptibility test results.[24] Fortunately, a literature survey shows that *S. aureus* is susceptible to aPDT and the PS efficiency and photosensitizing activity is independent of the antibiotic resistance spectrum of the isolates.[17, 25]

Among the PSs studied in the photoinactivation of microorganisms mediated by aPDT, porphyrins are, unquestionably, the most considered class of compounds.[17] Nowadays, several research groups are concerned in the study of new synthetic methodologies for porphyrin derivatives with appropriate features for their application in aPDT. One of the strategies used consists in coupling different compounds with well-established pharmacological activities.[26, 27]Under this context, porphyrins can be linked to other biologically active molecules, aiming to increase their biological efficacy.[28-32]

Having in mind the potentiality already known of porphyrin derivatives in aPDT and, since 4-oxoquinolines are associated with several biological activities, mainly as antibacterial agents,[33, 34] in this work it is reported the synthesis of new porphyrin/4-oxoquinoline conjugates through the Heck coupling and their potential photodynamic effect towards *S. aureus.* Heck coupling

[a] *QOPNA & LAQV-REQUIMTE and Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal, E-mail: gneves@ua.pt; jcavaleiro@ua.pt*
[b] *Programa de Pós-Graduação em Química, Instituto de Química, Universidade Federal Fluminense, 24010-141 Niterói, Rio de Janeiro, Brazil. E-mail: mceciliabvs@gmail.com*
[c] *Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal. E-mail: ana.peixoto@ua.pt; aalmeida@ua.pt*
† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

reaction methodology has been widely used and has already demonstrated its potential in the functionalization of tetrapyrrolic macrocycles, generating compounds with interesting properties.[35-38] The synthesis of other porphyrin/4-quinolone conjugates was already reported by our group,[28,32] but in those cases the coupling between the porphyrin macrocycle and the quinolone unit was made through the 6- and 7-positions of the quinolone nucleus (Figure 1). Since, N-R_1 position of the quinolone moiety is part of the enzyme-DNA binding complex and has a hydrophobic interaction with the major grove of DNA,[34] in this present work we had prepared novel porphyrin/4-quinolone derivatives linked by the *N*-position of the quinolone nucleus (Figure 1) and compared the aPDT results with the previously reported ones.[32]

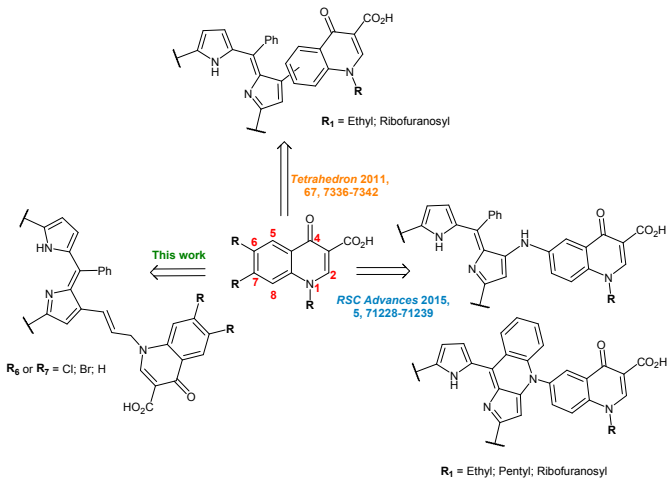


Figure 1. General structures of the new porphyrin/4-oxoquinoline conjugates and others previously studied.

Experimental

General ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C), Bruker Avance 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometers. $CDCl_3$ was used as solvent and tetramethylsilane (TMS) as internal reference. Chemical shifts are expressed in δ (ppm) and the coupling constants (*J*) are expressed in Hertz. Unequivocal ^{1}H assignments were made using 2D COSY, while ^{13}C assignments were made based on 2D HSQC and HMBC experiments. HMRS 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. Source and desolvation temperatures were 80 °C and 150 °C, respectively. Capillary voltage was 3000 V. The spectra were acquired at a nominal resolution of 9000 and at cone voltages of 30 V. Nebulisation and collision gases were N_2 and Ar, respectively. Porphyrin solutions in methanol were introduced at a 10 µL.min^{-1} flow rate. The UV–Vis spectra were recorded on an UV-2501 PC Shimadzu spectrophotometer using $CHCl_3$ as solvent. Flash chromatography was carried out using silica gel (230–400 mesh) and preparative thin-layer chromatography was carried out on 20 × 20 cm glass plates coated with silica gel (1 mm thick). The reactions were routinely monitored by thin layer chromatography (TLC) on silica gel precoated F_{254} Merck plates.

Coupling reactions of porphyrin 2 and 4-oxoquinolines 1a–e.

Study of the reaction conditions according with Table 1

A mixture of porphyrin **2**[39](10 mg, 13.2 mmol), palladium catalyst (10 mol %), ligand (20 mol %), base (26.4 mmol), 1-allyl-4-oxoquinoline **1b** (7.5 mg, 26.4 mmol) and dried toluene:DMF (1:0.5) was purged with N_2 and stirred at 120 ºC under N_2 atmosphere. The progress of the reaction was monitored by TLC (2-29 h) and it was stopped when no more evolution was observed (see Table 1). Then, after reaching room temperature, the mixture was washed with 100 mL of distilled water and extracted with 100 mL of CH_2Cl_2. The organic phase was separated and dried with anhydrous sodium sulphate. The solvent was evaporated, and the crude mixture was purified by preparative column chromatography using CH_2Cl_2 firstly to elute the unreacted porphyrin **2** and then a mixture of CH_2Cl_2: MeOH (20:1) to elute the reaction products. The combination of catalyst/ligand/base that promoted higher yields of porphyrin/4-oxoquinoline conjugates in the classic conditions was selected to perform the reaction under microwave (MW) irradiation (see below).

Synthesis of porphyrin/4-oxoquinoline conjugates under MW irradiation

The porphyrin **2** (10 mg, 13.2 mmol), $Pd(OAc)_2$ (0.3 mg, 10 mol %), PPh_3 (0.7 mg, 20 mol %), KOAc (2.5 mg, 26.4 mmol), 1-allyl-4-oxoquinoline **1a-e** (26.4 mmol) and toluene:DMF (1:0.5, 1.5 mL) were placed in a MW glass vessel and placed in the microwave reactor. MW irradiation (250 W) was applied for 15 minutes at 120 ºC. After this period, the reaction mixture was washed with 100 mL of distilled water and extracted with 100 mL of CH_2Cl_2. The organic phase was separated and dried with anhydrous sodium sulphate. The solvent was evaporated and the crude mixture was purified by preparative column chromatography under the experimental conditions already described.

The structure and numbering of each new derivatives is presented in Schemes 3 and 4.

2-[ethyl-1-(but-2-en-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate]-5,10,15,20-tetraphenylporphyrinatozinc(II) (5a) Yield: (67 %). ^{1}H NMR (500 MHz; $CDCl_3$) δ 8.77 (s, 1H, H-3), 8.71-8.70 (m, 3H, β-H), 8.67-8.66 (m, 2H, β-H), 8.55 (d, *J* = 4.6 Hz, 2H, β-H), 8.48 (s, 1H, H-2'), 8.38 (dd, *J* = 8.1, 1.2 Hz, 1H, H-5'), 8.06-8.04 (m, 6H, H-*o*-Ph-5,10,15), 7.92-7.90 (m, 2H, H-*o*-Ph-20), 7.62-7.55 (m, 10H, H-*m*,*p*-Ph-5,10,15, H-*p*-Ph-20), 7.47-7.43 (m, 4H, H-*m*-Ph-20, H-7', H-8'), 7.37-7.34 (m, 1H, H-6'), 6.33-6.24 (m, 2H, H-α, H-β), 4.63 (d, *J* = 4.7 Hz, 2H, CH_2), 4.31 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 1.31 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3). ^{13}C NMR (125 MHz; $CDCl_3$) δ 175.4 (C-4'), 165.7 (CO_2Et), 150.5, 150.5, 150.3, 150.3, 150.2, 150.0, 149.3 (C-2'), 147.6, 146.0, 143.6, 143.4, 143.1, 140.5, 139.2, 134.4, 133.9, 132.9, 132.3, 132.3, 131.9, 131.9, 131.8, 131.3, 130.6, 130.6 (C-3), 128.7, 128.5, 127.7, 127.4, 127.2, 127.2, 126.4, 126.3, 125.5, 121.4, 120.9, 120.6, 120.2, 120.1, 116.7, 110.4, 61.0 (OCH_2CH_3), 56.4 (CH_2), 14.2 (OCH_2CH_3). UV-vis (DMF): λ_{max} (log ε) 432 (5.38) 476 (4.15) 516

View Article Online DOI: 10.1039/C9PP00158E

(3.63) 564 (4.12) 605 (3.73) nm. HRMS (ESI) *m/z* calcd for $C_{59}H_{42}N_5O_3Zn$ $[M + H]^+$ 932.2579, found 932.2592.

2-[ethyl-1-(but-2-en-1-yl)-6-chloro-4-oxo-1,4-dihydroquinoline-3-carboxylate]-5,10,15,20-tetraphenylporphyrinatozinc(II) (5b) Yield: (42 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (s, 1H, H-3), 8.78-8.75 (m, 3H, β-H), 8.73-8.71 (m, 2H, β-H), 8.61 (d, *J* = 4.6 Hz, 1H, β-H), 8.52 (s, 1H, H-2'), 8.42 (d, *J* = 2.6 Hz, 1H, H-5'), 8.13-8.09 (m, 6H, H-*o*-Ph-5,10,15), 7.99-7.98 (m, 2H, H-*o*-Ph-20), 7.70-7.63 (m, 10H, H-*m,p*-Ph-5,10,15 e H-*p*-Ph-20), 7.61 (dd, *J* = 9.1, 2.6 Hz, 1H, H-7'), 7.54 (t, *J* = 7,6 Hz, 2H, H-*m*-Ph-20), 7.47 (d, *J* = 9.1 Hz, 1H, H-8'), 6.36-6.34 (m, 2H, H-α, H-β), 4.71 (d, *J* = 3.8 Hz, 2H, CH_2), 4.39 (q, J = 7.1 Hz, 2H, O*C*H_2CH$_3$), 1.37 (t, *J* = 7.1 Hz, 3H, OCH$_2$*C*H_3). ^{13}C NMR (125 MHz, $CDCl_3$+MeOD) δ 174.2 (C-4'), 165.6 (CO_2Et), 150.74, 150.67, 150.57, 150.4, 150.2, 149.6 (C-2'), 147.8, 146.1, 143.8, 143.5, 143.3, 140.5, 137.8, 134.6, 134.0, 133.2, 132.22 (C-α or C-β), 132.16, 131.98, 131.94, 131.5, 130.8 (C-3), 130.2, 127.9, 127.44, 127.39, 127.1 (C-5'), 126.8, 126.6 (C-7'), 126.5, 121.3, 121.1 (C-α or C-β) 120.8, 120.4, 120.2, 118.7 (C-8'), 111.1, 61.3 (O*C*H_2CH$_3$), 56.3 (CH_2), 14.4 (OCH$_2$*C*H_3). UV-vis (DMF): λ_{max} (log ε) 433 (5.56) 476 (4.38) 516 (3.81) 564 (4.25) 604 (3.87) nm. HRMS (ESI) *m/z* calcd for $C_{59}H_{41}ClN_5O_3Zn$ $[M + H]^+$ 966.2189, found 966.2207.

