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Synthesis and characterization of new porphyrin/4-quinolone conjugates

Ana T.P.C. Gomes [a], Anna C. Cunha [b], Maria do Rosário M. Domingues [a], Maria G.P.M.S. Neves [a,*], Augusto C. Tomé [a], Artur M.S. Silva [a], Fernanda da C. Santos [b], Maria C.B.V. Souza [b], Vitor F. Ferreira [b], José A.S. Cavaleiro [a,*]

[a] University of Aveiro, Department of Chemistry and QOPNA, 3810-193 Aveiro, Portugal
[b] Universidade Federal Fluminense, Departamento de Química Orgânica, 24020-141 Niterói, RJ, Brazil

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ABSTRACT

New porphyrin/4-quinolone conjugates were synthesized from the Suzuki–Miyaura coupling reaction of a β-borylated porphyrin with bromo-4-quinolones containing *N*-ethyl and *N*-D-ribofuranosyl substituents. The use of electrospray ionization tandem mass spectrometry showed important information about the fragmentation pathways of the new compounds. It was possible to distinguish between those compounds with the porphyrin moiety linked at the 6-position of the quinolone unit from their 7-substituted isomers. The new compounds showed to be good singlet oxygen generators.

1. Introduction

Since a few decades ago several research groups have been concerned with the establishment of new synthetic methodologies leading to new porphyrin macrocycles.[1] When envisaging synthetic and reactivity studies to be carried out, chemists might have in mind potential applications for the new products, mainly in the biomedical area. Porphyrin derivatives have demonstrated significant properties by acting as photosensitizers against cancer cells and in the treatment of the age-related macular degeneration. Although a few formulations are approved, it is known that new and better derivatives should become available by synthesis. Structural requirements of a new potential photosensitizer have to be considered in a synthetic plan. It is also required for a new synthesized derivative that it should have adequate photophysical properties to be used in photodynamic therapy of cancer cells.[2,3] This therapy combines the use of a photosensitizing drug, oxygen and visible light to produce lethal cytotoxic agents like singlet oxygen ($^1O_2$) and/or other reactive oxygen species, which are responsible for the destruction of the malignant tissues.[4–7] In such way a potential photosensitizer must give rise in high yield to such reactive oxygen species.

It is well-known that coupling molecules with well-established pharmacological activities might be a good strategy to develop new significant products. In this context, porphyrins linked to other biologically active molecules, like, e.g., quinolones, can give rise to new derivatives with potential biological applications. Quinolone derivatives can themselves act as antimicrobial[8,9] and antitumour agents.[10,11] Establishment of synthetic methodologies leading to porphyrin/quinolone conjugates can then be considered an important synthetic target. Our group has already reported two different procedures for the functionalization of porphyrins with that kind of molecules.[12,13]

This publication reports a new and efficient approach for the synthesis of novel porphyrin/quinolone conjugates through a Suzuki–Miyaura coupling reaction involving a β-borylated porphyrin and four bromo-4-quinolones. The structural characterization of the new derivatives has included the use of electrospray ionization tandem mass spectrometry (ESI-MS/MS). This is a technique, which can give significant structural information about porphyrinic compounds;[14–17] having in mind the possibility of biological significance for the new derivatives[18] such technique also allows the monitoring of a drug-related material being used.

* Corresponding authors. Tel.: +351 234 370 717; fax: +351 234 370 084; e-mail addresses: gneves@ua.pt (M.G.P.M.S. Neves), jcavaleiro@ua.pt (J.A.S. Cavaleiro).

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The rate of the singlet oxygen ($^1O_2$) production by the new deprotected and demetallated porphyrin/quinolone conjugates was also evaluated.

2. Results and discussion

2.1. Synthesis of β-substituted porphyrin/quinolone derivatives

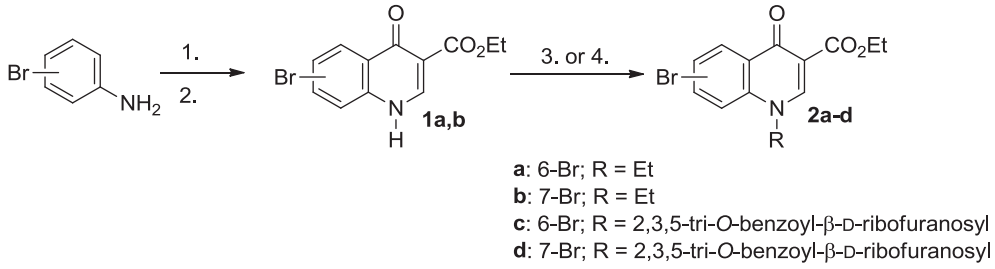
The required bromo-4-quinolones **2a**–**d** were conveniently prepared according to the synthetic strategy shown in Scheme 1. The reaction of *meta*- and *para*-bromoanilines with diethyl ethoxymethylenemalonate afforded, after cyclization, the corresponding derivatives **1a**,**b**.[19] Then, treatment of these intermediates with ethyl bromide in the presence of K_2CO_3 in DMF provided the *N*-protected ethyl 1,4-dihydro-1-ethyl-4-oxoquinoline-3-carboxylates **2a** and **2b**.[20] The synthesis of the protected ribonucleosides ethyl 1,4-dihydro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-4-oxoquinoline-3-carboxylates **2c** and **2d** required the previous silylation of the appropriate bromoquinolones **1a**,**b** with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylchorosilane (TMCS), followed by the condensation with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in acetonitrile using trimethylsilyltrifluoromethanesulfonate (TMSOTf) as catalyst.[19]

The β-bromoporphyrin required for the synthesis of **3** was prepared in good yield by reaction of *meso*-tetraphenylporphyrin (TPP) with *N*-bromosuccinimide in chloroform.[22]

The Suzuki–Miyaura coupling reaction of the β-borylated porphyrin **3** with bromo-4-quinolones **2a**–**d** was carried out in a mixture of toluene/DMF using $Pd(PPh_3)_4$ as the catalyst and Cs_2CO_3 as the base. The reactions were carried out in Schlenk tubes at 80 °C for 16 h. The workup involved the treatment of the reaction mixture with an aqueous solution of NaCl, extraction with dichloromethane, evaporation of the solvent and purification of the crude residue by column chromatography. The desired products **4a**–**d** were obtained in moderate to excellent (50–89%) yields.

