Efficiency of singlet oxygen generation of aminosquarylium cyanines

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Abstract

Several symmetrical *N*-methylamino- and *N*,*N*-diethylaminosquarylium cyanine dyes bearing benzothiazole, benzoselenazole and quinoline nuclei, displaying strong absorption within the "phototherapeutic window" (600–1000 nm), were investigated for the efficiency of singlet oxygen production, aiming their potential sensitising ability for photodynamic therapy (PDT). The assessment was performed determining the corresponding quantum yields of singlet oxygen generation, measuring the luminescence decay of the dyes in the near infrared. By combining the exhibited absorption and the obtained quantum yields, some of the dyes can be regarded as potential candidates as sensitizers for PDT.
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1. Introduction

Squarylium dyes, which are 1,3-disubstituted compounds resulting from the condensation of one equivalent of squaric acid (3,4-dihydroxy-1,2-dioxocyclobut-3-ene) with two molar equivalents of electron donating aromatic or heterocyclic methylene bases [1,2], typically exhibit special properties such as good photochemical stability, high photoconductivity and sharp and intense absorption in the visible and near infrared regions [3,4]. These and other properties have made this group of dyes very attractive for several photonics applications such as xerographic photoreceptors [5–7], organic solar cells [8–10] and optical recording media [11–13], and over the last years much attention has been focused onto it.

Recently, the use of squarylium dyes as long-wavelength probes in biological assay techniques has also been described [14–16], broadening the scope of utilisation of these compounds.

Some of the properties that account for the interest over this class of functional dyes also rends them potentially useful sensitizers for photodynamic therapy (PDT), a promising new treatment modality for cancer, that involves the combined use of a photosensitising drug and light of appropriate wavelength. The former, after being localized in the target tissue, gives rise, upon illumination, to a cascade of biochemical processes from which results the destruction of the abnormal cells. It is generally accepted that singlet oxygen, generated from ground-state triplet oxygen, is the main cytotoxic specie responsible for the biological effects displayed [17,18]. The innocuity of both the sensitizer and light by themselves, together with a preferential uptake or retention of the sensitizer by the neoplastic cells and the possibility of exposing to light only the diseased tissue, enable the photodynamic process to be selective enough to destroy tumour tissue while sparing the healthy one.

The 'first generation' photosensitizers, which are derivatives of haematoporphyrin and, until now, the most commonly used sensitizers for medical applications, exhibit some limitations that have somewhat hampered the development of PDT. The design of new drugs able to overcome the several drawbacks of the classic sensitizers is one of the major topics of research in the field and is considered to be essential for the clinical implementation of this therapeutic procedure [19,20].

Any new compound intended to be candidate as photosensitizer for PDT, should have several characteristics amongst which strong absorption ($>10^5 \, M^{-1} \, cm^{-1}$) within the so-called "phototherapeutic window" (600–1000 nm), in which tissue light scattering is minimised and penetration

1a-c (R_1 = H, R_2 = CH$_3$, R_3 = C$_2$H$_5$)
2a-c (R_1 = H, R_2 = CH$_3$, R_3 = n-C$_6$H$_{13}$)
3a-c (R_1 = C$_2$H$_5$, R_2 = C$_2$H$_5$, R_3 = C$_2$H$_5$)
4a-c (R_1 = C$_2$H$_5$, R_2 = C$_2$H$_5$, R_3 = n-C$_6$H$_{13}$)
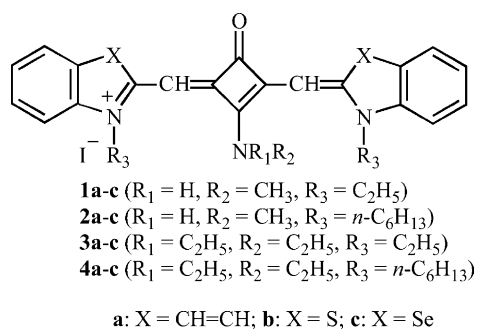
a: X = CH=CH; **b**: X = S; **c**: X = Se

Fig. 1. Aminosquarylium cyanine dyes **1**–**4**.

depth of light in tissues increases, and an inherent ability to produce reactive singlet oxygen [21].

So far, the potential of squarylium dyes as sensitizers for PDT has been poorly explored [22–24]. To the best of our knowledge, those of the cyanine type had not received attention concerning that purpose until recently, when we disclosed the singlet oxygen generation ability of simple squarylium cyanine dyes derived from benzothiazole, benzoselenazole and quinoline [25].

Herein we report the extension of such study to some aminosquarylium cyanines dyes (Fig. 1), though to have several advantages over their non-ionic analogues. The presence of an amine moiety, besides shifting advantageously the absorption maximum of the dye towards the red end of the spectrum, also provides a potential mean to influence conviniently the hydrophilicity of the dye and its interaction with cellular components. Furthermore, the cationic character of these dyes confers them additional interest since cationic cyanines have been regarded as very promising potential agents for PDT [26,27].

In this respect, some halogenated aminosquarylium dyes of the hemicyanine type have recently been referred as sensitizing agents for PDT [28].

