Singlet oxygen generation ability of squarylium cyanine dyes

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Abstract

The quantum yields for singlet oxygen generation of several squarylium cyanine dyes derived from benzothiazole, benzoselenazole and quinoline, displaying absorption within the so-called "phototherapeutic window" (600–1000 nm), were determined, envisioning their potential usefulness for photodynamic therapy (PDT). The determination was performed by a direct method measuring the luminescence decay of the dyes in the near infrared. Considering the absorption and the quantum yields displayed by some of the dyes, these seemed to be potential candidates as sensitizers for PDT.
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1. Introduction

Photodynamic therapy (PDT) of cancer is a recent and fast developing technique based on the use of drugs (photosensitizers), which are preferentially taken up or retained by neoplastic tissues, that acquire the desired activity only when excited by light of the appropriate wavelength. It is generally accepted that the photosensitising property of the drug involves predominantly the conversion of dissolved triplet oxygen to cytotoxic singlet oxygen, which is on the basis of tumour cells destruction [1,2].

Until now, for the vast majority of medical applications of PDT, it is used as photosensitizers, a mixture of hematoporphyrin derivatives, which, however, present several undesirable properties. Consequently, in the last years a great deal of research work on other different classes of photosensitizers has emerged, being the development of new sensitizers with better medical properties now considered to be essential for the clinical implementation of this therapeutic modality [3,4].

The success of any new drug as photosensitizer for PDT critically depends on its photophysical and photochemical properties. These include a strong absorption ($>10^5 \, M^{-1} \, cm^{-1}$) within the so-called "phototherapeutic window" (600–1000 nm), in which tissue light scattering is low and penetration depth of light in tissues increases and also a high quantum yield of the compound's triplet state formation, combined with high triplet lifetime. The reason for this lies in the fact that photochemical reactions predominantly occur in the excited state and a good efficiency of the conversion of triplet oxygen to cytotoxic singlet oxygen is crucial [5].

Squarylium dyes, which are 1,3-disubstituted compounds synthesized by condensation of one equivalent of squaric acid (3,4-dihydroxy-1,2-dioxocyclobut-3-ene) with two molar equivalents of electron rich aromatic or heterocyclic methylene bases [6,7], display sharp and intense absorption in the visible and near infrared region. Although these and other properties have attracted considerable interest to this class of dyes due their possible technological application as substrates for optical recording media [8–10], xerographic photoreceptors [11–13] and organic solar cells [14–16], the potential of squarylium dyes as sensitizers for PDT have scarcely been explored [17–19]. The incipient intersystem crossing efficiency usually observed for these dyes [20–22] is probably on the base of it.

Amongst the large group of squarylium dyes those of the cyanine type have received less attention, remaining, to the best of our knowledge, to be investigated with regard to their usefulness for PDT.

While the photophysical behaviour of squarylium cyanines has been scantily examined [23,24], application of some of these dyes as long-wavelength probes in biological assay techniques has been disclosed [25–27].

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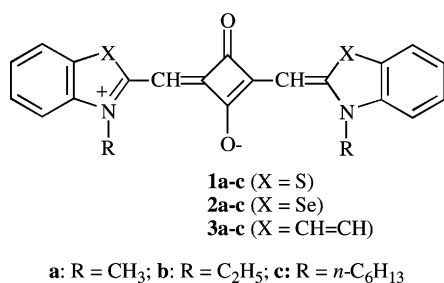*E-mail address:* psantos@utad.pt (P.F. Santos).

1a–c (X = S)
2a–c (X = Se)
3a–c (X = CH=CH)

a: R = CH₃; **b**: R = C₂H₅; **c**: R = n-C₆H₁₃

Fig. 1. Squarylium cyanine dyes **1–3**.

Herein we report the ability of several squarylium cyanine dyes (Fig. 1), displaying absorption near 700 nm, to generate singlet oxygen, envisioning their potential usefulness as PDT sensitizers. Some structural variations as the nature of the heteroaromatic ring and the length of the N,N'-dialkyl groups were made, in order to study their influence in the efficiency of singlet oxygen generation.

2. Experimental

2.1. Synthesis of squarylium dyes

Squarylium cyanine dyes **1–3** were readily prepared condensing a suitable N-alkyl-2-methylbenzothiazolium, N-alkyl-2-methylbenzoselenazolium or N-alkyl-2-methyl-quinolinium iodide and squaric acid in refluxing n-butanol/pyridine according to the literature procedure [28].