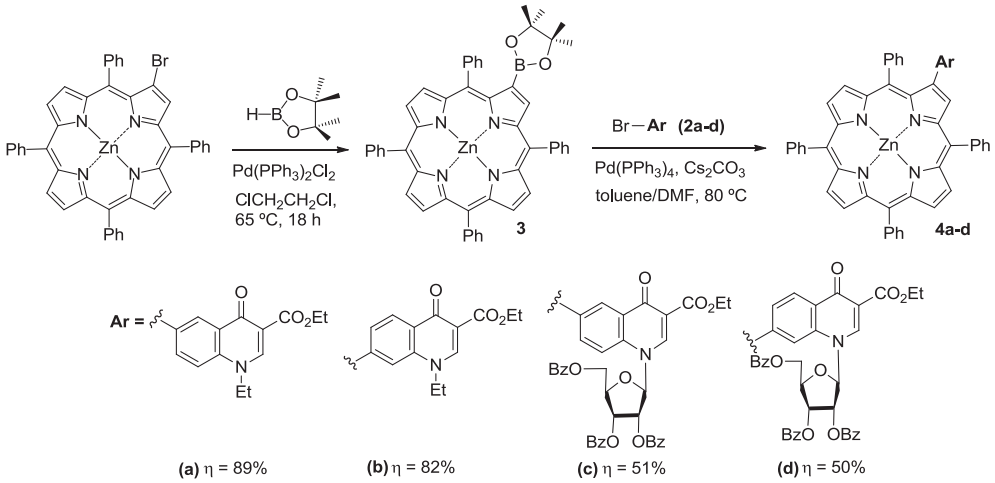
Quinolone derivatives **2a** and **2b** showed to be more reactive than the ribonucleosides **2c** and **2d** providing the corresponding porphyrin/quinolone conjugates **4a** and **4b** in 89% and 82% yields, respectively. The conjugates **4c** and **4d** were isolated in lower yields (~50%) but nearly 50% of the starting porphyrin **3** was recovered in both cases. Attempts to improve the outcome of the coupling process between **3** and **2c** or **2d** were not successful (e.g., by increasing the reaction times and by increasing the number of equivalents of the bromoquinolones); this fact might be due to steric effects caused by the ribofuranosyl group.

Basic hydrolysis of the ester groups of conjugates **4a**–**d** followed by the demetallation of the porphyrin nucleus under acidic conditions afforded efficiently conjugates **5a**–**d** (Scheme 3). The hydrolysis of the ester group in **4a** and **4b** was performed by heating each compound with a methanolic solution of potassium hydroxide at 80 °C for 1 h. Using similar reaction conditions, but extending the

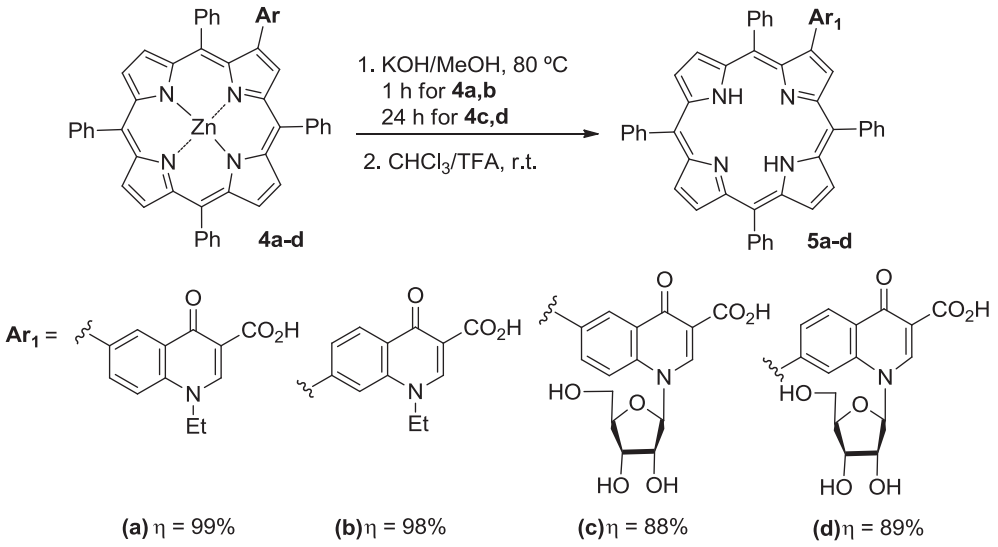


Scheme 1. Synthesis of the bromo-4-quinolones **2a**–**d**.

The synthesis of the novel porphyrin/quinolone conjugates **4a**–**d** is outlined in Scheme 2. The key β-borylated porphyrin **3** was obtained by reaction of 2-bromo-5,10,15,20-tetraphenylporphyrinatozinc(II) with pinacol borane in the presence of $Pd(PPh_3)_2Cl_2$.[21]



Scheme 2. Synthesis of porphyrin **3** and its transformation into products **4a**–**d**.



Scheme 3. Deprotection and demetallation of porphyrin/quinolone conjugates **4a**–**d**.

reaction time to 24 h, allowed the simultaneous hydrolysis of the ethyl ester groups and the removal of the protecting benzoyl groups of the ribose moieties in the porphyrin/quinolones **4c** and **4d**. In all cases, the reaction mixtures were neutralized with an aqueous solution of citric acid and extracted with dichloromethane. After the usual workup, the residues were taken into chloroform and trifluoroacetic acid (10:1) and, after stirring for 2 min at room temperature, the resulting green reaction mixtures were neutralized with sodium carbonate. After the workup, pure porphyrin/quinolone conjugates **5a** and **5b** were obtained in 99% and 98% yields, respectively, directly by crystallization while compounds **5c** and **5d** (obtained in 88% and 89% yields, respectively) required purification by preparative TLC using a mixture of $CHCl_3$/MeOH (1%) as the eluent.

2.2. Structural characterization

The structures of the new products **4a**–**d** and **5a**–**d** were assigned on the basis of their ^1H and ^{13}C NMR spectra and their molecular formulae were confirmed by HRMS. 2D NMR spectra (COSY, HMBC and HSQC) were also obtained in order to unequivocally identify the protons and carbons resonances.

The HRMS-ESI$^+$ of the isomeric porphyrin/quinole conjugates **4a** and **4b** show molecular ions at the *m*/*z* values 920.2574 and 920.2559 [$(M+H)^+$], confirming the success of the Suzuki–Miyaura coupling of porphyrin **3** with the bromo-4-quinolones **2a** and **2b**. The ^1H NMR spectra of the two isomers show the same type of profile, the principal difference being the signals due to the resonances of H-5′, H-6′/H-7′ and H-8′ of the quinolone moiety.