The dyes studied differ in the nature of both the heteroaromatic ring and the substituent amine group and in the length of the *N,N′*-dialkyl pendent groups, in order to assess the influence of each of those factors in the efficiency of singlet oxygen generation.

2. Experimental

2.1. Synthesis of aminosquarylium cyanine dyes

The *N*-methylamino- (**1**, **2**) and *N,N*-diethylaminosquarylium cyanine dyes (**3**, **4**) were prepared by methylation of the corresponding squarylium dyes, followed by nucleophilic substitution of the resulting central methoxide group with *N*-methylamine and *N,N*-diethylamine, according to the literature procedure [29].

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 degrees to the incident laser by a 5 MHz germanium photodiode (Judson, J16-8SP-R05M-HS) working at room temperature. Phenazine in dichoromethane (ϕ_Δ = 0.89) [30], 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. Each decay curve is an average of 100 measurements, except for the selenium dyes where the degradation of the sample was significant and only the initial 50 laser pulses (and decay measurements) were taken into account for average purposes.

3. Results and discussion

The singlet oxygen generation ability of the aminosquarylium cyanine dyes **1**–**4**, which absorb strongly in the range 659–722 nm, was found to be widely variable, going from poor to very good (Table 1).

As previously observed with parent non-substituted squarylium cyanine dyes [25], the efficiency of the compound to produce singlet oxygen depends markedly on the nature of the terminal heteroaromatic nuclei, following the increase of the weight of the atoms present in the heterocyclic rings. Accordingly, the higher values of ϕ_Δ are displayed by the dyes possessing benzoselenazole nuclei and the lowest by those incorporating quinoline moieties. The known heavy atom effect should be on the basis of it, since the heavy atom substitution ensures enhancement

Table 1
The UV spectral data and ϕ_Δ for aminosquarylium cyanine dyes **1**–**4**

Dye	X	NR$_1$R$_2$	R$_3$	λ_{max}[a] (nm) (log ε)	ϕ_Δ[b]
1a	CH=CH	NHCH$_3$	C$_2$H$_5$	707 (5.43)	0.01
1b	S	NHCH$_3$	C$_2$H$_5$	659 (5.23)	0.04
1c	Se	NHCH$_3$	C$_2$H$_5$	674 (5.26)	0.45
2a	CH=CH	NHCH$_3$	n-C$_6$H$_{13}$	710 (5.36)	0.03
2b	S	NHCH$_3$	n-C$_6$H$_{13}$	659 (5.22)	0.14
2c	Se	NHCH$_3$	n-C$_6$H$_{13}$	677 (5.23)	0.68
3a	CH=CH	N(C$_2$H$_5$)$_2$	C$_2$H$_5$	719 (5.55)	~0
3b	S	N(C$_2$H$_5$)$_2$	C$_2$H$_5$	668 (5.27)	~0
3c	Se	N(C$_2$H$_5$)$_2$	C$_2$H$_5$	686 (5.22)	0.26
4a	CH=CH	N(C$_2$H$_5$)$_2$	n-C$_6$H$_{13}$	722 (5.38)	~0
4b	S	N(C$_2$H$_5$)$_2$	n-C$_6$H$_{13}$	671 (5.33)	~0
4c	Se	N(C$_2$H$_5$)$_2$	n-C$_6$H$_{13}$	689 (5.26)	0.40

[a] Measured in MeOH/CH$_2$Cl$_2$ (99/1).
[b] Measured in CH$_2$Cl$_2$.

of intersystem crossing from the singlet to the triplet state of the sensitizer, and, consequently, of the efficiency of triplet-singlet interconversion of oxygen.

All the dyes bearing the *N*-methylamine group in the four-member ring of the polimethine chain exhibit higher singlet oxygen generation quantum yields than the corresponding *N,N*-diethylamine substituted ones, which possibly arises from a stronger intramolecular hydrogen-bonding in the molecules possessing the secondary amine. The following higher degree of rigidification of the dye's structure can lead therefore to a decrease of the efficiency of the non-radiative decay by photoisomerization and to an inherent rise of the efficiency of intersystem crossing.

The quantum yields of singlet oxygen generation are also affected very distinguishably by the length of the pendent *N*-alkyl chain, differing, for dyes incorporating the same heteroaromatic group, from 0 to ca. 0.20. When the effect is observed, the higher value of ϕ_Δ belongs invariably to the dye with the longer *N*-alkyl group, probably because a bulkier substituent hinders photoisomerization with more efficacy.

4. Conclusions

The quantum yields of singlet oxygen generation of several *N*-methylamino- (**1**, **2**) and *N,N*-diethylaminosquarylium cyanine dyes (**3**, **4**) derived from benzothiazole, benzoselenazole and quinoline were determined measuring the luminescence of the dyes in the near infrared, and were found to depend on the length of the pendent *N*-alkyl chains and on the nature of both the heteroaromatic nuclei and the central amine moiety.

For some of the dyes, especially those containing selenium atoms and the secondary amine group as substituent, the absorption and the quantum yields determined for the singlet oxygen production suggest that these may be potential candidates as sensitizers for photodynamic therapy.

Acknowledgements

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