All compounds showed spectral data, including high-resolution mass spectra, fully consistent with the assigned structures.

2.2. Quantum yields for singlet oxygen generation (ϕ_Δ)

Singlet oxygen experiments were performed with a PTI PL2300 nitrogen laser (337.1 nm, 0.60 ns pulses, 1.6 mJ/pulse) with air equilibrated samples. The emission from singlet oxygen was detected at 90° to the incident laser by a 5 MHz germanium photodiode (Judson, J16-8SP-R05M-HS) working at room temperature. Phenazine in dichloromethane ($\phi_\Delta = 0.89$) [29], with an optical density of 0.30 at the wavelength of excitation, was used as reference. Accurate calibration curves, with linear dependence of singlet oxygen emission intensity versus laser energy were obtained by placing neutral density filters in the excitation laser pathlength. A comparison of the slopes for the samples under study and for the reference yields ϕ_Δ in a straightforward manner.

3. Results and discussion

The squarylium cyanine dyes studied display strong absorption within the range 650–710 nm and were found to be able to produce singlet oxygen with reasonable yields

Table 1
The yield, UV spectral data and ϕ_Δ for squarylium cyanine dyes **1–3**

Dye	X	R	Yield (%)	λ_{max}[a] (nm) (log ε)	ϕ_Δ[b]
1a	S	CH₃	63	647 (5.44)	0.17
1b	S	C₂H₅	50	650 (5.24)	0.26
1c	S	n-C₆H₁₃	76	650 (5.41)	0.26
2a	Se	CH₃	79	662 (5.21)	0.31
2b	Se	C₂H₅	72	665 (5.49)	0.31
2c	Se	n-C₆H₁₃	74	668 (5.41)	0.31
3a	CH=CH	CH₃	67	704 (5.35)	0.08
3b	CH=CH	C₂H₅	45	707 (5.39)	0.10
3c	CH=CH	n-C₆H₁₃	42	710 (5.37)	0.13

[a] Measured in MeOH/CH₂Cl₂ (99/1).
[b] Measured in CH₂Cl₂.

(Table 1). The contrastive poor quantum yields of singlet oxygen generation reported for similarly N-substituted thiacarbo- and selenacarbocyanines [30–32] suggest, as it would be expected, that rigidification of the polimethine chain, achieved by the inclusion of the four member ring derived from squaric acid, inhibits the non-radiative decay by *trans-cis*-photoisomerization with a consequent rise of the efficiency of intersystem crossing.

It is clearly apparent that the efficiency of singlet oxygen production is strongly dependent on the nature of the heteroaromatic moiety of the dye, increasing in the order quinoline < benzothiazole < benzoselenazole. This is most probably a consequence of the enhancement of spin–orbital interaction arising from the presence of the heavy atom in the dye's structure, which leads to an increase in the intersystem crossing and triplet yield. Accordingly, it can be reasonably anticipated that the incorporation of heavier atoms, such as iodine or bromine, either in the rigidified polimethine chain or in the hetearomatic ring, should facilitate intersystem crossing and increase ϕ_Δ.

Although not always consistently, the length of the N-alkyl chain also seems to affect the observed quantum yields. Whenever those are different within a series of dyes bearing the same heterocycle, the larger value is displayed by the dye possessing the longer N-alkyl group. Such behaviour is consistent with the fact that a longer pendent alkyl chain implies a more difficult photoisomeration.

Exceptions to this general trend are seleno dyes **2a–c**, for which the value of ϕ_Δ is the same. For these dyes, that exhibit the larger quantum yield observed, intersystem crossing, enhanced by the internal heavy-atom effect, possibly becomes the main deactivation process, diminishing, consequently, the contribution of others to the overall excited-state relaxation process. The lack of differentiating decay pathways, namely internal conversion and photoisomerization, favourites then the equalisation of the ability of the dyes to convert triplet oxygen to singlet oxygen.

4. Conclusions

The quantum yields for singlet oxygen generation of several squarylium cyanine dyes derived from benzothiazole,

benzoselenazole and quinoline were determined, measuring the luminescence of the dyes in the NIR. In view of the absorption and the quantum yields for singlet oxygen generation displayed by some of the dyes studied, these seemed to be interesting potential candidates as sensitizers for PDT.

Conversion of these non-ionic dyes to cationic cyanines, which have been regarded as very promising potential agents for PDT [33,34], since being easily accomplished by substitution of an oxygen atom of the squaric ring by a neutral nucleophile [35], amends the interest for these class of dyes in the context of PDT.

Acknowledgements

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