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Synthesis, characterization and photophysical properties of luminescent non-symmetric 4-pyridyl benzothiadiazole derivatives

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ABSTRACT

Two nonsymmetrical π-extended 2,1,3-benzothiadiazoles (BTDs) bearing a 4-pyridyl moiety (**BTD-4pyr** and **BTD-Et4pyr)** were synthesized by sequential cross coupling reactions. The structural difference between the two dyes is the presence of a triple bond between the BTD core and the 4-pyridyl moiety in **BTD-Et4pyr**. The compounds architecture is similar to previously described selective mitochondrial biomarkers. Both compounds exhibit large Stokes shifts (93–137 nm), high fluorescence quantum yields and linear fluorescence response in the nanomolar range. The existence of the triple bond decreased in about 35% the fluorescence measured from **BTD-Et4pyr** in respect to **BTD-4pyr**. Solid-state fluorescence of the dyes were measured, producing considerable smaller Stokes shifts than the ones observed in solutions (74 nm for **BTD-4pyr** and 66 nm for **BTD-Et4pyr**). X-ray crystallography analysis of **BTD-4pyr** indicated a quinoid character for the BTD ring and a nonplanar relation between the BTD core and the aryl/heteroaryl groups. DFT calculations pointed out that the LUMO electron density is concentrated over the BTD core. In relation to HOMO, the electron density **is** distributed mainly at the methoxyphenyl group, in the hydrocarbon fraction of the BTD core and in ethynyl group for **BTD-Et4pyr**.

1. Introduction

Many efforts have been made in the design and synthesis of new π-conjugated molecules with luminescent properties. This search is stimulated by the large number of application of these compounds in different areas of light technology, including: OLEDs constituents [1,2]; luminescent probes for biological [3,4] and analytical chemistry [5–7]; organic sensitizers for solar cells [8], among others. In this context, 2,1,3-benzothiadiazole (BTD) derivatives with π-extended conjugation surge as privileged compounds for these applications, thanks to some relevant features, including: large Stokes shifts, BTD's strong electron withdrawing ability (which facilitates intramolecular charge transfer stabilizing processes), relatively high reduction potential and electron affinity [9].

Concerning the design of novel fluorescent probes, it is a matter of interest for both analytical chemistry and chemical biology. In the analytical point of view, the development of selective and sensitive dyes increases the range of options to perform tagging, derivatization and indirect probing for quantitative determinations. With respect to chemical biology, depending on the structure, the dyes can be used as bioprobes, capable to selective label cellular structures/macromolecules, as well as allow the study of dynamic cellular processes [3,4]. In this way, some π-extended benzothiadiazole (BTD) derivatives have emerged in last years as privileged scaffolds for selective staining organelles and cellular components. Representative examples of BTD-based bioprobes are displayed in Fig. 1.

The green emitters **1a** and **1b** selectively tagged mitochondria in three different cell lines. These compounds were designed to display two intramolecular H-bonds, which are responsible to keep the planarity e allow a putative ESIPT (excited state intramolecular proton transfer) as excited state stabilizing process [10]. The distribution of the fluorescent BTD-based glycoligand **2** was **s**tudied in living and artificial cell system. The compound is able to access the inner compartment passively and accumulates specifically in membranes [11]. The compound **3a** was produced as a probe for

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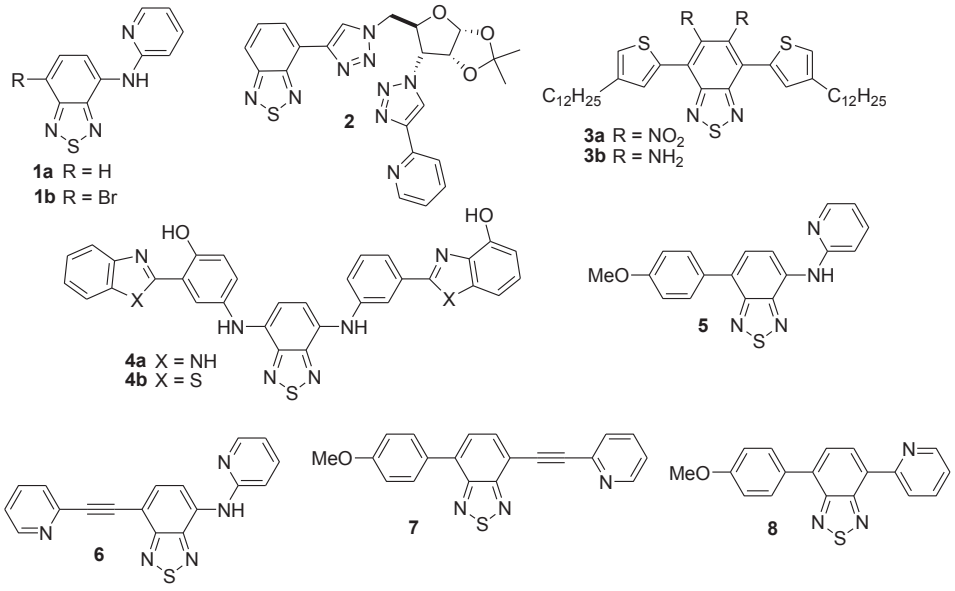