2-[ethyl-1-(but-2-en-1-yl)-7-chloro-4-oxo-1,4-dihydroquinoline-3-carboxylate]-5,10,15,20-tetraphenylporphyrinatozinc(II) (5c) Yield: (24 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.95 (s, 1H, H-3), 8.92-8.90 (m, 3H, β-H), 8.89-8.86 (m, 2H, β-H), 8.76 (d, *J* = 4.7 Hz, 1H, β-H), 8.38 (s, 1H, H-2'), 8.22- 8.16 (m, 6H, H-*o*-Ph-5,10,15), 8.13-8.08 (m, 3H, H-*o*-Ph-20, H-5'), 7.76-7.70 (m, 10H, H-*m,p*-Ph-5,10,15, H-*p*-Ph-20), 7.68-7.61 (m, 3H, H-*m*-Ph-20, H-8'), 7.49 (dd, *J* = 8.6, 1.5 Hz, 1H, H6'), 6.35 (s, 2H, H-α, H-β), 4.66 (d, *J* = 3.1 Hz, 2H, CH_2), 4.29 (q, *J* = 7.1 Hz, 2H, O*C*H_2CH$_3$), 1.35 (t, *J* = 7.1 Hz, 3H, OCH$_2$*C*H_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7 (C-4'), 165.4 (CO_2Et), 150.9, 150.8, 150.8, 150.7, 150.6, 150.4, 149.4 (C-2'), 147.9, 146.2, 143.3, 143.0, 142.8, 140.8, 139.9, 139.1, 134.6, 134.0, 132.5, 132.4, 131.8, 131.0 (C-3), 129.5, 128.2, 127.7, 127.6, 127.4, 126.8, 126.8, 126.7, 125.7 (C-6'), 121.9, 121.2, 120.8, 120.8, 116.2 (C-8'), 111.5, 61.1 (O*C*H_2CH$_3$), 56.7 (CH_2), 14.5 (OCH$_2$*C*H_3). UV-vis (DMF): λ_{max} (log ε) 432 (5.45) 476 (4.50) 515 (3.88) 564 (4.18) 604 (3.86) nm. HRMS (ESI) *m/z* calcd for $C_{59}H_{41}ClN_5O_3Zn$ $[M + H]^+$ 966.2189, found 966.2211.

2-[ethyl-1-(but-2-en-1-yl)-6-bromine-4-oxo-1,4-dihydroquinoline-3-carboxylate)-5,10,15,20-tetraphenylporphyrinatozinc(II) (5d) Yield: (46 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.83 (s, 1H, H-3), 8.80-8.77 (m, 3H, β-H), 8.75-8.73 (m, 2H, β-H), 8.62 (d, *J* = 4.7, 1H, β-H), 8.59 (d, *J* = 2.4 Hz, 1H, H-5'), 8.51 (s, 1H, H-2'), 8.13-8.10 (m, 6H, H-*o*-Ph-5,10,15), 8.00-7.98 (m, 2H, H-*o*-Ph-20), 7.74 (dd, *J* = 9.0, 2.4 Hz, 1H, H-7'), 7.70-7.62 (m, 10H, H-*m,p*-Ph-5,10,15, H-*p*-Ph-20), 7.57-7.52 (m, 2H, H-*m*-Ph-20), 7.39 (d, *J* = 9.1 Hz, 1H, H-8'), 6.35 (s, 2H, H-α, H-β), 4.69 (d, *J* = 2.6 Hz, 2H, CH_2), 4.39 (q, *J* = 7.1 Hz, 2H, O*C*H_2CH$_3$), 1.38 (t, *J* = 7.1 Hz, 3H, OCH$_2$*C*H_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4 (C-4'), 165.8 (CO_2Et), 150.8, 150.8, 150.6, 150.5, 150.3, 149.4 (C-2'), 147.9, 146.2, 143.5, 143.2, 143.0, 140.8, 138.2, 135.6, 134.6, 134.1, 132.4, 132.2, 132.2, 132.0, 131.7, 131.0 (C-3), 130.7, 130.6, 128.2, 127.6, 127.6, 126.7, 126.6, 121.7, 121.4, 121.1, 120.6, 119.3, 118.5 (C-8'), 111.6, 61.3 (O*C*H_2CH$_3$), 56.6 (CH_2), 14.6 (OCH$_2$*C*H_3). UV-vis (DMF): λ_{max} (log ε) 431 (5.53) 475 (3.68) 523 (3.55) 564 (4.22) 604 (3.82) nm. HRMS (ESI) *m/z* calcd for $C_{59}H_{41}BrN_5O_3Zn$ $[M + H]^+$ 1010.1684, found 1010.1697.

2-[ethyl-1-(but-2-en-1-yl)-7-bromine-4-oxo-1,4-dihydroquinoline-3-carboxylate]-5,10,15,20-tetraphenylporphyrinatozinc(II) (5e) Yield: (24 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.97 (s, 1H, H-3), 8.93-8.91 (m, 3H, β-H), 8.89-8.87 (m, 2H, β-H), 8.77 (d, *J* = 4.7 Hz, 1H, β-H), 8.44 (s, 1H, H-2'), 8.27 (d, *J* = 8.6 Hz, 1H, H-5'), 8.21-8.18 (m, 6H, H-*o*-Ph-5,10,15), 8.10-8.08 (m, 2H, H-*o*-Ph-20), 7.77-7.72 (m, 10H, H-*m,p*-Ph-5,10,15), 7.68-7.65 (m, 3H, H-*m,p*-Ph-20, H-8'), 7.53 (dd, *J* = 8.6, 1.6 Hz, 1H, H-6'), 6.41-6.38 (m, 2H, H-α,H-β), 4.69 (d, *J* = 3.9 Hz, 2H, CH_2), 4.38 (q, *J* = 7.1 Hz, 2H, O*C*H_2CH$_3$), 1.40 (t, *J* = 7.1 Hz, 3H, OCH$_2$*C*H_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0 (C-4'), 165.7 (CO_2Et), 150.9, 150.9, 150.8, 150.7, 150.6, 150.4, 149.5 (C-2'), 148.0, 146.3, 143.2, 143.0, 142.8, 141.0, 140.2, 134.6, 134.1, 132.6, 132.4, 131.9, 131.2 (C-3), 129.7 (C-5'), 128.7, 128.3, 128.0, 127.8, 127.7, 126.9, 126.8, 126.7, 122.0, 121.7, 121.3, 120.9, 119.3, 111.7, 61.2 (O*C*H_2CH$_3$), 56.4 (CH_2), 14.6 (OCH$_2$*C*H_3). UV-vis (DMF): λ_{max} (log ε) 433 (5.67) 475 (3.74) 524 (3.58) 574 (4.29) 604 (3.86) nm. HRMS (ESI) *m/z* calcd for $C_{59}H_{41}BrN_5O_3Zn$ $[M + H]^+$ 1010.1684, found 1010.1708.

3-[(*E*)-4-(3-oxoprop-1-en-1-yl]-5,10,15,20 tetraphenylporphyrinatozinc(II) (6) ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.11 (s, 1H, H-3), 9.08 (d, *J* = 8.0 Hz, 1H, CHO), 8.91-8.89 (m, 6H, β-H), 8.20-8.18 (m, 6H, H-*o*-Ph-5,10,15), 8.11-8.09 (m, 2H, H-*o*-Ph-20), 7.83-7.71 (m, 12H, H-*m,p*-Ph-5,10,15,20), 7.04 (d, *J* = 15.4 Hz, 1H, H-α), 6.77 (dd, *J* = 15.4, 8.0 Hz, 1H, H-β). ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.2 (CHO), 151.2, 151.16, 151.12, 151.1, 150.7, 150.1 (C-β), 149.8 (C-2'), 147.4, 146.4, 142.9, 142.5, 142.4, 138.9, 134.43, 134.41, 134.39, 134.1, 133.9, 132.8, 132.63, 132.61, 132.56, 132.51, 132.3, 132.0, 129.6 (C-α), 129.0, 128.5, 128.4, 128.2, 127.9, 127.6, 127.1, 126.6, 121.9, 121.4, 121.3, 120.8. HRMS (ESI) *m/z* calcd for $C_{47}H_{31}N_4OZn$ $[M + H]^+$ 731.1789, found 731.1770.

3-[4-(2-(carboxyamino)vinyl]-5,10,15,20-tetraphenylporphyrinatozinc(II) (7) ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (s, 1H, H-3), 8.93-8.90 (m, 3H, β-H), 8.89-8.87 (m, 2H, β-H), 8.83 (d, *J* = 4.7 Hz, 1H, β-H), 8.21-8.18 (m, 6H, H-*o*-Ph-5,10,15), 8.08-8.05 (m, 2H, H-*o*-Ph-20), 7.77-7.70 (m, 12H, H-*m,p*-Ph-5,10,15,20), 6.36-6.33 (m, 2H, H-α, H-β), 4.53 (d, *J* = 5.0 Hz, 1H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1 (NH*C*O$_2$H), 156.5, 153.4, 150.9, 150.7, 150.5, 150.4, 150.4, 150.3, 148.5, 146.9, 143.1, 143.0, 142.9, 142.7, 137.8, 134.5, 134.1, 132.5, 132.3, 132.2, 132.1, 131.7, 131.2 (C-α or C-β), 131.0, 130.8, 128.1, 127.6, 127.6, 126.9, 127.7, 124.4 (C-α or C-β), 121.6, 121.2, 121.1, 120.6, 118.8, 65.7 (CH_2). HRMS (ESI) *m/z* calcd for $C_{48}H_{33}N_5O_2Zn^{\cdot}$ $M^{+\bullet}$ 775.1926, found 775.2043.

Hydrolysis and demetallation of conjugates 5a-e. General Procedure

Into a flask with reflux condenser were added the conjugates **5a-e** (10 mg), NaOH (160 mg, 4000 mmol), EtOH (4 mL). The reaction was kept under stirring and reflux for 2 hours. After that time the mixture was neutralized with 50 mL of dilute citric acid solution and extracted with 100 mL of CH_2Cl_2. The organic phase was dried with anhydrous Na_2SO_4 and the solvent was evaporated. To the obtained residue was added CH_2Cl_2 (4 mL) and TFA (0.5 mL). The mixture was vigorously stirred at room temperature for 30 minutes, after which it was neutralized with 100 mL of saturated aqueous solution of $NaHCO_3$. The organic extracts were dried over anhydrous Na_2SO_4.

The solvent was evaporated under reduced pressure and the product was obtained pure after purification by preparative TLC using a mixture of CH_2Cl_2/MeOH (20:1).

