



Multiple fluorescent behaviors of phenothiazine-based organic molecules



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ABSTRACT

We have designed a conventional one-step Suzuki coupling synthetic method to prepare 3,7-di-aryl substituted 10H-phenothiazine derivatives and investigated their optical behaviors. The compound 3,7-Bis (4-aminophenyl) phenothiazine (compound **1**), substituting with electron-donating aniline, can exhibit photodamage behavior toward cancer cells. Furthermore, the compound **1** can form fluorescent organic nanoparticle (FON) in acidic aqueous whereas can emit red fluorescence in alkaline organic solvent. More importantly, compound **1** can be oxidized to manufacture a stable near-IR dye (>950 nm). Alternatively, the control compound 3,7-Bis (4-nitrophenyl) phenothiazine (compound **2**) enabled us to determine that an electron-withdrawing group, when attaching on the phenothiazine, is unfavorable for molecular design to manufacture a cation form of NIR dye but is favorable to stabilize the phenothiazine core and manufacture an anion form of NIR dye.

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1. Introduction

The phenothiazine (PTZ) core scaffold is a class of electron-rich tricyclic nitrogen–sulfur heterocycles, which exhibits relatively intense luminescence, high photoconductivities and undergoes reversible oxidation processes. In general, PTZ derivatives (PTZs) have a low oxidation potential and a high propensity to form stable radical cations [1,2]. Moreover, the well-defined electron-donating properties of PTZs [3–6] can be partially associated with electrophores to alter the oxidation potentials of PTZs [7,8], especially electronic substitutions in the 3- or 3,7-positions. Thus, because of its ground state intramolecular charge transfer (ICT) and excited-state photoinduced electron transfer (PET) properties, PTZs are widely used as organic light-emitting diodes (OLEDs) [3,9], acid–base dyes and pigments [10,11], semiconductors [12,13], chemical sensors [14] or near-IR dyes [15,16]. However, most of these applications use the protected PTZ structure with covalent substitutions on the nitrogen atom at the 10-position (10N–H becomes 10N–R). There is a particular examples, such as the methylene blue (MB) derivatives, the important pharmacological applications of PTZs

are attributed to its stable radical cation heterocyclic form, which can be used for photodynamic therapy and exhibit near infrared fluorescence [17–19]. In this case, the proton 10N–H of PTZ is indispensable in the formation of a stable radical cation, but this leads to certain limitations for structural modifications. Thus, few reports on NIR chromophores from PTZs have been published and most of the above applications are based on the use of a protected PTZ structure with covalent substitutions on the nitrogen atom at the N-10-position.

Recently, we described the convenient preparation of divinyl-substituted 10H-phenothiazines at the 3- or 3,7-positions without a protecting group at the N-10 position and concluded that the proton detachments from the 10N–H of the PTZs can be used to construct NIR ionic sensors dyes with dual photophysical properties, red emission in DMSO solution with NIR absorptive energy and green emission in aqueous solution [20,21]. These findings indicate that the deprotonation of these PTZs on the nitrogen atom at the 10 position (10N–H) are critically important and that these fluorophores and NIR chromophores can eventually be switched on by the proton loss. However, we also described the aggregation-induced emission enhancement (AIEE) properties and developed fluorescent organic nanoparticle (FON) models of anionic and cationic PTZs [21,22]. In particular, the AIEE properties of protonated PTZs were observed for both in vitro spectral studies and cellular staining and can eventually be used in cancer cell recognizer fluorophores.

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In the present study, we describe the convenient preparation of diaryl-substituted 10H-phenothiazines at the 3,7-positions (Scheme 1). The optical properties of protonated, deprotonated and oxidated PTZs were examined, illustrating spectral diversities including (1) visible fluorophores and NIR chromophores switched on by the loss of protons at 10N–H, (2) FON based on the AIEE properties of protonated (cationic) 10H-phenothiazine derivatives, (3) a stable phenothiazinium product with an absorption maxima of more than 950 nm after an oxidation reaction and (4) the ability to support extra phototoxicity to cancer cells with super oxide generation.

2. Experimental

2.1. Material

The general chemicals employed in this study were the highest grade available and were obtained from Acros Organic Co., Merck Ltd., or Aldrich Chemical Co. and were used without further purification. All of the solvents were of spectrometric grade.

2.2. Apparatus

The absorption spectra were generated using a Thermo Genesys 6 UV–visible spectrophotometer, and the fluorescence spectra were recorded using a HORIBA JOBIN-YVON Fluoromas-4 spectrofluorometer with a 1 nm band-pass and a 1 cm cell length at room temperature. The cellular fluorescence image was taken using a Leica AF6000 fluorescence microscope with a DFC310 FX digital color camera. The Xenon light source LAX-cute (Asahi Spectra) was used to measure the singlet oxygen yield and PDT cell death.