Fig. 1. Representative bioprobes based on BTD core.

hypoxic tumor cells. This NO_2-BTD derivative is nonfluorescent, however, when it was added to culture of MG63 cancer cells in hypoxic conditions, a strong red fluorescence was observed. This emission is due to the reduction of **3a** to its fluorescent NH_2-derivative **3b**. Moreover, it was determined that the presence of **3a** in cells stimulates the expression of the nitroredutase, which could improve the conversion of **3a** to **3b** [12]. The symmetric BTD derivatives **4** enable high quality images and selective staining for nuclear dsDNA in human stem cells. The compounds exhibited large stoke shifts and high stability, representing an interesting alternative to the commercial imaging probes [13]. The compounds **5** and **6** were evaluated as mitochondrial marker in cancer cells [14]. The results were not satisfactory, however it was observed that the presence of the donator group 4-methoxyphenyl in **5** was capable to increase the emission intensity and, the presence of the ethynylpyridyl group of **6** slightly enhanced the mitochondrial selectivity. Bearing these results in mind, authors synthesized the bioprobe **7**, which possess both 4-methoxyphenyl and ethynylpyridyl groups. This compound proved to be a remarkable mitochondrial dye for bioimaging experiments, allowing organelle imaging and revealing the mitochondrial dynamics during the whole cell division cycle. Another efficient and selective bioprobe for mitochondrial staining is **8** [15]. This compound structurally resembles **7**, however, lacking the triple bond spacer. Similarly to observed for **7**, the 4-methoxyphenyl group is responsible for the increasing of the fluorescence intensity whereas the 2-pyridyl group is related to organelle selection.

Taking into account the outstanding results observed for mitochondrial selection observed for **7** and **8**, this work describes the synthesis and the photophysycal characteristics, in solution and in solid phase, of the fluorescent compounds **BTD-4pyr** and **BTD-Et4pyr.** These dyes were designed considering the structural features that ensured intense emission (4-methoxyphenyl group) and selectivity (pyridyl group) to the previously described BTD-based bioprobes **7** and **8**. The compounds were obtained with good yields through sequential cross-coupling reactions, display large Stokes shifts, high fluorescence quantum yields and linear fluorescence response in the nanomolar range. Moreover, the dyes exhibit absorption/emission spectra and HOMO/LUMO electronic maps very similar to previous reported dyes.

2. Experimental

2.1. Material and instruments

All solvents (acetonitrile, methanol, acetone, ethyl acetate, tetrahydrofuran, propan-2-ol, dichloromethane, toluene, pentane and diethyl ether) were of analytical grade and purchased from Merck (Germany). Fluorescein sodium salt was form Fluka, Gemany, and NaOH was from Merck. The monoarylated intermediate **3** was synthesized using procedures described in literature [16]. Cesium fluoride (CsF) and potassium carbonate (K_2CO_3) were dried under vacuum, at 100 °C during 1 h before the use. Arilboronic acid/ester were obtained from Sigma-Aldrich and used as received. All cross-coupling reactions were performed under argon atmosphere. Toluene, tetrahydrofuran and dioxane were distillated under sodium/benzophenone before the use in reactions.

UV–vis absorption spectra were acquired on a Cary 50 single beam spectrophotometer using scan velocity of 1200 nm min^{-1}, spectral bandpass of 10 nm and 1 cm path length quartz cuvettes (two optically clear faces). Photoluminescence spectra and steady state photoluminescence measurements were performed on a PerkinElmer model LS 45 luminescence spectrophotometer with 10 nm excitation and emission spectral bandpass. Scanning was made at 1500 nm min^{-1} with solutions placed in 1 cm optical pathlength quartz cuvettes (four optically clear faces). Photoluminescence lifetimes were obtained from decay measurements using a Horiba-Jobin Ivon-IBH time correlated single photon counting fluorimeter with a 330 nm nanoLED source (N-16, 1.0 ns nominal pulse duration at 1 MHz repetition rate). Computer programs supplied by the manufacturer were employed to process the time resolved data. Nuclear resonance magnetic spectra were recorded in a Bruker (Germany) model Avance III HD 400 MHZ spectrometer. Electron spray ionization high-resolution mass spectrometric (ESI-(+) HRMS) analyses were performed on a Q-TOF (Micromass) mass spectrometer (Waters, USA) with the compounds dissolved in a 10% solution of formic acid in acetonitrile. A Bruker D8 VENTURE diffractometer outfitted with a PHOTON 100 area detector and a graphite monochromator (Mo-Kα, $\lambda = 0.71{,}073$ Å) was used to collect the X-ray data for the structural analysis of **BTD-4pyr**.