2-[1-(but-2-en-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid]-5,10,15,20-tetraphenylporphyrin (9a) Yield: (53 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.06 (s, 1H, OH), 8.83-8.81 (m, 3H, β-H, H-3), 8.74 (d, *J* = 4.8, 2H, β-H), 8.75 (s, 1H, H-2'), 8.67 (d, *J* = 4.8, 2H, β-H), 8.57 (dd, *J* = 8.1, 1.4 Hz, 1H, H-5'), 8.21-8.17 (m, 6H, H-*o*-Ph-5,10,15), 8.05-8.02 (m, 2H, H-*o*-Ph-20), 7.83-7.72 (m, 10H, H-*m,p*-Ph-5,10,15, H-*p*-Ph-20), 7.68-7.58 (m, 4H, H-*m*-Ph-20, H-7'e H-8'), 7.50-7.46 (m, 1H, H-6'), 6.53-6.34 (m, 2H,H-α, H-β), 4.81 (d, *J* = 5.3 Hz, 2H, CH_2), -2.72 (s, 2H, N*H*s). UV-vis (DMF): λ_{max} (log ε) 422 (5.50) 518 (4.27) 555 (3.87) 595 (3.76) 651 (3.52) nm. HRMS (ESI) m/z calcd for $C_{57}H_{40}N_5O_3$ $[M + H]^+$ 842.3131, found 842.3124.

2-[1-(but-2-en-1-yl)-7-chloro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid]-5,10,15,20-tetraphenylporphyrin (9b) Yield: (38 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.72 (s, 1H, OH), 8.82-8.73 (m, 7H, β-H, H-3, H-2'), 8.66 (d, *J* = 4.8, 1H, β-H), 8.54 (d, *J* = 2.3 Hz, 1H, H-5'), 8.20-8.18 (m, 6H, H-*o*-Ph-5,10,15), 8.06-8.04 (m, 2H, H-*o*-Ph-20), 7.81-7.74 (m, 10H, H-*m,p*-Ph-5,10,15, H-*p*-Ph-20), 7.69-7.63 (m, 3H, H-*m*-Ph-20, H-8'), 7.59 (dd, *J* = 9.1, 3.1 Hz, 1H, H-7'), 6.49-6.35 (m, 2H, H-α, H-β), 4.82 (d, *J* = 4.9 Hz, 2H, CH_2), -2.72 (s, 2H, N*H*s). UV-vis (DMF): λ_{max} (log ε) 426 (5.39) 519 (4.18) 555 (3.80) 596 (3.69) 652 (3.46) nm. HRMS (ESI) m/z calcd for $C_{57}H_{39}ClN_5O_3$ $[M + H]^+$ 876.2741, found 876.2744.

2-[1-(but-2-en-1-yl)-7-chloro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid]-5,10,15,20-tetraphenylporphyrin (9c) Yield: (47 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.75 (s, 1H, OH), 8.84-8.81 (m, 4H, β-H, H-3), 8.79 (d, *J* = 5.0, 2H, β-H), 8.74 (s, 1H, H-2'), 8.68 (d, *J* = 5.0, 1H, β-H), 8.49 (d, *J* = 8.7 Hz, 1H, H-5'), 8.21-8.17 (m, 6H, H-*o*-Ph-5,10,15), 8.09-8.07 (m, 2H, H-*o*-Ph-20), 7.80-7.74 (m, 10H, H-*m,p*-Ph-5,10,15, H-*p*-Ph20), 7.69-7.55 (m, 2H, H-*m*-Ph-20), 7.62 (d, *J* = 1.7 Hz, 1H, H-8'), 7.52 (dd, *J* = 8.7, 1.7 Hz, 1H, H-6'), 6.49-6.32 (m, 2H, H-α+H-β), 4.78 (d, *J* = 5.0 Hz, 2H, CH_2), -2.71 (s, 2H, N*H*s). UV-vis (DMF): λ_{max} (log ε) 427 (5.36) 518 (4.18) 554 (3.78) 595 (3.67) 651 (3.44) nm. HRMS (ESI) *m/z* calcd for $C_{57}H_{39}ClN_5O_3$ $[M + H]^+$ 876.2741, found 876.2753.

2-[1-(but-2-en-1-yl)-6-bromine-4-oxo-1,4-dihydroquinoline-3-carboxylic acid]-5,10,15,20-tetraphenylporphyrin (9d) Yield: (32 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.59 (s, 1H, OH), 8.93-8.72 (m, 7H, β-H, H-3), 8.62 (d, *J* = 2.3 Hz, 1H, H-5'), 8.50 (s, 1H, H-2'), 8.22-8.14 (m, 6H, H-*o*-Ph-5,10,15), 8.06-8.02 (m, 2H, H-*o*-Ph-20), 7.78-7.59 (m, 13H, H-*m,p*-Ph-5,10,15,20, H-8'), 7.38 (dd, *J* = 9.3, 2.2 Hz, 1H, H-7'), 6.44-6.41 (m, 2H, H-α, H-β), 4.85 (d, *J* = 5.0 Hz, 2H, CH_2), -2.72 (s, 2H, N*H*s). UV-vis (DMF): λ_{max} (log ε) 428 (5.37) 520 (4.21) 554 (3.80) 598 (3.69) 653 (3.48) nm. HRMS (ESI) *m/z* calcd for $C_{57}H_{39}BrN_5O_3$ $[M + H]^+$ 920.2236, found 920.2249.

2-[1-(but-2-en-1-yl)-7-bromine-4-oxo-1,4-dihydroquinoline-3-carboxylic acid]-5,10,15,20-tetraphenylporphyrin (9e) Yield: (69 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.75 (s, 1H, OH), 8.84-8.79 (m, 4H, β-H, H-3), 8.78-8.75 (m, 3H, β-H, H-2'), 8.68 (d, *J* = 4.9, 1H, β-H), 8.45 (d, *J* = 8.7 Hz, 1H, H-5'), 8.22-8.17 (m, 6H, H-*o*-Ph-5,10,15), 8.10-8.07 (m, 2H, H-*o*-Ph-20), 7.83 (d, *J* = 1.6 Hz, 1H, H-8'), 7.78-7.74 (m, 12H, H-*m,p*-Ph-5,10,15,20), 7.70 (dd, *J* = 8.6, 1.5 Hz, 1H, H-6'), 6.51-6.34 (m, 2H, H-α, H-β), 4.84 (d, *J* = 5.1 Hz, 2H, CH_2), -2.70 (s, 2H, N*H*s). UV-vis (DMF): λ_{max} (log ε) 422 (5.39) 518 (4.16) 554 (3.75) 595 (3.65) 651 (3.43) nm. HRMS (ESI) *m/z* calcd for $C_{57}H_{39}BrN_5O_3$ $[M + H]^+$ 920.2236, found 920.2242.

Singlet oxygen generation studies

A stock solution of each 4-oxoquinoline porphyrin derivative **9a-e** at 0.1 mM in DMF and a stock 10 mM solution of 1,3-diphenylisobenzofuran (DPiBF) in DMF were prepared. Aliquots of 2.5 mL of a solution of each porphyrin (0.5 μM) and DPiBF (50 μM) in DMF were irradiated at an irradiance of 20.0 mW.cm^{-2}, in a glass cuvette, at room temperature and under gentle magnetic stirring. As light source it was used an illumination system (LC-122 LumaCare, London) equipped with a halogen/quartz 250 W lamp coupled to an interchangeable optic fibre probe (400–800 nm) and using a dichroic cut-off filter (λ > 550 nm) to achieve an emission radiation from 550 to 800 nm. The absorption decay of DPiBF at 415 nm was measured at different irradiation times. The production of singlet oxygen was evaluated qualitatively through the DPiBF, a singlet oxygen ($^1O_2$) quencher, since DPiBF decays in a first reaction order manner during continuous irradiation in a photosensitised experiment. The irradiation of the PS in the presence of dissolved oxygen will result in the formation of $^1O_2$, which is trapped by DPiBF resulting in colourless *o*-dibenzoylbenzene, after the Diels–Alder like reaction with $^1O_2$.

Photodynamic inactivation studies

Photosensitizers stock solutions

Stock solutions of 500 μM of porphyrin derivatives **9a-e** were prepared in dimethyl sulfoxide (DMSO), protected from light and were sonicated for 30 min at 25 °C previously to each assay.

Light source

The samples and light control were exposed to white light (400-750 nm) delivered by a LED system (ELMARK – VEGA20, 20W, 1400 lm) with fluence rate of 25 mW.cm^{-2}.

Bacterial Strains and Growth Conditions

The genetically transformed bioluminescent *Staphylococcus aureus* Xen 31 (*S. aureus* Xen31)[40] was grown on Tryptic Soy Agar (TSA, Merck) supplemented with 50 mg/mL of ampicillin (Amp) and with 34 mg/mL of chloramphenicol (Cm). Before each assay, one isolated colony was transferred to 10 mL of Luria Bertani medium (Liofilchem, Italy) previously supplemented with Amp and Cm and was grown overnight at 37 °C under stirring (120 rpm). An aliquot was transferred into 10 mL Tryptic Soy Broth (TSB, Merck) under the same growth conditions till stationary growth phase was achieved [≈10^6 relative light unit (RLU). An optical density at 600 nm (OD600) of 1.6 ± 0.1 corresponded to ≈10^6 colony forming units (CFU)/mL. The correlation between CFU/mL and the bioluminescent

View Article Online
DOI: 10.1039/C9PP00102F

signal (in RLUs) of bioluminescent *S. aureus* strain was evaluated. A fresh overnight bacterial culture was serially diluted 10^{-1} to 10^{-5} in phosphate-buffered saline (PBS.) Non-diluted and diluted aliquots were pour plated on TSA medium (0.5 mL) and, simultaneously, were read on a luminometer (0.5 mL) (TD-20/20 Luminometer, Turner Designs, Inc., Madison, WI, United States) to determine the bioluminescence signal. The results obtained are presented in Figure 2.

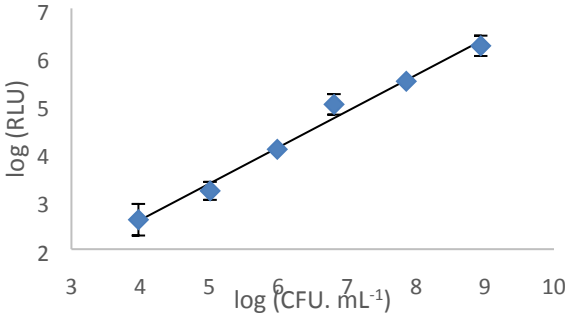


Figure 2. Relationship between the bioluminescence signal and viable counts of an overnight culture of *S. aureus* (< 10^6 CFU. mL) serially diluted in PBS. All values are the mean of three independent assays. The error bars represent the standard deviation.

Antimicrobial Photodynamic Therapy (aPDT) Procedure

Bioluminescent *S. aureus* culture was grown overnight and was tenfold diluted in PBS (pH 7.49) to a final concentration of ≈10^9 CFU/mL, which corresponds approximately to 10^6 RLU. The bacterial suspension was equally distributed in 50 mL sterilized and acid-washed beakers.

Bioluminescence Monitoring

All the experiments were carried out under white light (400-750 nm) and the *S. aureus* bioluminescence signal was measured in the luminometer (TD-20/20 Luminometer, Turner Designs, Inc., Madison, WI, United States) at different times of light exposure. The assays were finished whenever the detection limit of the luminometer was achieved (*ca* 2.3 log). Light control (LC) comprised bacterial suspension exposed to the light and dark control (DC) comprised bacterial suspension with each PS at the same concentration (1.0 µM) protected from the light with aluminium foil, were also evaluated.