In the ^1H NMR spectrum of the porphyrin/quinolone conjugate **4a** it is possible to identify the two AB spin systems related to the resonances of four β-pyrrolic protons: one at 8.95 and 8.93 ppm (*J*=4.7 Hz) due to H-7 and H-8 and the other at 8.85 and 8.76 ppm (*J*=4.7 Hz) assigned to H-17 and H-18. The two singlets at 8.93 and 8.91 ppm are due to the resonances of the H-12,13 and H-3, respectively. The protons of the *meso*-phenyl groups appear as two multiplets, one at 8.23–8.21 ppm due to *ortho*-protons and the other at 7.78–7.72 ppm due to the *meta*- and *para*-protons. The protons of the phenyl group nearby the quinolone unit appear as multiplets at 8.02–7.86 ppm (H-*ortho*), 7.49–7.48 ppm (H-*meta*) and at 7.11–7.09 ppm (H-*para*).

The unequivocal assignments of the quinolone proton resonances were based on COSY correlations. Therefore, the doublet at 8.70 ppm (*J*=2.1 Hz) due to H-5′ correlates with the double doublet at 7.55 ppm (*J*=2.1 Hz and *J*=8.6 Hz) and consequently it was attributed to the resonance of H-7′. Also the correlation of H-7′ with the doublet at 7.08 ppm (*J*=8.6 Hz) allowed to assign this signal to H-8′. The singlet at 8.52 ppm was identified as being due to the resonance of H-2′.

A careful analysis of the HMBC spectrum of **4a** allowed the unequivocal assignment of the carbonyl carbon resonances. The resonance of H-2′ correlates with both carbonyl signals at 166.3 and 174.2 ppm. However, the resonance of protons H-5′ only correlates with the signal at 174.2 ppm allowing obviously its assignment to the C-4′ resonance; the other one at 166.3 ppm was assigned to the ester carbonyl group. The correlation observed between the quartet at 4.42 ppm and the signal at 166.3 ppm confirms the assignment of this quartet to the methylene protons resonance of the ester group.

As referred previously, the main difference between **4a** and **4b** is given by the resonances of the quinolone moiety. In the case of **4b**, the COSY analysis shows that the doublet at 7.57 ppm (*J*=8.1 Hz), assigned to the resonance of H-6′, correlates with the signal of H-5′, that appears under the multiplet at 8.24–8.20 ppm, due to the H-*ortho* protons of the 5,10,15-phenyl groups. The resonance of H-8′ appears as a singlet at 7.29 ppm and, as in the case of derivative **4a**, the singlet at 8.48 ppm was assigned to the resonance of H-2′.

In the case of the porphyrin/ribonucleoside conjugates **4c** and **4d**, the *m*/*z* values 1336.3452 and 1336.3468 $[M+H]^+$ observed for both isomers in the HRMS-ESI$^+$ spectra confirmed their molecular formulae. The ^1H NMR spectra of these compounds show a more complex pattern due to presence of the protected ribose unit. However, the resonances due to the H-β, *meso*-phenyl and quinolone protons show similar features to the ones observed for the corresponding porphyrin/quinolone conjugates **4a** and **4b**. The COSY spectra were fundamental for the unequivocal assignment of all sugar protons and of their protecting groups. For instance, for compound **4c** a careful analysis of the COSY correlations allowed to assign the doublet at 6.39 ppm (*J*=5.4 Hz) to the resonance of H-1″, the triplets at 6.07 ppm (*J*=5.4 Hz) and 5.93 ppm (*J*=5.4 Hz) to H-2″ and H-3″, respectively, and the multiplet at 4.94–4.75 ppm to the resonance of H-4″ and H-5″. Considering the proton resonances of the benzoyl groups, the protons H-2‴ appear as two double doublets at 8.11 and 7.97 ppm (*J*=1.4 and 7.8 Hz) and a multiplet at 7.15–6.90 ppm, while one H-4‴ appears as a triple triplet at 7.56 ppm (*J*=1.4 and 6.1 Hz); the others H-4‴ and H-3‴ appear as

a multiplet at 7.43–7.34 ppm together with the resonances of H-7′ and H-8′ of the quinolone moiety. The correlation of these signals with the triple triplet at 7.56 ppm (J=1.2, 1.4 and 6.1 Hz) and with the multiplet at 7.43–7.34 ppm allowed to assign these signals to the resonances of, respectively, two and four H-3‴. Finally the signals of the three H-4″ appear also in the multiplet at 7.43–7.34 ppm together with the resonance of H-7′ and H-8′ of the quinolone moiety.

Based on the heteronuclear (^{1}H–^{13}C) HSQC and HMBC spectra it was possible to identify the resonances of the carbonyl and aliphatic carbons. For example, for compound **4c** the carbonyl carbons were identified as the signals at 174.3 (C-4′), 166.0 (C=O of the ethyl ester group) and 165.1, 165.0 and 164.6 ppm (carbonyl carbon of the benzoyl groups). In the aliphatic region of this spectrum the carbon resonances of C-1″ (91.2 ppm); C-2″ (74.3 ppm), C-3″ (70.3 ppm), C-4″ and C-5″ (80.3 and 63.0 ppm) as well the carbon resonances of the ethyl group (60.4 and 14.3 ppm) can be found. It is important to note that HMBC correlations allow us to confirm the structure of the compound **4c**, showing correlations between all the subunits of this molecule (Fig. 1). The resonance of H-1″ of the ribose moiety correlates with C-2′ of the quinolone unit at 131.9 ppm and that due to H-2″ correlates with the carbonyl group of the benzoyl group at 165.1 ppm. The resonance of H-5′ of the quinolone unit correlates with the C-2 of the porphyrin moiety at 135.5 ppm.

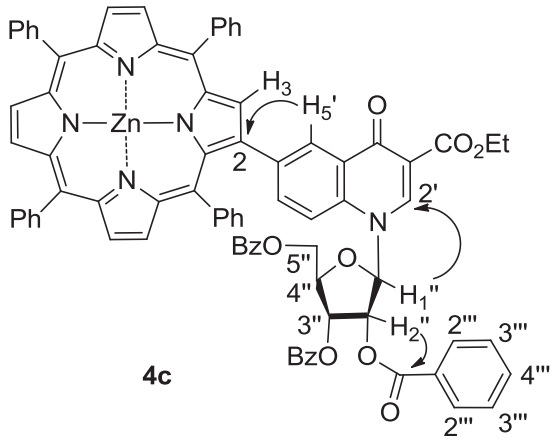


Fig. 1. Main HMBC correlations of compound **4c**.

