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A versatile fluorescent molecular probe endowed with singlet oxygen generation under white-light photosensitization

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ABSTRACT

Despite fluorescent photodynamic therapy (fluorescent-PDT) dyes are promising theranostic agents, current approaches unfortunately involve crucial shortcomings (such as, narrow absorption bands, high cost, low bio-compatibility and specificity, low dual efficiency) making difficult their clinical translation. Particularly, efficient fluorescent-PDT agents triggered under white-light, with potential application in topic solar treatments, are scarce. Here, we describe the rational development of a novel fluorescent-PDT molecular biomaterial based on BODIPY building blocks able to sustain, simultaneously, synthetic accessibility, high fluorescence and phototoxicity within a broad spectral window, biocompatibility, including low dark toxicity and high cell permeability with selective accumulation in lysosomes and, what is more important, excellent efficient activity triggered under white light. These all-in-one combined properties make the new dye a valuable ground platform for the development of future smarter theranostic agents.

1. Introduction

Theragnosis defines an emerging medical protocol combining imaging and therapy using a single biomaterial displaying both capacities, which is valuable to advance in the establishment of patient-customized treatments [1–3]. Photodynamic therapy (PDT) using $^1O_2$ photosensitizers (i.e., dyes able to generate cytotoxic singlet oxygen under light irradiation) suited to bring fluorescence (i.e., imaging capability) are currently undergoing intensive investigations as promising theranostic biomaterials [2,4–6].

Although conceptually inspiring, fluorescent-PDT agents are still at an early stage of development, since their dual activity demands requirements of photophysically-opposed processes into a single dye: high enough fluorescence signal and photostability allowing bioimaging under severe irradiation conditions and/or prolonged times; fluorescence quenching via triplet-state population enabling efficient production of $^1O_2$ [7–9]. The incorporation of heavy atoms into the molecular structure of fluorescent dyes is one of the most common approaches to enhance intersystem crossing coupling and, therefore, to improve the generation of $^1O_2$ in organic dyes [7–9]. However, this approach reduces drastically its fluorescence efficiency and increases the dye cytotoxicity, which is an important limitation when developing a photosensitizer (preferably with null cytotoxicity under dark conditions to avoid unwanted side effects when administered to patients) [10]. In this regard, the last generation of fluorescent-PDT agents based on halogen-free organic dyes has reduced the undesired dark toxicity while enhancing the required phototoxicity, but brightness, absorption spectral broadness and biospecificity remain low [2].

Therefore, the development of smarter organic dyes that successfully integrate, all-in-one, the required complex multifunctionality (low toxicity, high phototoxicity, high-enough fluorescence,

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biospecificifity, and robustness and versatility from both photophysical and biological points of view) is demanded to overcome the above-mentioned main shortcomings. Moreover, fluorescent-PDT agents designed "ad hoc" to be efficiently triggered under white light radiation are unknown even though white-light PDT has been proposed as an effective therapeutic approach for topic daylight treatments on fungal diseases, cariogenic microorganisms and dermatologic lesions (acne, keratosis, skin tumors and so forth) [11–16]. Photosensitizers activated under white light radiation have been already reported although their absorption profiles mismatch the spectral emission of the light source (i.e., solar radiation) [11–19]. In fact, they exhibit just one and narrow absorption band in the visible spectral region allowing white-light trigger activation but reducing the harvested light over the entire wavelength region (400–750 nm) and, consequently, limiting significantly the therapeutic efficiency.

To this goal, we have judiciously define a new strategy to develop, through a straightforward and cost-effective synthetic protocol, a multichromophoric organic system based solely on BODIPY chromophores (**1** in Fig. 1) with the following properties: (1) wide and strong absorption over the UV–visible spectrum to enable white-light triggered activity; (2) high-enough fluorescence to make bioimaging possible; (3) efficient triplet-state population to enable generation of $^1O_2$ without using heavy atoms; (4) robustness to endure relatively-high density photo-excitation; (5) absence of dark toxicity (6) high biocompatibility to promote specific localization into cell. All these combined properties in a single fluorescent-PDT agent are a novelty, and should make the desired dye a promising ground platform towards the development of theranostic agents for clinical applications. In particular, those related with treatment of skin disease by means of daylight irradiation, in which deep penetration of the incoming light are not required.

2. Experimental

2.1. General methods

2.1.1. Synthesis

All starting materials and reagents were obtained commercially, unless otherwise indicated, and used without further purifications.