Photodynamic inactivation assay

Bacterial suspensions were prepared from cultures (≈10^6 RLU) and ten-fold diluted in PBS and distributed in sterilized glass beakers. The appropriate volume of each PS (**9a-e**) was added to the suspensions to reach a final concentration of 1.0 µM. Samples and controls (LC and DC) were incubated under stirring for 15 min and protect from light. After this period, the samples and LC were irradiated for 90 min under stirring and the temperature was controlled at ≈ 37 °C. Aliquots of the treated and control samples were collected at time 0 min and after predefined irradiation times and the bioluminescence was read in the luminometer. Simultaneously, aliquots of treated and control samples were collected at time 0 and 90 min, then serially diluted and plated in duplicate in Tryptic Soy Agar medium. The petri plates were incubated for 24 h at 37 °C. Three independent experiments were done.

Statistical analysis

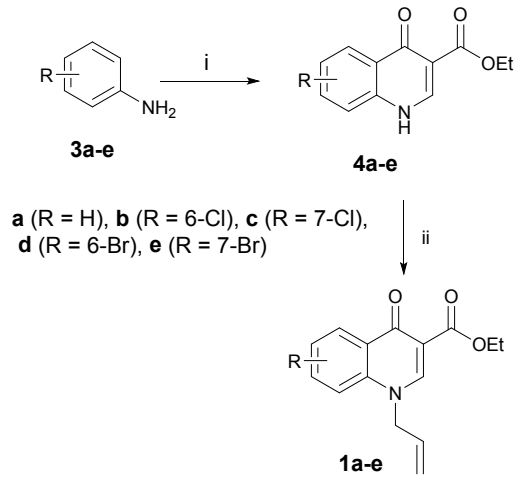
Statistical analysis was performed with GraphPad Prism 6. Normal distributions were checked by the Kolmogorov–Smirnov test and the homogeneity of variance was verified with the Brown Forsythe test. For the bioluminescence monitor assay, three independent experiments with two replicates per assay for each condition were performed and the differences between the results were analyzed by two-way analysis of variance (ANOVA) and Dunnet's multiple comparison tests. The value of $p < 0.05$ was considered significant. For the study of reduction of CFU/mL through the pour plated methodology, three independent experiments with two replicates per assay for each condition were performed and the results were analyzed by two-way analysis of variance (ANOVA) and Bonferroni's multiple comparison tests. The value of $p < 0.0001$ was considered significant.

Results and Discussion

Synthesis of porphyrin/4-oxoquinoline conjugates

The synthetic access to the desired porphyrin-4-oxoquinoline derivatives **9a**-**e** (Scheme 4) required the preparation of the precursors **5a**-**e** (Schemes 2 and 3). The key step in the proposed strategy is the Heck coupling reaction between 1-allyl-4-oxoquinoline substrates **1a-e** and the 2-bromo-5,10,15,20-tetraphenylporphyrinatozinc(II) (**2**).[39] These oxoquinoline derivatives were prepared by the reaction of the adequate anilines **3a-e** with diethyl ethoxymethylene malonate (EMME), followed by thermic cyclization (Scheme 1).[41, 42] The subsequent reaction of **4a-e** with allyl chloride afforded the 1-allyl-4-oxo-1,4-dihydroquinoline-3-carboxylates.[43] The porphyrin macrocycle precursor **2** was prepared according to literature by a controlled bromination of 5,10,15,20-tetraphenylporphyrin (**TPP**) with *N*-bromosuccinimide (**NBS**), followed by metalation with $Zn(OAc)_2$, affording 2-bromo-5,10,15,20-tetraphenylporphyrinatozinc(II) (**2**) (Scheme 2).[39]

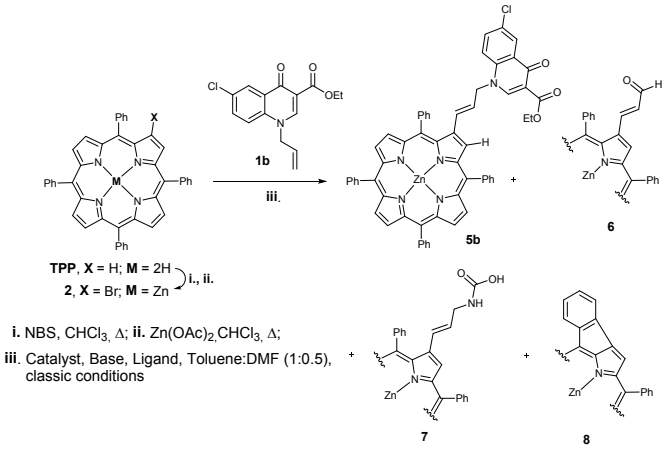
View Article Online
DOI: 10.1039/C9PP00102F



(i) (1) EMME, EtOH, reflux, 2 h. (2) diphenyl ether, 270 ºC;
(ii) (1) DMF, K_2CO_3, r.t., 15 min. (2) allyl chloride, 80 ºC, 24 h.

Scheme 1. Synthesis of 4-oxoquinoline substrates **1a–e.**

In order to select the best reaction conditions for the Heck coupling reaction, an optimization was planned using the Zn(II) β-bromoporphyrin **2** and the 1-allyl-4-oxo-1,4-dihydroquinoline-3-carboxylate **1b** (Scheme 2 and Table 1). The catalysts selected for this study were palladium(II) acetate [$Pd(AcO)_2$] or $Pd(PPh_3)_4$ and the couplings were performed in the presence of the different bases K_2CO_3, Cs_2CO_3 and KOAc using as ligands tetra-*n*-butylammonium bromide (TBAB) or triphenylphosphine (PPh_3). All reactions were performed using a solvent system comprised by dry toluene and DMF (1:0.5) at 120 °C under nitrogen atmosphere and the progress of the reactions were monitored by TLC (Scheme 2 and Table 1). After the workup the crude residue was fractionated by silica gel column and all the fractions were further purified by preparative thin layer chromatography.



Scheme 2. Heck coupling reaction between β-bromoporphyrin **2** and 1-allyl-4-oxoquinoline derivative **1b**

Table 1. Catalyst, base and ligand effects on the time and yield of the Heck reaction between quinoline derivative **1b** and β-bromo porphyrin **2**.

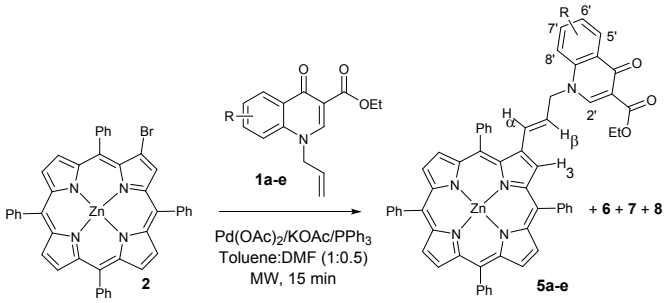
E ntry	Cat.	Base	Lig.	Tim e	% 5b	%	% 7	%	% 2 6	% 8	rec ov.
1	$Pd(OAc)_2$	K_2CO_3	TBAB	3 h	13	15	5	6	5
2	$Pd(OAc)_2$	Cs_2CO_3	TBAB	29 h	traces	19	trace	10	32
3	$Pd(OAc)_2$	KOAc	TBAB	2 h	33	1	19	10	6
4	$Pd(OAc)_2$	KOAc	PPh_3	2 h	42	3	9	10	5
5	$Pd(PPh_3)_4$	KOAc	TBAB	3 h	20	18	13	12	14
6*	$Pd(OAc)_2$	KOAc	PPh_3	15 min	42	Traces	4	8	1

* MW radiation

The results obtained show that the yield of the expected porphyrin/4-oxoquinoline conjugate **5b** is strongly dependent of the reaction conditions and regardless the reaction conditions, its formation is accompanied by the formation of three unexpected products: the aldehyde porphyrin derivative **6**, the porphyrin derivative **7** and the fused porphyrin **8**. The formation of the by-products **6** and **7** can be explained by the decomposition of porphyrin/4-oxoquinoline conjugate **5b** and their establishment will be discussed in the mechanistic consideration part. The formation of fused porphyrin **8** is due to the thermal cyclization and it was already reported in the literature.[44] From Table 1, it possible to observe that the combination $Pd(OAc)_2/Cs_2CO_3$/TBAB (entry 2) is the one that produced the porphyrin/4-oxoquinoline conjugate **5b** in lower yield (only traces were detected) followed by the combination of $Pd(OAc)_2/K_2CO_3$/TBAB (entry 1) that afforded the desired conjugate in 13% yield. A better performance was observed when $Pd(OAc)_2$ was used in the presence of KOAc and using TBAB or PPh_3 as ligands (entries 3 and 4). Under these conditions and just after two hours of reaction, the yield of porphyrin derivative **5b** was dramatically increased to 33% in the presence of TBAB and to 42 % in the presence of PPh_3. No improvement was observed in the presence of $Pd(PPh_3)_4$/KOAc/TBAB (entry 5) where the yield of conjugate **5b** did not go beyond 20 %, even after 3 hours of reactions.

In the attempt to minimize the formation of the secondary products by reducing the reaction time, an extra assay was performed under MW radiation using the best catalytic combination, $Pd(OAc)_2/K_2CO_3/PPh_3$. Under these conditions the porphyrin derivative **5a** was also isolated in 42 % but a considerable reduction on the reaction time from 2h to 15 min was achieved (Table 1, entry 6) So, these conditions were selected to couple the porphyrin derivative **2** with the remaining 4-oxoquinoline substrates **1a, 1c–e** (Table 2, Scheme 3)

DOI: 10.1039/C9PP00102F



a (R = H), **b** (R = 6-Cl), **c** (R = 7-Cl), **d** (R = 6-Br), **e** (R = 7-Br)

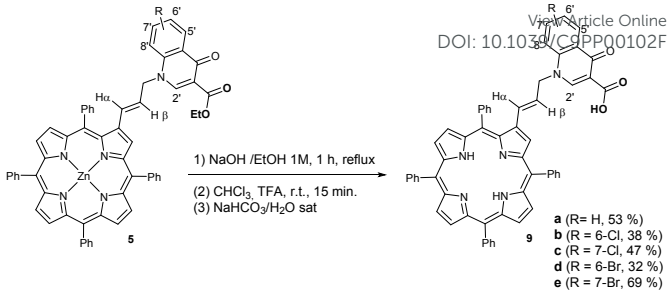
Scheme 3. Heck coupling reaction between β-bromo porphyrin **2** and 1-allyl-4-oxoquinoline derivatives **1a-e**.

Table 2. Results obtained in the Heck coupling reactions between quinoline derivative **1a-e** and β-bromoporphyrin **2**.