For the conjugate **4d** the chemical shifts of the protons and carbons of the ribonucleoside unit are similar to the ones described for **4c**. Presumably due to an aggregation effect, the corresponding spectrum does not show the same resolution as it was observed for compound **4c**, even when other deuterated solvents (DMF, THF, DMSO, TFA) and also different temperatures were tested.

The most important features of the ^{1}H NMR spectra of conjugates **5a**–**d** are the following: (i) the presence of characteristic signals at $\delta \sim -2.50$ due to the resonances of the inner N–H protons; (ii) the absence of the signals due to the resonances of the $CO_2CH_2CH_3$ group, and in the case of compounds 5c and 5d, the absence of signals due to the benzoyl groups. These spectra confirm the success of the demetallation and deprotection steps.

The HRMS-ESI$^+$ spectra of the isomeric porphyrin/quinolone conjugates **5a**, **5b**, **5c** and **5d**, with molecular ions $[M+H]^+$ at m/z 830.3097, 830.3095, 934.3192 and 934.3206, respectively, are also in agreement with the corresponding molecular formulae.

Considering the ESI-MS studies, the spectra of all studied porphyrin derivatives **4a**–**d** and **5a**–**d**, obtained in the positive mode, show the corresponding protonated molecules $[M+H]^+$. The ESI-MS/MS spectra of these ions show that the fragmentation pattern is similar for each pair of isomers (Figs. S1 and S2 in Supplementary data).

In the case of compounds **4a** and **4b** the MS/MS spectra (Table 1 and Fig. S1) show that the major product ion (100% relative abundance) is due to the loss of CH_3CH_2OH (−46 Da) from the ethoxycarbonyl group. Minor ions formed by loss of CH_2=CH_2 at m/z 892.4 and by the combined loss of CH_2=CH_2 and CH_3CH_2OH, at m/z 846.4, are also detected for both isomers. But in case of **4a** the combined loss of CH_2=CH_2 and $CH_3CH_2CO_2H$ is responsible for a minor product ion at m/z 818.4 (the proposed structures of molecular ions $[M+H]^+$ and main fragmentation ions of compounds **4a** and **4b** are shown in Scheme S1, SD).

Table 1
Main fragment ions observed in the ESI-MS/MS spectra of the $[M+H]^+$ ions of porphyrin/quinolone conjugates **4a**–**d** and their relative abundances (RA%)

	Compound			
	4a	**4b**	**4c**	**4d**
$[M+H]^+$	920.5	920.5	1336.3	1336.3
$-CH_2$=CH_2	892.4 (<5)	892.4 (<5)	—	—
$-HOCH_2CH_3$	874.4 (100)	874.4 (100)	—	—
$-CH_2$=CH_2 and $-CH_3CH_2OH$	846.4 (20)	846.2 (<5)	—	—
$-CH_3CH_2CO_2H$ and $-CH_2$=CH_2	818.4 (<5)	—	—	—
−(Sugar−H)			892.4 (10)	892.5 (<**5**)
$-C_{56}H_{37}N_5O_3Zn$			445.2 (100)	445.2 (100)

Interestingly, in the case of porphyrin/ribonucleoside derivatives **4c** and **4d** the major fragmentation pathway is due to the loss of the subunits porphyrin/quinolone (−890 Da, loss of $C_{56}H_{37}N_5O_3Zn$) with the formation of abundant product ions at m/z 445.2 (the proposed structures of molecular ions $[M+H]^+$ and main fragmentation ions of compounds **4c** and **4d** are shown in Scheme S2, SD). In this case, the charge is retained in the sugar unit. The complementary cleavage of the *N*-sugar bond [loss of the ribose moiety (−444 Da, $C_{26}H_{20}O_7$) and the charge retained in the porphyrinic unit] is responsible for the minor ions detected at m/z 892.5 for both isomers.

Although the fragmentation pathways for each pair of isomeric porphyrin/quinolone conjugates **4a**/**4b** and **4c**/**4d** are similar, the relative abundance of certain minor fragment ions is different and this suggests that the position of the linkage porphyrin/quinolone affects the stability of the product ions. For instance, for derivatives **4a**/**4b** the combined loss of CH_2=CH_2 and CH_3CH_2OH (m/z 846.4) occurs with higher relative abundance for derivative **4a** (porphyrin moiety linked at C-6 position of the quinolone unit) than for **4b** (analogue linkage at C-7) as indicated in Table 1. A similar situation occurs when compounds **4c** and **4d** are compared; the relative abundance of the minor fragment due to the loss of the sugar unit (m/z 892.4) is higher for **4c** than for **4d**.

Considering now the results obtained for the deprotected and demetallated porphyrin/quinolone conjugates **5a**–**d**, shown in Table 2 and Fig. S2, it is possible to observe a facile decarboxylation of the quinolone moiety for all the compounds. In fact, all spectra show that the major product ion is due to the loss of CO_2 (−44 Da). This fragmentation pathway is typical of compounds with a carboxylic group, as observed for porphyrin/amino acid conjugates.[15] Minor ions due to the loss of the quinolone unit ($C_{12}H_{10}NO_3$) and to the combined loss of CO_2 with CH_2=CH_2 are detected, respectively, at m/z 615.3 and at m/z 758.4 for compound **5a** (the proposed structures of molecular ions $[M+H]^+$ and main fragmentation ions of compounds **5a** and **5b** are shown in SD, Scheme S3-A). For compound **5b** only a minor ion at m/z 812.5 due to the loss of H_2O is detected. Again, for derivatives **5a** and **5b** the position of the linkage porphyrin/quinolone seems to affect slightly the fragmentation pattern and the stability of the minor product ions formed.

Table 2
Main fragment ions observed in the ESI-MS/MS spectra of porphyrin/quinolone conjugates **5a**–**d** and their relative intensities (RA%)

	Compound			
	5a	**5b**	**5c**	**5d**
$[M+H]^+$	830.3	830.3	934.3	934.3
$-H_2O$	—	812.5 (<5)	—	—
$-CO_2$	786.5 (100)	786.2 (100)	890.5 (100)	890.5 (100)
$-CO_2-R$ ($CH_2=CH_2$ or sugar)	758.4 (<5)	—	—	758.5 (70)
$-C_{12}H_{10}NO_3$ (quinolone)	615.3 (<5)	—	—	—
$-H_2O$ and −sugar	—	—	784.4 (5)	784.4 (20)

Interestingly, in the case of porphyrin/ribonucleoside derivatives **5c** and **5d** the difference between the relative abundance (RA) of the less abundant fragment ions is considerable. In fact, the combined loss of CO_2 and the sugar unit for **5d** is responsible for an important fragment ion (RA 70%) at *m*/*z* 758.5, while for **5c** this fragment shows low relative abundance (<5% RA). The combined loss of the sugar unit and H_2O (−150 Da), responsible for the ions at *m*/*z* 784.4, is observed for both isomers, but again it occurs with a higher abundance (20%) than for derivative **5d** (5%) (the proposed structures of molecular ions $[M+H]^+$ and main fragmentation ions of compounds **5c** and **5d**, are shown in SD, Scheme S3-B).