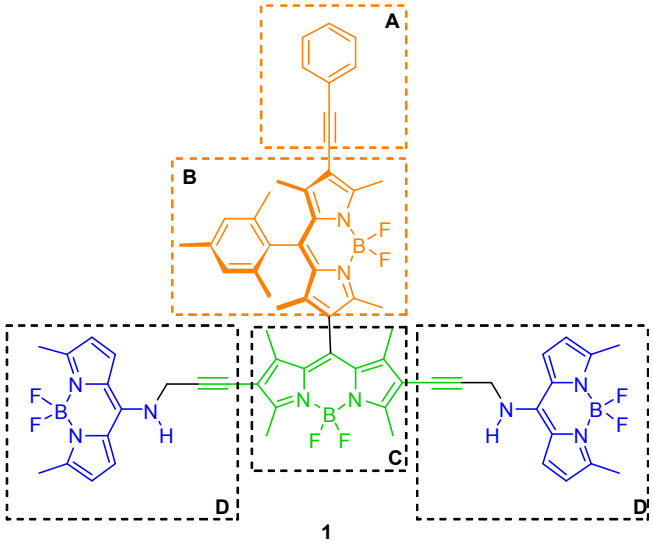

Fig. 1. Developed fluorescent-PDT molecule (key BODIPY moieties in different colors and key building blocks fragments into dotted windows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Common solvents were dried and distilled by standard procedures. Flash chromatography was performed using silica gel (230–400 mesh). Melting points were determined on a melting point apparatus and are uncorrected. NMR spectra were recorded at 20 °C, and the residual solvent peaks used as internal standards. FTIR spectra were obtained from neat samples using the ATR technique. High resolution mass spectrometry (HRMS) was performed using the EI technique.

2.1.1.1. General procedure for iodination reactions. A solution of ICl in CH_2Cl_2 was added dropwise to a solution of the corresponding BODIPY dye in CH_2Cl_2/MeOH (1:1, v/v). Amounts are indicated in each case. The mixture was stirred at r.t. for 10–20 min (the reaction was monitored by TLC). Then, the reaction solvent was evaporated under vacuum, and the resulting mixture dissolved in CH_2Cl_2. The obtained solution was washed with H_2O, dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. The obtained iodinated BODIPY was purified by flash chromatography on silica gel.

2.1.1.2. General procedure for Sonogashira reactions. The corresponding alkyne (2 mol equiv) and CuI (amount indicated in each case) were added to a solution of the corresponding haloBODIPY dye in tetrahydrofuran/diisopropylamine (THF/DIPA, 2:1, v/v) under argon. The mixture was stirred at r.t. for 5 min and, then, $Pd(PPh_3)_2Cl_2$ (10 mol%) was added, maintaining the stirring under argon. The reaction was monitored by TLC. CH_2Cl_2 was finally added to the mixture, and the obtained organic layer washed with 10% HCl, dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. The obtained Sonogashira product was purified by flash chromatography on silica gel.

2.1.2. Photophysical properties and singlet oxygen production

Photophysical signatures were measured in diluted solutions ($2 \cdot 10^{-6}$ M) in ethyl acetate (spectroscopic grade) and 1 cm path length quartz cuvettes. The absorption spectra were registered in a Varian spectrophotometer (model Cary 4E). The steady-state fluorescence and singlet oxygen emission spectra, as well as the fluorescence decay curves were recorded in an Edinburgh Instruments spectrofluorimeter (model FLSP920). The emission spectra were corrected from the monochromator wavelength dependence, the lamp profile and the photomultiplier sensitivity. Fluorescence quantum yields (ϕ_{fl}) for compounds **1**, **5** and **7** were calculated upon excitation at 500 nm using commercial PM597 (ϕ_{fl}^r = 0.43 in ethanol) as reference, while for the blue-edge emitting compound **9**, Coumarin 1 (ϕ_{fl}^r = 0.75 in ethanol) upon excitation at 400 nm, was considered. The energy transfer efficiency was checked measuring the absolute photoluminescence quantum yield of the "orange" acceptor upon selective excitation (400 nm) of the first "blue" donor by means of an integrating sphere coupled to the aforementioned spectrofluorimeter. The integrating sphere was also used to measure the fluorescence efficiency of compound **1** in the culture media. Radiative-decay curves were registered through time-correlated single-photon counting technique with picosecond time-resolution. Fluorescence emission was monitored at the maximum upon excitation at 500 nm by a wavelength-tunable laser (Fianium) with 150 ps full width at half maximum (FWHM) pulses. Lifetime values (τ) were obtained after the deconvolution of the instrumental response signal from the recorded decay curves by means of an iterative method. The goodness of the exponential fit was controlled by statistical parameters (chi-square and Durbin-Watson) and analysis of the residuals. The radiative (k_{fl}) and non-radiative (k_{nr}) rate constants were calculated from the fluorescence quantum yield and lifetime: $k_{fl} = \phi_{fl}/\tau$ and $k_{nr} = (1\text{-}\phi_{fl})/\tau$.

The production of $^1O_2$ was determined by direct measurement of the luminescence at 1270 nm with a NIR detector integrated in the spectrofluorimeter (InGaAs detector, Hamamatsu G8605-23).