Entry	1-allyl-4-oxoquinoline derivative	% 5	% 6	% 7	% 8	% 2 recov.
1	**1a**	67	9	5	13	5
2	**1b**	42	Traces	4	8	1
3	**1c**	24	1	1	10	22
4	**1d**	46	3	2	2	25
5	**1e**	24	8	3	8	37

Also, with these 4-oxoquinoline substrates the formation of the expected conjugates **5a, 5c-e** was accompanied by the formation of the by-products **6**-**8** with total yields varying between 7 and 27%. The yields obtained for the desired porphyrin-quinoline derivatives 5a-e show that the best performance was achieved with the non-substituted oxoquinoline **1a** (67%), followed by the substituted oxoquinolines **1b** and **1d** with the halogen atoms (chloro and bromo) in position 6 that afforded the conjugates **5b** and **5d** in moderate yields (42 and 46% of yield, respectively). The presence of the halogen atom at position **7** is responsible for a decrease in the efficiency of the coupling since both derivatives **5c** and **5e** were isolated in 24% yield. So, these results suggest that the electronic features of the oxoquinoline moiety are affecting the reactivity of these quinolones in the Heck coupling.

Finally, the porphyrinic acid derivatives **9a-e** were obtained in moderate yields (32-69%) after submitting the porphyrin/4-oxoquinoline conjugates **5a-e** to basic hydrolysis and demetallation under acidic conditions (Scheme 4).



Scheme 4. Deprotection and demetallation of porphyrin/4-oxoquinoline conjugates **5a-e**.

The structures of new conjugates **5a-e** and the by-products **6**-**8** were confirmed by using 1D (^{1}H and ^{13}C spectra) and 2D [(^{1}H,^{1}H) COSY, (^{1}H,^{13}C) HSQC and (^{1}H,^{13}C) HMBC] NMR techniques, high-resolution mass spectrometry (HRMS-ESI) and UV-Vis spectroscopy; the structures of the free-bases **9a-e** were confirmed by ^{1}H NMR and confirmed by UV-Vis spectroscopy, ^{1}H NMR and HRMS-ESI (see experimental part).

The HRMS-ESI$^+$ of the porphyrin/4-oxoquinoline conjugates **5a-e** show molecular ions at the *m/z* values 932.2592 for **5a**, 966.2207 for **5b**, 966.2211 for **5c**, 1010.1697 for **5d** and 1010.1708 for **5e** corresponding to their $[M+H]^+$, confirming the success of the Heck coupling reaction of porphyrin **2** with the 1-allyl-4-oxoquinoline derivative **1a-e**.

The ^{1}H NMR spectra of the porphyrin/4-oxoquinoline conjugates **5a-e** show a similar profile, being the principal difference the signals due to the resonances of H-5', H-6'/H-7' and H-8' of the oxoquinoline moiety.

For example, the ^{1}H NMR spectra of substance **5b** (Figure 3) is in accordance with an asymmetric molecule. The resonances of the seven β-pyrrolic protons appear as a singlet at 8.79 ppm (H-3), two multiplets at 8.78-8.75 ppm (3 β-H) and 8.73-8.71 ppm (2 β-H) and a doublet at 8.61 ppm (J = 4.6 Hz, 1 β-H). The *meso*-phenyl protons appeared as four multiplets: at 8.13-8.09 ppm due to the resonance of the *ortho* protons at 5, 10 and 15 positions; at 7.99 -7.98 ppm due to the resonance of the *ortho* protons at 20 position; at 7.70-7.63 ppm due to the resonance of the *meta*-phenyl and *para*-phenyl protons at 5, 10 and 15 positions and the resonance of the *para*-phenyl protons at 20 position and a triplet at 7.54 ppm (J = 7.6 Hz, 2H), due to the resonance of the *meta*-phenyl protons at 20 position.

In what concerns to the signals related to oxoquinoline ring, the multiplet at 6.36-6.34 ppm is related to the resonances of H-α and H-β and the doublet at 4.71 ppm (J = 3.8 Hz) is related to the resonance of the CH_2, which confirms the success of the Heck reaction. The resonance of H-5' appeared as a doublet at 8.42 ppm (J = 2.6 Hz), as confirmed by the correlation with the double doublet at 7.61 ppm (J = 9.1 and 2.6 Hz), assigned to the resonance of H-7'. The correlation observed between the resonance of H-7' and the doublet at 7.47 ppm (J = 9.1 Hz) allowed the assignment of this signal to H-8' proton. Furthermore, the signals related to the ethyl group of the ester appear as a quartet at 4.39 ppm (J = 7.1 Hz)

related to OCH_2 protons, correlated with a triplet at 1.37 ppm (J = 7.1 Hz), related to the CH_3 protons.

Based on the heteronuclear (^{1}H-^{13}C) HSQC and HMBC spectra it was possible to identify the resonances of some carbons. For example, for compound **5b** the signals at 174.2 and 165.6 ppm were related to the carbon of the ketone carbonyl (C-4') and of the ethyl ester group (CO_2Et), respectively; In the aliphatic region of this spectrum, the carbon resonances of the ethyl group were identified at 61.3 ppm (OCH_2CH_3) and 14.5 ppm (OCH_2CH_3). Through these spectra it was also possible to identify the resonances of the carbons C-α and C-β at 132.22 and 121.1 ppm, although it was not possible to reach their specific assignments. The resonances of the methylene carbon (CH_2) appears at 56.3 ppm and the signals at 149.6, 127.1, 126.6 and 118.7 ppm were related to the hydrogenated carbons of the 4-oxoquinoline nucleus (C-2', C-5', C-7 'and C-8', respectively). The signal at 130.8 ppm was unequivocally assigned to the C-3 carbon of the porphyrin macrocycle.

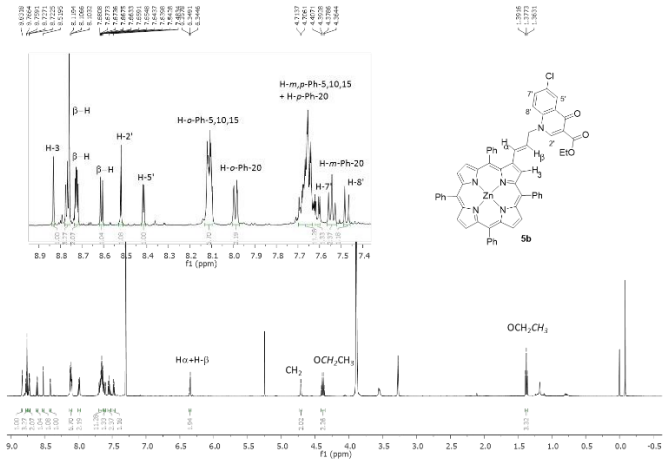


Figure 3. ^{1}H NMR of porphyrin/4-oxoquinoline conjugate **5b**.

In what concerns the deprotected and demetallated derivatives **9a-e**, it was possible to confirm the success of both processes by the appearance of the signal at 14.72 ppm assigned to the proton of the carboxylic group and the signal at -2.72 ppm due to the inner *N*H protons of the porphyrinic macrocycle.

The structures of the by-products were elucidated by adequate spectroscopic techniques, namely ^{1}H and ^{13}C NMR spectroscopy. The resonance of the protons was unequivocally assigned according with the signal multiplicity and the correlations observed in the COSY spectrum. In the NMR spectrum of the by-product **6**, it was possible to identify a singlet at 9.11 ppm related to the resonance of H-3. The doublet at 7.04 ppm (J = 15.4 Hz) and a double doublet at 6.77 ppm (J = 15.4 and 8.0 Hz) are related to H-α and H-β, respectively. The signal related to the resonance of H-β was also unequivocally assigned due to its correlations with the doublet at 9.08 ppm (J = 8.0 Hz), related to the proton of the CHO group. In the ^{13}C NMR spectrum of this by-product it was possible to identify a signal at 194.3 ppm due to the carbonyl carbon of the aldehyde group. According to the HSQC spectra, it was also possible to assign the signals at 150.1 and 129.6 ppm to the carbons C-β and C-α, respectively.

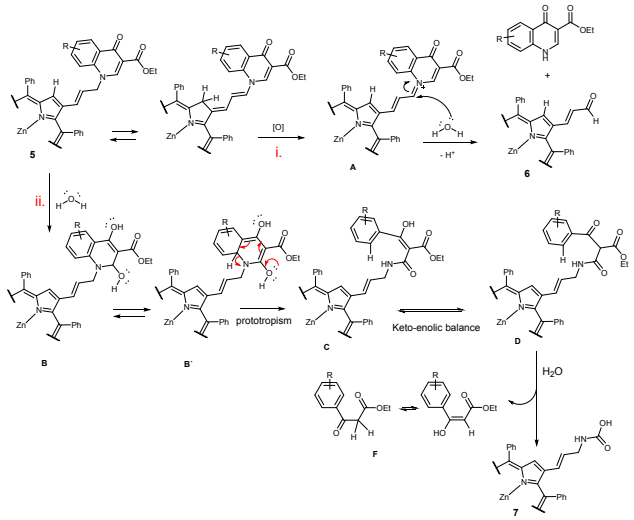
The structure of the by-product **7** was also confirmed by ^{1}H and ^{13}C NMR and two bidimensional studies (COSY and HSQC). From the ^{1}H NMR and COSY studies it was possible to assign in the aromatic region the signals related to the β-pyrrolic protons and the H-*o,m,p*-Ph protons at the *meso*-positions of the porphyrin macrocycle (see experimental part). The most important feature of these spectra is the multiplet at 6.36-6.33 ppm, related to the resonance of H-α and H-β) and the doublet at 4.53 ppm (J = 5.0 Hz) related to the resonance of CH_2. ^{13}C, HSBC and HMQC NMR studies allowed the unequivocally assignment of the signal at 171.1 ppm to the carbonyl carbon of the amide group and the correlation between these carbonyl group with the signal at 65.7 ppm, that was assigned to CH_2. However, it was not possible to distinguish one from the other, carbons C-α and C-β were assigned to signals at 131.2 and 124.4 ppm.

Mechanistic considerations

The mechanism of the formation of the porphyrin/4-oxoquinoline conjugates **5a-e** followed the catalytic cycle described for the Heck coupling reaction.[45, 46] However, in all the reactions the formation of the unexpected aldehyde **6** and of the allylcarbamic acid **7** was observed**.** We believe that the formation of these compounds is related to the thermal decomposition of the porphyrin/4-oxoquinolineconjugates **5a-e** and followed the mechanism proposed in Scheme **5**. In fact, when porphyrin/4-oxoquinolineconjugates **5b** was submitted to same temperature conditions used for the Heck coupling reactions, it was observed the formation of the by-products **6** and **7**.

The formation of derivative **6** can be justified by the oxidative step i of the porphyrin/4-oxoquinolineconjugates **5** leading to intermediate **A**. The hydrolysis of this iminium intermediate can then afford the aldehyde **6** and the non-substituted oxoquinoline **4** (Scheme 5, via i.).

View Article Online
DOI: 10.1039/C9PP00102F



Scheme 5. Proposed mechanism for formation of derivatives **6** and **7**.

In the case of the formation of derivative **7** the Michael type addition of water to the C-2 carbon of the 4-oxoquinoline nucleus of conjugate **5** would lead to intermediate **B** (Scheme 5, via ii.), which by prototropism affords intermediate **B`**. This intermediate after the ring-opening of the hydroxyquinoline ring affords tautomers **C** and **D**. The hydrolysis of these intermediates furnished the by-product **7** accompanied by the elimination of the ketoester **F** (Scheme 5, via ii.).