2.3. Singlet oxygen generation studies

Photodynamic activity is significantly related to the singlet oxygen production ($^1O_2$).[23] Photosensitizers that form singlet oxygen are important for photoinduced reaction processes that are responsible for the destruction of tissues.[23,24] In this context, we decided to determine the photosensitizing properties of compounds **5a**–**d** since that might be considered as an extra added-value to the new products. The capacity to generate singlet oxygen was then qualitatively evaluated by monitoring the photodecomposition of a singlet oxygen quencher agent, the 1,3-diphenylisobenzofuran (DPiBF). In this process $^1O_2$ produced by the photosensitizer reacts with the yellow DPiBF in a [4+2] cycloaddition, affording the colourless *ortho*-dibenzoylbenzene. 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/quinolone conjugates **5a**–**d** 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.[25] The results were compared with those obtained using *meso*-tetraphenylporphyrin (TPP), a well-known singlet oxygen generator.[26] The obtained results are shown in Fig. 2. The curves in the graphic represent the % of decay of absorption of DPiBF versus illumination time; a more pronounced decay corresponds to a higher rate of singlet oxygen production.

It is evident from Fig. 2 that in all cases a significant photodegradation of DPiBF is observed. This indicates that all porphyrin/quinolone conjugates **5a**–**d** demonstrate a photoxidising ability. It is also evident that all conjugates **5a**–**d** are better singlet oxygen generators than TPP. It is interesting to note that the best singlet oxygen generators are compounds **5a** and **5d**. This indicates that a direct relationship between the position of the porphyrin moiety in the quinolone (positions 6 or 7) or the type of *N*-substituent, and the ability of the conjugates to generate singlet oxygen cannot be established.

3. Conclusions

In this work it is shown that β-borylated porphyrin **3** reacts with several bromoquinolone derivatives in a Suzuki–Miyaura reaction affording novel porphyrin/quinolone conjugates **4a**–**d**. Also it is possible to prepare β-substituted porphyrin/quinolone conjugates **5a**–**d**, in excellent yields, through alkaline hydrolysis of **4a**–**d**, followed by demetallation. The new derivatives have been fully characterized by NMR and mass spectrometry techniques. The electrospray tandem mass spectrometry (MS/MS) of derivatives **4a**–**d** and **5a**–**d** shows several interesting features. Isomeric porphyrin/quinolone conjugates **4a**,**b** and **5c**,**d**, as well as their deprotected forms **5a**,**b** and **5c**,**d**, give rise to similar fragmentation pathways, confirming their analogous structures. Due to the RA of some peaks it is possible to distinguish between derivatives with the porphyrin moiety linked at C-6 of the quinolone unit (**4a** and **4c**) from those derivatives having the porphyrin moiety linked to the 7-position of the quinolone moiety (**4b** and **4d**). This study gives important information about the fragmentation pathways of these derivatives, allowing the possibility of following the metabolic degradation of a drug with the same structural features.

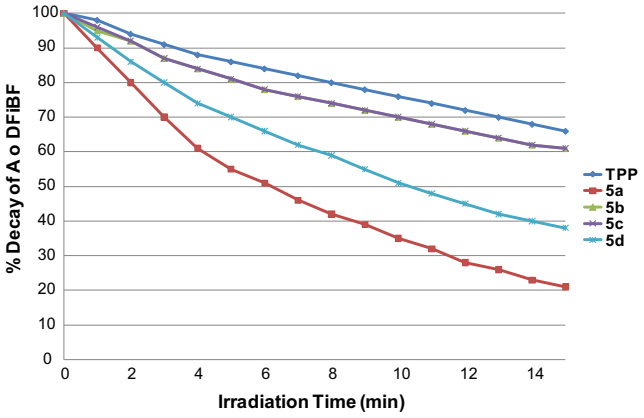


Fig. 2. Photodecomposition of DPiBF by singlet oxygen generated by **TPP**, **5a**, **5b**, **5c** and **5d** after irradiation with white light filtered through a cut-off filter for wavelength <550 nm (25 mW cm^{-2}).

Singlet oxygen studies show that all the β-substituted porphyrin/quinolone conjugates **5a**–**d** are better singlet oxygen generators than TPP and that the efficiency of these compounds to generate singlet oxygen is affected by the linkage position between the porphyrin and the quinolone moieties, as well by the *N*-substituent of the quinolone group.

In conclusion, the reaction of bromoquinolones with borylated porphyrin **3** can be considered as a new synthetic methodology leading to new porphyrin/quinolone conjugates.

4. Experimental

4.1. General

^1H and ^{13}C NMR spectra were recorded on Bruker Avance 300 (300 MHz for ^1H and 75 MHz for ^{13}C) and Bruker Avance 500 (500 MHz for ^1H and 125 MHz for ^{13}C) spectrometers. $CDCl_3$, $CDCl_3$/CD_3OD or DMSO-d_6 were used as solvents and TMS as internal reference and the chemical shifts are expressed in δ (ppm). Unequivocal ^1H assignments were made using 2D COSY and NOESY experiments (mixing time of 800 ms), while ^{13}C assignments were made on the basis of 2D HSQC and HMBC experiments (the delay for long-range *J* C/H couplings were optimized for 7 Hz). HRMS were recorded on a VG AutoSpec M mass spectrometer using $CHCl_3$ as solvent and 3-nitrobenzyl alcohol (NBA) as matrix. Positive ion ESI mass spectra and tandem mass spectra were acquired using a Q-TOF 2 instrument (Micromass, Manchester, UK). The samples for ESI analyses were prepared by diluting 1 μL of the porphyrin solutions in chloroform ($\sim 10^{-5}$ M) in 200 μL of methanol. Nitrogen was used as the

nebulizer gas and argon was used as the collision gas. Samples were introduced into the mass spectrometer using a flow rate of 10 μL/min, the needle voltage was set at 3000 V, with the ion source at 80 °C and needle temperature at 150 °C. Cone voltage was 30 V. Collision-induced decomposition mass spectra (MS/MS) were acquired by selecting the desired precursor ion with the quadrupole section of the mass spectrometer and using collision energy of 25–40 eV. The UV–vis spectra were recorded on an UV-2501 PC Shimatzu spectrophotometer using $CHCl_3$ as solvent. Flash chromatography was carried out using silica gel (230–400 mesh). Preparative thin-layer chromatography was carried out on 20×20 cm glass plates coated with silica gel (1 mm thick). Analytical TLC was carried out on precoated plastic sheets with silica gel (Merck 60, 0.2 mm thick). Porphyrin **3**, 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,10,15,20-tetraphenylporphyrinatozinc(II), was prepared according to the literature.[27]