2.3. Cell culture conditions and compound incubation

The HeLa human cervical cancer cells were maintained in Modified Eagle's Medium (MEM) containing non-essential amino acids, Earle's salts, L-glutamine, 1 mM sodium bicarbonate, 1 mM sodium pyruvate, 1% (penicillin + streptomycin) and 10% fetal bovine serum (FBS). Cells were cultured at 37 °C in a humid atmosphere with 95% air and 5% CO₂. Before the microscopic observation, cells were seeded onto coverslips and incubated for 24 h. The next day, cells were incubated with various concentrations of

compounds for 8 h, for which the DMSO stock solutions were diluted in serum-free medium before use (1/100, v/v).

2.4. Light-induced cytotoxicity assay

The cancer cells were examined in the assay. Varying concentrations of compounds were incubated with the cells in the dark for 8 h. Subsequently, the culture medium was removed and fresh culture medium was added to each well. The plates were irradiated using a light source, which is described in the next section, and then incubated overnight at 37 °C. All the assays were performed in triplicate, and the average of the three to five individual runs is presented.

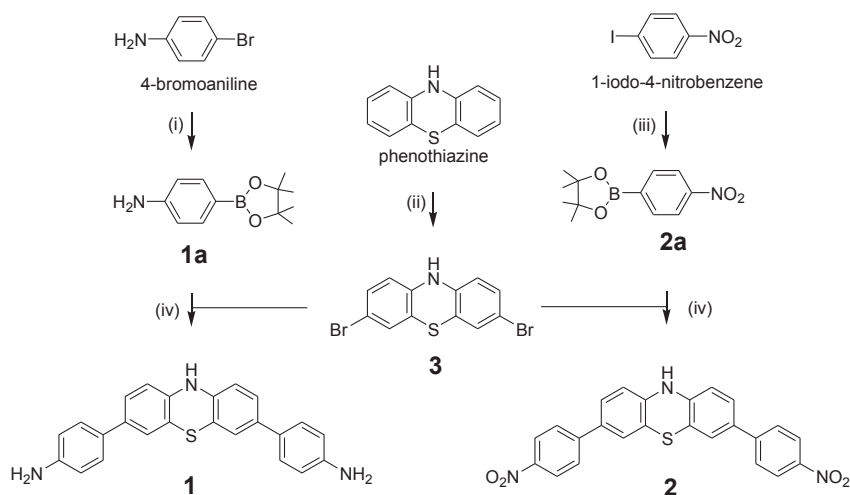
2.5. Measurement

Preparation of mixed solutions: first, HCl aqueous solutions with a pH of 2 were prepared and then directly mixed with THF at volume ratios of 0, 25, 50, 75, and 100 percent. The light source for the measurement of reactive oxygen species (ROS) and photodamage was a UV light (a 20-W power tunable Xenon lamp that passes through a UV-a mirror module). The light output from the optional light guide and collimator lens was an average of 3 mW/cm² on the dish surface. The singlet oxygen yields test from the compounds that were dissolved in DMSO was determined using a photo-steady-state method using 1,3-diphenylisobenzofuran (DPBF) as the scavenger. The superoxide generation from the compounds in an aqueous solution was determined using the photo-steady-state method using 4-((9-acridinacarbonyl) amino)-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO-9-ac) as the radical detector. For the fluorescence microscope observation, to collect blue emission of the compounds, a UV light cube, in which light passed through a 370 ± 10 nm bp filter and the emission was collected through a 440 nm lp filter.

2.6. Synthesis

2.6.1. 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)aniline: (1a)

4-Bromoaniline (1 mmol) was added into a high pressure bottle containing the catalyst of bis(triphenylphosphine) palladium(II) chloride (5 mg, Strem Chemicals), followed by the addition of the solvent pair (20 mL of dioxane/1 mL of Et₃N) and pinacolborane



Scheme 1. Synthetic route to produce phenothiazine-based photosensitizers: (i) pinacolborane, Pd(PPh)₃Cl₂, dioxane/Et₃N, N₂. (ii) NBS (N-bromosuccinimide), THF, ice bath. (iii) bis(pinacolato)diboron, Pd(PPh)₃Cl₂, dioxane/Et₃N, N₂. (iv) K₂CO₃, (o-tol)₃P, Pd(OAc)₂, DME/H₂O = 5/1, N₂, reflux.

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