Singlet oxygen generation studies

The photodynamic activity of a PS is strongly related to its capability to produce reactive oxygen species (ROS), namely the $^1O_2$. The PSs that produce ROS induce processes that are responsible for oxidative reactions at cellular components level, causing function loss, structure disruption and, consequently, cell death.[3-5]

Hence, the potential photodynamic effect of the new porphyrin/4-oxoquinoline conjugates **9a-e** was first analysed by evaluating their ability to generate $^1O_2$. To monitor the generation of this cytotoxic species was used an indirect method based on the photodecomposition of 1,3-diphenylisobenzofuran (DPiBF). In this method, DPiBF, a yellow compound, reacts with the $^1O_2$ produced through a [4+2] cycloaddition process being converted to 1,2-dibenzoylbenzene, which is colourless. Since DPiBF absorbs at 415 nm, it is possible to follow the capability of the photosensitizer to generate $^1O_2$ by measuring the absorption decay at that wavelength.[23,25] Hence, aerated solutions of the porphyrin/4-oxoquinolineconjugates **9a-e** and DPiBF (100-fold molar excess) in DMF/H_2O (9:1) were exposed to filtered white light (cut-off < 550 nm) while monitoring the 415 nm absorption of DPBF.[29, 47, 48]

The results of the photodecomposition of DFiBF during a period of irradiation in the presence of each one of the compounds **9a-e** are shown in Figure 4.

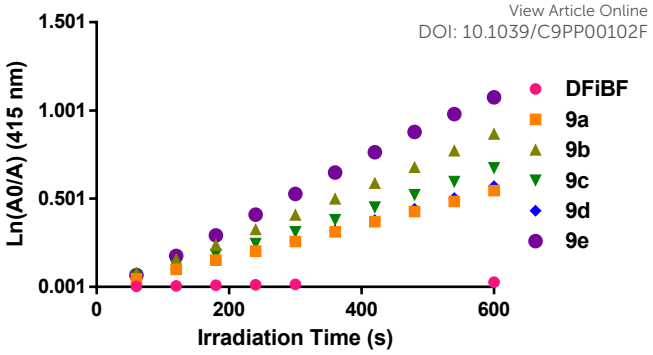


Figure 4. Time-dependent photodecomposition of DPiBF at 50 µM photosensitized by porphyrin/4-oxoquinolone derivatives **9a-e** at 0.5 µM in DMF:H_2O (9:1) upon irradiation with white light filtered through a cut-off filter for wavelengths < 550 nm, with an irradiance of 20.0 mW.cm^{-2}.

It is evident from figure 4 that in all cases a significant photodegradation of DPiBF is observed. This indicates that all porphyrin/4-oxoquinolinederivatives demonstrate a photooxidizing ability. Despite the slight differences, it is possible to highlight that derivative **9e** (R = 7-Br) is the best generator of $^1O_2$, followed by **9b** (R = 6-Cl) and **9c** (R = 7-Cl). Porphyrin/4-oxoquinolones **9d** (R = 6-Br) and **9a** without any substituent at 6- and 7- position of the quinolone nucleus, were the less efficient $^1O_2$ generators. These results suggest that the positive effect in the generation of $^1O_2$ induced by the presence of the halogen atom, in the case of bromine is correlated with its position in 4-oxoquinoline nucleus.

Photodynamic effect of porphyrin/4-oxoquinoline derivatives 9a-e in *S. aureus*

The photodynamic efficiency of the porphyrin/4-oxoquinoline derivatives **9a-e** was evaluated in the presence of the recombinant bioluminescent *S. aureus*. This Gram-positive bacterium is an excellent bacterial model to monitor the efficiency of a photoinactivation process since its light output is a highly sensitive reporter of its metabolic activity.[49] This bioluminescent strain possesses a stable copy of the *Photorhabdus luminescens luxABCDE* operon on the bacterial chromosome.[40] In order to evaluate the effect of each PS on the *S. aureus* photoinactivation efficiency, the porphyrin/4-oxoquinolinederivatives **9a-e** were tested at the PS concentration of 1.0 µM. The photodynamic experiments were carried out under PAR white light (380–700 nm) irradiation at an irradiance of 250 W.m^{-2} for 90 min. The results obtained are summarized in Figure 5.

View Article Online
DOI: 10.1039/C9PP00102F

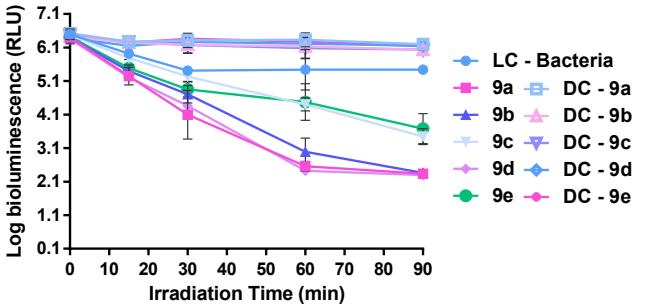


Figure 5. Bioluminescence monitoring of *S. aureus* treated with porphyrin/4-oxoquinolinederivatives **9a-e** at 1.0 µM after 10, 30, 60 and 90 min of irradiation with white light at an irradiance of 250 W.m^{-2}. The values are expressed as the means of three independent experiments; error bars indicate the standard deviation; DC dark control; LC light control.

The results confirmed that the porphyrin/4-oxoquinoline derivatives **9a-e** are able to cause a significant decrease in the bioluminescent signal emitted by *S. aureus* ($p < 0.05$). In fact, porphyrin derivatives **9a**, **9b** and **9d** caused a reduction of the bioluminescence signal of 4.1 log ($p < 0.05$) after 90 min of irradiation, reaching the detection limit of the method. Under the same light conditions, porphyrin derivatives **9c** and **9e** showed to be slightly less effective, inducing reductions of 3.2 and 2.7 log ($p < 0.05$) on bacterial bioluminescence, respectively. The control samples (light and dark controls) did not affect the bioluminescence signal, showing that the viability of *S. aureus* is not affected by irradiation or by the presence of any porphyrin/4-oxoquinolinederivatives **9a-e** tested in the dark.

The results also shown that the halogen atom localization at the 4-oxoquinoline nucleus in derivatives **9b**-**9e** seems to be an important feature for their photodynamic efficacy: the ones with the halogen atom at the 6- position in quinolone nucleus (**9b** and **9d**) were the most efficient in the photoinactivation of *S. aureus*. On the contrary, porphyrin/4-oxoquinolinederivatives **9c** and **9e**, with the halogen atom at the 7- position in quinolone nucleus, were the less efficient ones. It is important to highlight the result obtained for derivative **9a**, since this compound is also one of the best PSs in the inactivation of *S. aureus*. Since this efficacy is not directly related with the ability of these compounds to generate oxygen singlet and also considering their amphiphilicity (miliLoP: **9a**: 9.75; **9b** and **9c**: 9.86; **9d** and **9e**: 9.88)[50] we believe that compounds **9a**, **9b** and **9d** are the ones with the better adequate structural features to generate the toxic singlet oxygen in close proximity to the target cells.

In order to confirm the bioluminescence reduction of *S. aureus* with the reduction of CFU/mL, the previous study was accomplished with the pour plated methodology. Thus, aliquots of the samples and controls were collected at times 0 and 90 min of the aPDT protocol, serially diluted in PBS and pour plated in duplicate in TSA. The results obtained are presented in Figure 6. LC and DC were also evaluated and no significant variation in the *S. aureus* viability was observed, indicating that *S. aureus* was not affected either by irradiation (light control) or by any of the tested compounds (dark control).

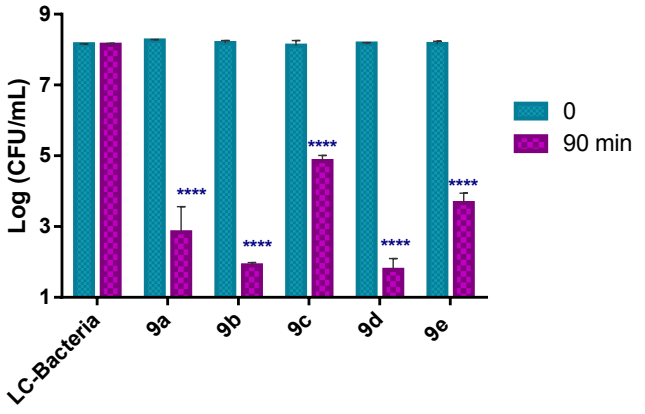


Figure 6. Photodynamic inactivation of *S. aureus* treated with porphyrin/4-oxoquinoline derivatives **9a-e** at 1.0 µM after 90 min of irradiation with white light at an irradiance of 250 W.m^{-2}. The values are expressed as the means of three independent experiments; error bars indicate the standard deviation; DC dark control; LC light control. ****($p < 0.0001$) significantly different from time 0.

The results obtained showed that, as previously mentioned, **porphyrin/4-oxoquinoline derivatives 9a, 9b and 9d were the most efficient PSs, causing a viability bacterial decrease of 5.4, 6.3 and 6.4 log (**$p<0.0001$**) of *S. aureus,* respectively, after 90 min of irradiation (135 J.cm^{-2}). Also, in this case, conjugates 9c and 9e promoted the lowest decrease in photoinactivation of *S. aureus*, 3.2 and 4.7 log (**$p < 0.0001$**), respectively. These results also confirm that the irradiation (light control) or any of the tested compounds (dark control) does not affect the viability of the microorganisms. These results prompt us to consider porphyrin/4-oxoquinolinederivatives 9a-e at 1.0 µM to be effective PS, since, according to the American Society for Microbiology, a compound can be designated antimicrobial if achieve a reduction of at least 3 log10 CFU.**[51]

As already mentioned, our research group recently reported the synthesis and the photodynamic effect of porphyrin/4-quinolone conjugates with *N*-ethyl, *N*-pentyl and *N*-ribofuransyl substituents.[32] These derivatives were tested also against *S. aureus* bacteria and it was found that using a 10 µM concentration of each PS and an irradiance of 15.0 mW cm^{-2} (135-162 J.cm^{-2}), the viability of the bacteria was reduced about 4.9, 3.4 and 2.1 log CFU, for *N*-ethyl, *N*-pentyl and *N*-ribofuransyl-substituted derivatives, respectively.[32] The results now achieved with these new porphyrin/4-oxoquinoline derivatives **9a-e** are very encouraging, since with a 10-fold lower concentration it was achieved better inactivation rates against that Gram-positive bacterium.