4.2. Coupling reactions of porphyrin 3 with bromoquinolones 2a–d. General procedure

Porphyrin **3** (10.0 mg, 12.4 μmol), bromoquinolones **2a–d** (33.5 μmol), Cs_2CO_3 (6.5 mg, 19.9 μmol), $Pd(PPh_3)_4$ (11.0 mg, 9.6 μmol), DMF (0.5 mL) and toluene (1 mL) were brought together in a 25 mL Schlenk tube. The solvent was degassed by repeated sonication for 0.5–1 min under reduced pressure. The reaction mixture was stirred for 16 h at 80 °C, the mixture was quenched with aq. NaCl and extracted with CH_2Cl_2. The organic layer was washed with water, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography using a mixture of toluene/ethyl acetate as the eluent.

4.2.1. 2-(3-Ethoxycarbonyl-1-ethyl-4-oxo-1,4-dihydroquinolin-6-yl)-5,10,15,20-tetraphenylporphyrinatozinc(II), ***4a****.* Yield: 89%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 and 8.93 (AB, 2H, J=4.7 Hz, H-7 and H-8), 8.93 (s, 2H, H-12 and H13), 8.91 (s, 1H, H-3), 8.85 and 8.76 (AB, 2H, J=4.7 Hz, H-17 and H-18), 8.70 (d, 1H, J=2.1 Hz, H-5′), 8.52 (s, 1H, H-2′), 8.26–8.17 (m, 6H, H*o*-Ph-5,10,15), 8.02–7.86 (m, 2H, H*o*-Ph-20), 7.81–7.69 (m, 9H, H*m*,*p*-Ph-5,10,15), 7.50 (dd, 1H, J=2.1 and 8.6 Hz, H-7′), 7.11–7.06 (m, 2H, H*m*-Ph-20), 7.49–7.48 (m, 1H, H*p*-Ph-20), 7.08 (d, 1H, J=8.6 Hz, H-8′), 4.42 (q, 2H, J=7.2 Hz, $CO_2CH_2CH_3$), 4.28–4.27 (m, 2H, NCH_2CH_3), 1.61 (t, 3H, J=7.2 Hz, NCH_2CH_3), 1.45 (t, 3H, J=7.2 Hz, $CO_2CH_2CH_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.2 (C-4′), 166.3 ($CO_2CH_2CH_3$), 151.0, 150.5, 150.4, 150.3, 150.2, 148.3 (C-2′), 147.6, 145.2, 142.65, 142.65, 141.4, 137.0, 136.3, 135.7, 134.8 (C-7′), 134.4, 134.3, 132.5, 132.2, 132.1, 132.0, 131.5, 131.4, 128.6, 128.38 (C-5′), 128.34, 128.2, 127.5, 127.4, 126.6, 126.8, 126.6, 121.8, 121.7, 121.4, 121.0, 120.8, 114.3 (C-8′), 110.9, 60.9 (NCH_2CH_3), 48.9 ($CO_2CH_2CH_3$), 14.7 (NCH_2CH_3), 14.5 ($CO_2CH_2CH_3$) ppm. UV–vis ($CHCl_3$): λ_{max} (log ε)=423 (5.47), 511 (3.37), 550 (4.10), 587 (3.32) nm; HRMS (ESI) m/z calcd for $C_{58}H_{42}N_5O_3Zn$ $[M+H]^+$ 920.2579, found 920.2574.

4.2.2. 2-(3-Ethoxycarbonyl-1-ethyl-4-oxo-1,4-dihydroquinolin-7-yl)-5,10,15,20-tetraphenylporphyrinatozinc(II), ***4b****.* Yield: 82%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 and 8.93 (AB, 2H, J=4.6 Hz, H-7 and H-8), 8.94–8.93 (m, 3H, H-3, H-12 and H-13), 8.87 and 8.78 (AB, 2H, J=4.6 Hz, H-17 and H-18), 8.48 (s, 1H, H-2′), 8.24–8.20 (m, 7H, H*o*-Ph-5,10,15 and H-5′), 7.91–7.84 (m, 2H, H*o*-Ph-20), 7.77–7.74 (m, 9H, H*m*,*p*-Ph-5,10,15), 7.57 (d, 1H, J=8.7 Hz, H-6′), 7.29 (s, 1H, H-8′), 7.17–6.94 (m, 3H, H*m*,*p*-Ph-20), 4.41 (q, 2H, 7.2 Hz, $CO_2CH_2CH_3$), 4.26–4.05 (m, 2H, NCH_2CH_3), 1.45 (t, 3H, J=7.1 Hz, NCH_2CH_3), 1.40 (t, 3H, J=7.1 Hz, $CO_2CH_2CH_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3 (C-4′), 166.3 ($CO_2CH_2CH_3$), 151.0, 150.7, 150.57, 150.53, 150.4, 150.3, 150.2, 148.4 (C-2′), 147.2, 145.1, 142.6, 142.5, 141.3, 137.4, 135.2, 134.4, 134.3, 132.6, 132.3, 132.2, 131.7, 127.54, 127.49, 127.5, 127.2, 126.8, 126.6, 122.1, 121.7, 121.6, 121.2, 120.9, 117.2, 110.7, 60.9 (NCH_2CH_3), 48.4 ($CO_2CH_2CH_3$), 14.6 (NCH_2CH_3), 14.4 ($CO_2CH_2CH_3$) ppm. UV–vis ($CHCl_3$): λ_{max} (log ε)=423 (5.34), 558 (4.14), 598 (3.10), 667 (3.07) nm; HRMS (ESI) m/z calcd for $C_{58}H_{42}N_5O_3Zn$ $[M+H]^+$ 920.2579, found 920.2559.