Conclusions

In this work new porphyrin/4-oxoquinoline conjugates **5a-e** were obtained by Heck reaction between β-bromoporphyrin **2** and 1-allyl-4-oxoquinolines **1a-e**. The best conditions for these reactions were established using $Pd(OAc)_2$ as catalyst, PPh_3 as ligand and KOAc as base. The use of MW radiation allowed the reduction of the reaction time to 15 minutes without changes in yields. In these reactions the aldehyde porphyrin derivative **6**, porphyrin allylcarbamic acid **7** and the fused porphyrin **8** were also obtained and their mechanisms of formation were assigned. The ester group of the 4-oxoquinolines conjugates **5a-e** were deprotected trough alkaline hydrolysis and demetallated affording porphyrin/4-oxoquinoline acids **9a-e** in good yields. The new conjugates were fully characterized, and the evaluation of singlet oxygen production showed that these compounds are capable to generate that cytotoxic species. The photodynamic effect of these derivatives in the photoinactivation of bioluminescent *S. aureus*, was evaluated and the results demonstrate that all compounds are capable to inactivate *S. aureus*, in particular, porphyrin/4-oxoquinoline derivatives **9a**, **9b** and **9d** promoted the higher photoinactivation rates (up to 5 log).

In summary, the methodology herein described allows the synthesis of promising porphyrin/4-oxoquinoline conjugates that have potential applications or could be prototypes for future photosensitizers in the aPDT of *S. aureus*.

Author contributions

Fernanda Sagrillo performed the synthesis and the structural characterization of porphyrin/4-oxoquinoline derivatives and contributed to the manuscript preparation. Cristina Dias performed the photodynamic evaluation assays of all derivatives, the analysis of biological results and contributed to the manuscript preparation. Ana Gomes performed the design of the synthesis protocol of porphyrin/4-oxoquinoline derivatives, the structural and photo-physical characterization of porphyrin/4-oxoquinoline analysis, interpretation of the biological results and contributed to the manuscript preparation. Amparo Faustino performed the design of the photodynamic evaluation of porphyrin/4-oxoquinoline derivatives experiments and manuscript preparation. Alan Souza and Amanda Costa performed the synthesis of the 4-oxoquinoline precursors. Fernanda Boechat and Maria Cecília Souza were responsible for the supervision of the synthesis of the 4-oxoquinoline precursors and the design of the synthesis protocol of porphyrin/4-oxoquinoline derivatives. Maria Neves and José Cavaleiro were responsible for the supervision of the synthesis of the conjugates, contributed to the analysis and interpretation of the results and in the manuscript preparation. Adelaide Almeida was responsible for the supervision and the design of the antimicrobial photodynamic experiments, contributed in the analysis and interpretation of the biological results and in the manuscript preparation.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Thanks are due to the University of Aveiro, to FCT/MEC for the financial support to QOPNA Research Unit (FCT UID/QUI/00062/2019) and Centre for Environmental and Marine Studies (CESAM) Unit (UID/AMB/50017/2019) and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020, and also to the Portuguese NMR Network. Thanks are also due to Universidade Federal Fluminence, FAPERJ, CNPQ, CAPES (finance Code 001), and UFF for financial support and fellowships. Fernanda Savacini Sagrillo thanks CAPES for granting the scholarship for a sandwich doctorate (PDSE) at the University of Aveiro. C. J. Dias also thanks FCT for her research fellow (BI/UI51/7965/2017).

References

1. J. R. C. Moellering, Vancomycin: A 50-Year Reassessment, *Clin. Infect. Dis.,* 2006, **42**, S3-S4.
2. R. O. A. Resistance, Tackling Drug-resistant Infections Globally: Final Report and Recommendations, 2016.
3. E. Alves, M. A. F. Faustino, M. G. P. M. S. Neves, Â. Cunha, H. Nadais, A. Almeida, Potential applications of porphyrins in photodynamic inactivation beyond the medical scope, *J. Photochem. Photobiol., C,* 2015, **22**, 34-57.
4. M. R. Hamblin, Upconversion in photodynamic therapy: plumbing the depths, *Dalton Trans.,* 2018, **47**, 8571-8580.
5. G. Jori, M. Camerin, M. Soncin, L. Guidolin, O. Coppellotti, Antimicrobial Photodynamic Therapy: Basic Principles. *In Photodynamic Inactivation of Microbial Pathogens: Medical and Environmental Applications,* The Royal Society of Chemistry, 2011, Chap. 1, Vol. 11, pp 1-18.
6. A. Tavares, S. R. S. Dias, C. M. B. Carvalho, M. A. F. Faustino, J. P. C. Tomé, M. G. P. M. S. Neves, A. C. Tomé, J. A. S. Cavaleiro, Â. Cunha, N. C. M. Gomes, E. Alves, A. Almeida, Mechanisms of photodynamic inactivation of a Gram-negative recombinant bioluminescent bacterium by cationic porphyrins, *Photochem. Photobiol. Sci.,* 2011**, 10**, 1659-1669.
7. A. Tavares, C. M. B. Carvalho, M. A. Faustino, M. G. P. M. S. Neves, J. P. C. Tomé, A. C. Tomé, J. A. S. Cavaleiro, A. Cunha, N. C. M. Gomes, E. Alves, A. Almeida, Antimicrobial photodynamic therapy: study of bacterial recovery viability and potential development of resistance after treatment. *Marine drugs* 2010**, 8**, 91-105.
8. F. Giuliani, M. Martinelli, A. Cocchi, D. Arbia, L. Fantetti, G. Roncucci, *In Vitro* Resistance Selection Studies of RLP068/Cl, a New Zn(II) Phthalocyanine Suitable for Antimicrobial Photodynamic Therapy, *Antimicrob. Agents Chemother.,* 2010**, 54**, 637-642.
9. L. Costa, J. P. C. Tomé, M. G. P. M. S. Neves, A. C. Tomé, J. A. S. Cavaleiro, M. A. F. Faustino, Â. Cunha, N. C. M. Gomes, A. Almeida, Evaluation of resistance development and viability recovery by a non-enveloped virus after repeated cycles of aPDT, *Antiviral Res.,* 2011**, 91**, 278-282.
10. A. Minnock, D. I. Vernon, J. Schofield, J. Griffiths, J. H. Parish, S. B. Brown, Mechanism of Uptake of a Cationic Water-Soluble

Pyridinium Zinc Phthalocyanine across the Outer Membrane of *Escherichia coli*, *Antimicrob. Agents Chemother.,* 2000**, 44**, 522-527.

11. E. Alves, C. M. B. Carvalho, J. P. C. Tomé, M. A. F. Faustino, M. G. P. M. S. Neves, A. C. Tomé, J. A. S. Cavaleiro, Â. Cunha, S. Mendo, A. Almeida, Photodynamic inactivation of recombinant bioluminescent Escherichia coli by cationic porphyrins under artificial and solar irradiation, *J. Ind. Microbiol. Biotechnol.,* 2008, **35**, 1447.

12. D. Vecchio, T. Dai, L. Huang, L. Fantetti, G. Roncucci, M. R. Hamblin, Antimicrobial photodynamic therapy with RLP068 kills methicillin-resistant *Staphylococcus aureus* and improves wound healing in a mouse model of infected skin abrasion., *J. Biophotonics,* 2013, **6**, 733-742.

13. K. P. Francis, D. Joh, C. Bellinger-Kawahara, M. J. Hawkinson, T. F. Purchio, P. R. Contag, Monitoring Bioluminescent *Staphylococcus aureus* Infections in Living Mice Using a Novel luxABCDEConstruct. *Infection and Immunity* 2000**, 68**, 3594-3600.

14. S. Beirão, S. Fernandes, J. Coelho, M. A. F. Faustino, J. P. C. Tomé, M. G. P. M. S. Neves, A. C. Tomé, A. Almeida, A. Cunha, Photodynamic Inactivation of Bacterial and Yeast Biofilms With a Cationic Porphyrin, *Photochem. Photobiol.,* 2014**, 90**, 1387-1396.

15. F. Freire, C. Ferraresi, A. O. C. Jorge, M. R. Hamblin, Photodynamic therapy of oral Candida infection in a mouse model. *J. Photochem. Photobiol., B,* 2016, **159**, 161-168.

16. P. G. Calzavara-Pinton, M. T. Rossi, B. Ortel, Photodynamic Antifungal Chemotherapy. In Photodynamic Inactivation of Microbial Pathogens: Medical and Environmental Applications, The Royal Society of Chemistry, 2011, Vol. 11, Chap. 14, pp 361-375.

17. A. Almeida, A. Cunha, M. A. F. Faustino, A. C. Tomé, M. G. P. M. S. Neves, Porphyrins as Antimicrobial Photosensitizing Agents. In Photodynamic Inactivation of Microbial Pathogens: Medical and Environmental Applications, The Royal Society of Chemistry, 2011, Chap. 5, Vol. 11, pp 83-160.

18. J. P. C. Tome, E. M. P. Silva, A. Pereira, C. M. A. Alonso, M. A. F. Faustino, M. G. P. M. S. Neves, A. C. Tome, J. A. S. Cavaleiro, S. A. P. Tavares, R. R. Duarte, M. F. Caeiro, M. L. Valdeira, Synthesis of neutral and cationic tripyridylporphyrin-D-galactose conjugates and the photoinactivation of HSV-1. *Bioorganic Med. Chem.,* 2007, **15**, 4705-4713.

19. J. P. C. Tome, M. G. P. M. S. Neves, A. C. Tome, J. A. S. Cavaleiro, A. F. Mendonca, L. S. N. Pegado, R. Duarte, M. L. Valdeira, Synthesis of glycoporphyrin derivatives and their antiviral activity against herpes simplex virus types 1 and 2. *Bioorganic Med. Chem.,* 2005, **13**, 3878-3888.

20. E. M. P. Silva, F. Giuntini, M. A. F. Faustino, J. P. C. Tome, M. G. P. M. S. Neves, A. C. Tome, A. M. S. Silva, M. G. Santana-Marques, A. J. Ferrer-Correia, J. A. S. Cavaleiro, M. F. Caeiro, R. R. Duarte, S. A. P. Tavares, I. N. Pegado, B. d'Almeida, A. P. A. De Matos, M. L. Valdeira, Synthesis of cationic beta-vinyl substituted meso-tetraphenylporphyrins and their in vitro activity against herpes simplex virus type 1. *Bioorg. Med. Chem. Lett.,* 2005, **15**, 3333-3337.

21. J.-P. Rasigade, F. Vandenesch, *Staphylococcus aureus*: A pathogen with still unresolved issues. *Infect. Genet. Evol.,* 2014, **21**, 510-514.

22. R. L. Skov, K. S. Jensen, Community-associated meticillin-resistant *Staphylococcus aureus* as a cause of hospital-acquired infections. *J. Hosp. Infect.,* 2009, **73**, 364-370.

23. R. Klevens, M. A. Morrison, J. Nadle, Invasive methicillin-resistant *staphylococcus aureus* infections in the united states. *JAMA* 2007, **298**, 1763-1771.

24. J. J. Mendes, A. Marques-Costa, C. Vilela, J. Neves, N. Candeias, P. Cavaco-Silva, J. Melo-Cristino, Clinical and bacteriological survey of diabetic foot infections in Lisbon. *Diabetes Res. Clin. Pract.,* 2012, **95**, 153-161.

25. J. Pratten, V. Benhamou, C. Street, Antimicrobial Photodynamic Therapy (aPDT) for Oral Infections. In Photodynamic Inactivation of Microbial Pathogens: Medical and Environmental Applications, The Royal Society of Chemistry, 2011, Chap. 13, Vol. 11, pp 327-360.