4.2.3. 2-[3-Ethoxycarbonyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranos-1-yl)-4-oxo-1,4-dihydroquinolin-6-yl]-5,10,15,20-tetraphenylporphyrinatozinc(II), ***4c****.* Yield: 51%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.94 and 8,83 (AB, 2H, J=4.6 Hz, H-7 and H-8), 8.96 (s, 1H, H-3), 8.92 (s, 2H, H-12 and H-13), 8.86 (s, 1H, H-2′), 8.84 and 8.74 (AB, 2H, J=4.7 Hz, H-17 and H-18), 8.57 (d, 1H, J=2.0 Hz, H-5′), 8.26–8.16 (m, 6H, H*o*-Ph-5,10,15), 8.11 (dd, 2H, J=1.4 and 7.8 Hz, H-2‴), 7.99–8.03 (m, 2H, H*o*-Ph-20), 7.97 (dd, 2H, J=1.4 and 7.8 Hz, H-2‴), 7.77–7.74 (m, 12H, H*m*,*p*-Ph-5,10,15,20), 7.56 (tt, 2H, J=1.4 and 6.1 Hz, H-4‴), 7.43–7.34 (m, 9H, 6× H-3‴, H-4‴, H-7′ and H-8′), 7.15–6.90 (m, 2H, H-2‴), 6.39 (d, 1H, J=5.1 Hz, H-1″), 6.07 (t, 1H, J=5.1 Hz, H-2″), 5.93 (t, 1H, J=5.1 Hz, H-3″), 4.96–4.79 (m, 3H, H-4″ and H-5″), 4.33–4.14 (m, 1H, $CO_2CH_2CH_3$), 1.32 (t, 1H, J=7.1 Hz, $CO_2CH_2CH_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3 (C-4′), 166.0 ($CO_2CH_2CH_3$), 165.1 (C=O Bz), 165.0 (C=O Bz), 164.6 (C=O Bz), 150.9, 150.5, 150.4, 150.33, 150.30, 150.2, 147.5 (C-2′), 146.1, 142.6, 141.1, 135.7, 135.5 (C-2), 134.4 (C-7′), 134.1, 133.8, 133.6, 132.6, 132.19, 132.12, 131.9 (C-2′), 131.5, 129.9, 129.8, 129.7, 129.2, 128.9 (C-5′), 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 127.5, 127.4, 126.9, 126.5, 121.9, 121.4, 121.0, 120.8, 113.5 (C-8′), 91.2 (H-1″), 80.3 (H-4″ or H-5″), 70.5, 70.4, 63.2 (H-4″ or H-5″), 62.8, 60.9, 29.7, 29.5 ($CO_2CH_2CH_3$), 14.29 ($CO_2CH_2CH_3$) ppm. UV–vis ($CHCl_3$): λ_{max} (log ε)=428 (5.35), 558 (4.25), 598 (3.81), 673 (2.89) nm; HRMS (ESI) m/z calcd for $C_{82}H_{58}N_5O_{10}Zn$ $[M+H]^+$ 1336.3470, found 1336.3452.

4.2.4. 2-[3-Ethoxycarbonyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranos-1-yl)-4-oxo-1,4-dihydroquinolin-7-yl]-5,10,15,20-tetraphenylporphyrinatozinc(II), ***4d****.* Yield: 50%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.92 (s, 1H, H-2′), 8.81–8.76 (m, 4H, β-H), 8.69 (d, 1H, J=4.6 Hz, H-17 or H-18), 8.63–8.58 (m, 2H, β-H), 8.23–8.08 (m, 8H, H*o*-Ph-5,10,15), 7.98–7.95 (m, 2H, H-2‴), 7.81–7.79 (m, 9H, H*m*,*p*-Ph-5,10,15,20), 7.72–7.70 (m, 2H, H-2‴), 7.66–7.55 (m, 9H, 4× H-3‴, 3× H-4‴, H-5′ and H-8′), 7.40 (t, 2H, J=7.7 Hz, H3‴), 7.09 (t, 2H, J=7.5 Hz, H*m*-Ph-20), 6.97–6.92 (m, 1H, H-2″), 6.77 (br s, 2H, H-2‴), 6.54–6.49 (m, 1H, H-1″), 6.20–6.06 (m, 1H, H*p*-Ph-20), 5.84–5.93 (m, 1H, H-3″), 5.01–4.97 (m, 1H, H-4″), 4.82–4.79 (m, 3H, H-5″), 4.19–4.05 (m, 2H, $CO_2CH_2CH_3$), 1.22 (t, 3H, J=7.0 Hz, $CO_2CH_2CH_3$) ppm. ^{13}C RMN (125 MHz, DMSO): 172.5 (C-4′), 165.5 ($CO_2CH_2CH_3$), 164.6 (C=O Bz), 164.2 (C=O Bz), 163.5 (C=O Bz), 149.5, 146.4 (C-2′), 142.9, 142.5, 139.3, 134.1, 133.6, 131.6, 129.5, 129.3, 128.9, 128.7, 128.5, 128.1, 127.5, 126.7, 126.5, 120.1, 119.2, 111.5, 88.7, 80.8, 75.1, 70.6, 64.0, 59.8, 13.9 ($CO_2CH_2CH_3$). UV–vis ($CHCl_3$): λ_{max} (log ε)=429 (5.37), 559 (4.07), 600 (3.63), 666 (3.18) nm; HRMS (ESI) m/z calcd for $C_{82}H_{58}N_5O_{10}Zn$ $[M+H]^+$ 1336.3470, found 1336.3468.

4.3. Deprotection and demetallation of porphyrin/quinolone conjugates 4a–d. General procedure

A solution of the porphyrin/quinolone conjugate **4a–d** (7.5 μmol) in 1.78 M methanolic potassium hydroxide solution (2.5 mL) and THF/Py (0.5 mL/50 μL) was stirred at 80 °C for 1 h (compounds **4a**,**b**) or 24 h (compounds **4c**,**d**) in a sealed tube.[28] The resulting solution was neutralized with aqueous solution of citric acid. The mixture was extracted with chloroform and then the organic phase was washed with water and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to dryness. The residue was dissolved in $CHCl_3$ (1 mL) and neat TFA (0.1 mL) was added. This mixture was stirred in the dark at room temperature for 2 min. Chloroform and water were then added and the mixture was neutralized with aqueous sodium carbonate. The mixture was extracted with chloroform, the organic phase was washed with water, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure to dryness. Pure porphyrin/quinolone conjugates

5a,**b** were obtained directly by crystallization of the residue from chloroform/hexane. The porphyrin/quinolone conjugates **5c**,**d** were first purified by preparative TLC using $CHCl_3$/MeOH (1%) as the eluent and then were crystallized from chloroform/hexane.

4.3.1. 2-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydroquinolin-6-yl)-5,10,15,20-tetraphenylporphyrin, ***5a****.* Yield: 99%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.14 (s, 1H, CO_2H), 8.87 and 8.84 (AB, 2H, J=4.7 Hz, H-7 and H-8), 8.83 (s, 1H, H-3), 8.82 (s, 2H, 12 and H-13), 8.80 (s, 1H, H-2′), 8.78 and 8.69 (AB, 2H, J=4.8 Hz, H-17 and H-18), 8.67 (d, J=2.0 Hz, H-5′), 8.25–8.20 (m, 6H, H*o*-Ph-5,10,15), 7.94–7.92 (m, 2H, H*o*-Ph-20), 7.78–7.69 (m, 9H, H*m*,*p*-Ph-5,10,15), 7.55–7.54 (m, 1H, H-7′), 7.21 (d, 1H, J=8.8 Hz, H-8′), 7.10–7.06 (m, 3H, H*m*,*p*-Ph-20), 4.37–4.35 (m, 2H, NCH_2CH_3), 1.63 (t, 3H, J=7.1 Hz, NCH_2CH_3), -2.62 (s, 2H, NH) ppm. UV–vis (DMF/H_2O (9:1)): λ_{max} (log ε)=421 (5.07), 519 (5.06), 553 (3.86), 595 (3.74), 651(3.61) nm; HRMS (ESI) m/z calcd for $C_{56}H_{40}N_5O_3$ $[M+H]^+$ 830.3126, found 830.3097.

4.3.2. 2-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydroquinolin-7-yl)-5,10,15,20-tetraphenylporphyrin, ***5b****.* Yield: 98%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.15 (s, 1H, CO_2H), 8.90 and 8.87 (AB, 2H, J=4.7 Hz, H-7 and H-8), 8.83–8.82 (m, 3H, H-3, H-12 and 13), 8.76 (s, 1H, H-2′), 8.81 and 8.73 (AB, 2H, J=4.9 Hz, H-17 and H-18), 8.34 (d, 1H, J=8.2 Hz, H-6′), 8.26–8.20 (m, 6H, H*o*-Ph-5,10,15),7.93–7.92 (m, 2H, H*o*-Ph-20), 7.82–7.71 (m, 9H, H*m*,*p*-Ph-5,10,15), 7.40 (s, 1H, H-8′), 7.16–7.07 (m, 3H, H*m*,*p*-Ph-20), 4.27–4.24 (m, 2H, NCH_2CH_3), 1.44 (t, 3H, J=7.1 Hz, NCH_2CH_3), -2.63 (s, 2H, NH) ppm. UV–vis (DMF/H_2O (9:1)): λ_{max} (log ε)=421 (5.26), 517 (3.96), 553 (3.60), 593 (3.47), 649 (3.40) nm; HRMS (ESI) m/z calcd for $C_{56}H_{40}N_5O_3$ $[M+H]^+$ 830.3126, found 830.3095.

4.3.3. 2-[3-Carboxy-1-(β-D-ribofuranos-1-yl)-4-oxo-1,4-dihydroquinolin-6-yl]-5,10,15,20-tetraphenylporphyrin, ***5c****.* Yield: 88%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 15.19 (s, 1H, CO_2H), 9.59 (s, 1H, H-2′), 8.87–8.70 (m, 7H, β-H), 8.35–8.33 (m, 1H, H-5′), 8.32–8.23 (m, 6H, H*o*-Ph-5,10,15), 7.98–7.87 (m, 2H, H*o*-Ph-20), 7.86–7.83 (m, 12H, H*m*,*p*-Ph-5,10,15,20), 6.58–6.56 (m, 1H, H-1″), 6.20–6.18 (m, 1H, H-2″), 5.37–5.31 (m, 1H, H-3″), 4.23–4.21 (m, 2H, H-5″), 4.15–4.10 (m, 1H, H-4″), -2.76 (s, 2H, NH) ppm. UV–vis (DMF/H_2O (9:1)): λ_{max} (log ε)=421 (4.47), 517 (3.29), 552 (2.94), 593 (2.81), 648 (2.72) nm; HRMS (ESI) m/z calcd for $C_{59}H_{44}N_5O_7$ $[M+H]^+$ 934.3235, found 934.3192.

4.3.4. 2-[3-Carboxy-1-(β-D-ribofuranos-1-yl)-4-oxo-1,4-dihydroquinolin-7-yl]-5,10,15,20-tetraphenylporphyrin, ***5d****.* Yield: 89%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.06 (s, 1H, H-2′), 8.98–8.42 (m, 7H, β-H), 8.24–8.20 (m, 8H, H*o*-Ph-5,10,15,20), 7.84–7.79 (m, 12H, H*m*,*p*-Ph-5,10,15,20), 7.60–7.39 (m, 2H, H-5′ and H-8′), 7.22–6.94 (m, 1H, H-1″), 6.95–6.75 (m, 1H, H-2″), 6.58–6.50 (m, 1H, H-3″), 6.50 (m, 1H, H-4″), 6.04–6.00 (m, 2H, H-5″), -2.62 (s, 2H, NH) ppm. UV–vis (DMF/H_2O (9:1)): λ_{max} (log ε)=427 (4.86), 517 (3.48), 560 (3.45), 600 (3.23), 647 (3.06) nm; HRMS (ESI) m/z calcd for $C_{59}H_{44}N_5O_7$ $[M+H]^+$ 934.3235, found 934.3206.

4.4. Singlet oxygen generation studies

Stock solutions of the porphyrin/quinolone conjugates **5a**–**d** at 0.1 mM in DMSO and a stock solution of 1,3-diphenylisobenzofuran (DPiBF) at 10 mM in DMSO were prepared. The reaction mixture of 50 μM of DPiBF and 0.5 μM of each porphyrin/quinolone derivative in DMF/H_2O (9:1) in glass cells (2 mL) was irradiated with light (550–800 nm) with a fluence rate of 25.0 mW cm^{-2}. The solutions were stirred at room temperature while being irradiated. The generation of singlet oxygen was followed by its reaction with DPiBF. The breakdown of DPiBF was monitored by measuring the decreasing of the absorbance at 415 nm. The obtained values were compared with those obtained when using 0.5 μM *meso*-tetraphenylporphyrin.

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Supplementary data

Supporting data associated with this article can be found in the online version at doi:10.1016/j.tet.2011.07.025.

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