26. A. A. Aksenova, Y. L. Sebyakin, A. F. Mironov, Conjugates of Porphyrins with Carbohydrates. *Russ. J. Bioorgan. Chem.* 2003, **29**, 201-219.

27. J. A. S. Cavaleiro, J. P. C. Tomé, M. A. F. Faustino, Synthesis of Glycoporphyrins. In Heterocycles from Carbohydrate Precursors, El Ashry, E. S. H., Ed. Springer Berlin Heidelberg: Berlin, Heidelberg, 2007, pp 179-248.

28. A. T. P. C. Gomes, A. C. Cunha, M. d. R. M. Domingues, M. G. P. M. S. Neves, A. C. Tomé, A. M. S. Silva, F. d. C. Santos, M. C. B. V. Souza, V. F. Ferreira, J. A. S. Cavaleiro, Synthesis and characterization of new porphyrin/4-quinolone conjugates. *Tetrahedron,* 2011, **67**, 7336-7342.

29. A. T. P. C. Gomes, R. A. C. Leão, F. C. d. Silva, M. G. P. M. S. Neves, M. A. F. Faustino, A. C. Tomé, A. M. S. Silva, S. Pinheiro, M. C. B. V. d. Souza, V. F. Ferreira, J. A. S. Cavaleiro, Synthesis of new glycoporphyrin derivatives through carbohydrate-substituted α-diazoacetates, *J. Porphyr. Phtholocyanine* 2009, **13**, 247-255.

30. M. M. Bastos, A. T. P. C. Gomes, M. G. P. M. S. Neves, A. M. S. Silva, O. A. Santos-Filho, N. Boechat, J. A. S. Cavaleiro, Synthesis of β-Substituted Porphyrin Derivatives Containing Heterocyclic Moieties as Potential Photosensitizers Against Cutaneous Leishmaniasis. *Eur. J. Org. Chem.,* 2013, 1485-1493.

31. A. T. P. C. Gomes, M. A. F. Faustino, M. G. P. M. S. Neves, V. F. Ferreira, A. Juarranz, J. A. S. Cavaleiro, F. Sanz-Rodríguez, Photodynamic effect of glycochlorin conjugates in human cancer epithelial cells. *RSC Adv.,* 2015, ***5***, 33496-33502.

32. P. N. Batalha, A. T. P. C. Gomes, L. S. M. Forezi, L. Costa, M. C. B. V. de Souza, F. d. C. S. Boechat, V. F. Ferreira, A. Almeida, M. A. F. Faustino, M. G. P. M. S. Neves, J. A. S. Cavaleiro, Synthesis of new porphyrin/4-quinolone conjugates and evaluation of their efficiency in the photoinactivation of *Staphylococcus aureus*. *RSC Adv.,* 2015, **5**, 71228-71239.

33. L. R. Peterson, Quinolone Molecular Structure-Activity Relationships: What We Have Learned about Improving Antimicrobial Activity. *Clin. Infect. Dis.,* 2001, **33**, S180-S186.

34. L. A. Mitscher, Bacterial Topoisomerase Inhibitors: Quinolone and Pyridone Antibacterial Agents, *Chem. Rev.* 2005, **105**, 559-592.

35. V. R. Campos, A. T. P. C. Gomes, A. C. Cunha, M. G. P. M. S. Neves, V. F. Ferreira, J. A. S. Cavaleiro, Efficient access to β-vinylporphyrin derivatives via palladium cross coupling of β-bromoporphyrins with N-tosylhydrazones. *Beilstein J. Org. Chem.,* 2017, **13**, 195-202.

36. L. d. S. M. Forezi, A. T. P. C. Gomes, M. G. P. M. S. Neves, V. F. Ferreira, F. d. C. S. Boechat, M. C. B. V. de Souza, J. A. S. Cavaleiro, Synthesis of β-Substituted meso-Tetraaryl-21,23-dithiaporphyrins by Heck Reaction, *Eur. J. Org. Chem.,* 2015, 5909-5913.

37. K. B. Fields, J. V. Ruppel, N. L. Snyder, X. P. Zhang, Porphyrin Functionalization *via* Palladium-Catalyzed Carbon–Heteroatom Cross-Coupling Reactions. In *Handbook of Porphyrin Science*, 2010, pp 367-427.

38. J. A. S. Cavaleiro, A. C. Tomé, M. G. P. M. S. Neves, Tetraarylporphyrin Derivatives: New Synthetic Methodologies. In *Handbook of Porphyrin Science*, 2010, pp 193-294.

View Article Online
DOI: 10.1039/C9PP00102F

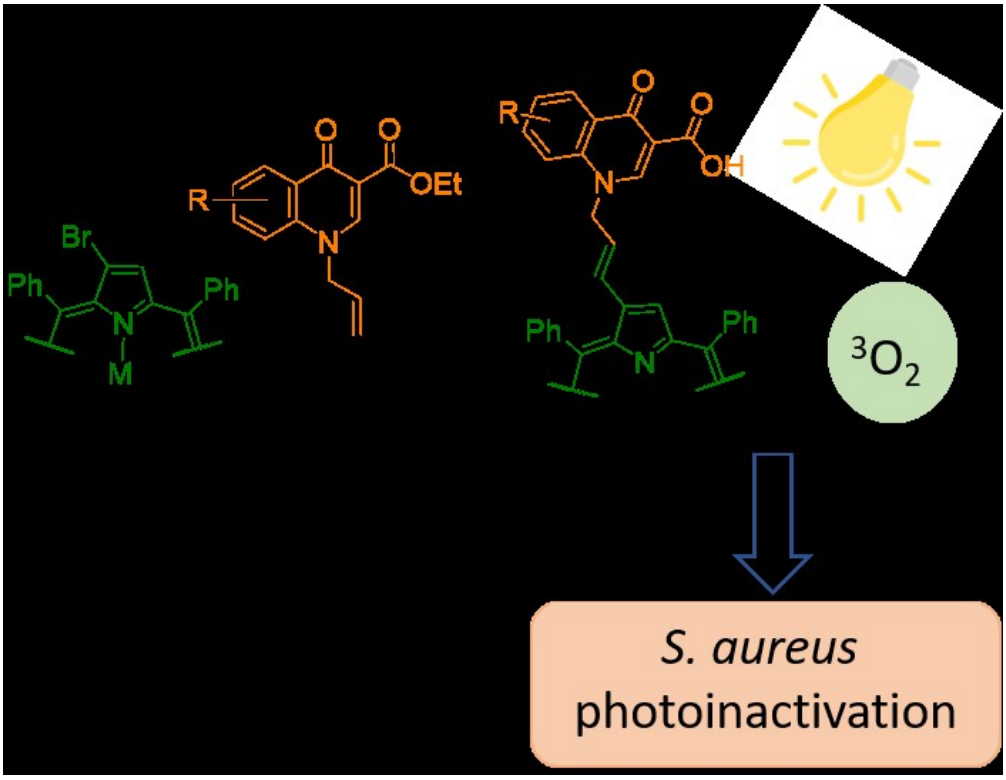
39. H. J. Callot, Bromuration de la meso-tetraphenylporphine.: Structure et reactivite des produits. *Tetrahedron Lett.,* 1973, **14**, 4987-4990.
40. M. Grinholc, J. Nakonieczna, G. Fila, A. Taraszkiewicz, A. Kawiak, G. Szewczyk, T. Sarna, L. Lilge, K. P. Bielawski, Antimicrobial photodynamic therapy with fulleropyrrolidine: photoinactivation mechanism of *Staphylococcus aureus*, *in vitro* and *in vivo* studies. *Appl. Microbiol. Biotechnol.,* 2015, **99**, 4031-4043.
41. R. G. Gould, W. A. Jacobs, The Synthesis of Certain Substituted Quinolines and 5,6-Benzoquinolines. *J. Am. Chem. Soc.,* 1939, **61**, 2890-2895.
42. B. Riegel, G. R. Lappin, B. H. Adelson, R. I. Jackson, C. J. Albisetti, R. M. Dodson, R. H. Baker, The Synthesis of Some 4-Quinolinols and 4-Chloroquinolines by the Ethoxymethylenemalonic Ester Method1. *J. Am. Chem. Soc.,* 1946, **68**, 1264-1266.
43. J. M. Ruxer, C. Lachoux, J. B. Ousset, J. L. Torregrosa, G. Mattioda, Synthesis of 1,4-dihydro-4-oxopyrrolo[1,2-b]pyridazine-3-carboxylic acids and 1,4-dihydro-4-oxoimidazo[1,5-b]pyridazine-3-carboxylic acids as potential antibacterial agents. *J. Heterocycl. Chem.,* 1994, **31**, 409-417.
44. T. Ishizuka, Y. Saegusa, Y. Shiota, K. Ohtake, K. Yoshizawa, T. Kojima, Multiply-fused porphyrins—effects of extended π-conjugation on the optical and electrochemical properties. *Chem. Commun.,* 2013, **49**, 5939-5941.
45. M. Tsutomu, M. Kunio, O. Atsumu, Arylation of Olefin with Aryl Iodide Catalyzed by Palladium. *B. Chem. Soc. Jpn.,* 1971, **44**, 581-581.
46. R. F. Heck, J. P. Nolley, Palladium-catalyzed vinylic hydrogen substitution reactions with aryl, benzyl, and styryl halides, *J. Org. Chem.,* 1972, **37**, 2320-2322.
47. P. Zimcik, M. Miletin, H. Radilova, V. Novakova, K. Kopecky, J. Svec, E. Rudolf, Synthesis, Properties and In Vitro Photodynamic Activity of Water-soluble Azaphthalocyanines and Azanaphthalocyanines, *Photochem. Photobiol.,* 2010, **86**, 168-175.
48. M. B. Spesia, M. E. Milanesio, E. N. Durantini, Synthesis, properties and photodynamic inactivation of *Escherichia coli* by novel cationic fullerene C_{60} derivatives, *Eur. J. Med. Chem.,* 2008, **43**, 853-861.
49. E. Alves, L. Costa, Â. Cunha, M. A. F. Faustino, M. G. P. M. S. Neves, A. Almeida, Bioluminescence and its application in the monitoring of antimicrobial photodynamic therapy, *Appl. Microbiol. Biotechnol.,* 2011, **92**, 1115-1128.
50. The parameters for drug-likeness were evaluated according to the Lipinski's 'rule-of-five', using the MolinspirationWebME Editor 3.81., *http://www.molinspiration.com* (accessed February 2019).
51. G. A. Pankey, L. D. Sabath, Clinical Relevance of Bacteriostatic versus Bactericidal Mechanisms of Action in the Treatment of Gram-Positive Bacterial Infections, *Clin. Infect. Dis.,* 2004, **38**, 864-870.

Contents List

View Article Online
DOI: 10.1039/C9PP00102F

New porphyrin/4-oxoquinoline conjugates were synthesized and revealed to be excellent photosensitizer agents in the inactivation of S. aureus after the antimicrobial photodynamic therapy protocol.

View Article Online
DOI: 10.1039/C9PP00102F



Graphical Abstract

118x91mm (150 x 150 DPI)