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A naphthalene-based two-photon fluorescent probe for selective and sensitive detection of thiophenols

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ABSTRACT

A naphthalene-based two-photon fluorescent probe for thiophenols has been reported in this work. The probe can be applied to the quantification of thiophenols with a linear range covering from 2.0×10^{-8} to 7.0×10^{-6} mol L^{-1}. It exhibited a high selectivity and excellent sensitivity with a detection limit of 9.6 nM. Moreover, it was successfully used for practical detection of thiophenol in water samples with a good recovery, and two-photon imaging of thiophenol in live cells and tissues at a depth of 40–155 μm.

1. Introduction

Thiophenols are the crucial reaction intermediates for organic synthesis, and can be widely employed in the production of pesticides, pharmaceuticals, and agrochemicals [1–3]. However, they are also a class of highly toxic and pollutant compounds and the medium lethal dose (LC_{50}) ranges from 0.01 to 0.4 mM for fish [4]. Prolonged exposure to thiophenols can lead to severe damage to the central nervous and other nervous systems including increased respiratory, muscular weakness, hind limb paralysis, coma, and even death [5]. Thiophenols have been listed as one of the prioritized pollutants by the United States Environmental Protection Agency (USEPA waste code: P014) [6]. Moreover, thiophenols are much more toxic than biologically important aliphatic thiols such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) [7–9]. Thus, rapid, sensitive and selective detection of thiophenols is of considerable interest in the fields of chemical, environmental and biological sciences.

In the past few years, many analytical methods such as high-performance liquid chromatography [10], gas chromatography-mass spectrometry [11] and UV–vis spectrometry [12] have been reported to detect the concentration of thiophenols. Due to their advantages of simplicity, high sensitivity and low lost, a large number of fluorescent probes have been developed for the determination of thiophenols so far [13–25]. However, most of them work under one-photon excitation, which limits their biological applications. An effective alternative to circumvent the drawbacks of one-photon excitation probing is the utilization of two-photon microscopy (TPM) with two-photon excitable probes. TPM has more advantageous features over one-photon microscopy including reduction of photodamage and photobleaching, better three dimensional spatial localization, deeper penetration depth, lower tissue autofluorescence and self-absorption [26–30]. However, only very few two-photon fluorescent probe for thiophenols have been reported [31]. Thus, searching for two-photon fluorescent probe for thiophenols is still an active field as well as a challenge for the analytical chemistry research effort.

Naphthalene derivative with a donor-π-acceptor (D-π-A) structure has a large two-photon active absorption cross-section and many other excellent characteristics, such as high fluorescence quantum yield and good photo- and chemo-stability. Thus, naphthalene derivative has been employed extensively as an efficient TP platform for designing TP probes for various targets [32–40]. In this work, we develop a highly sensitive naphthalene-based two-photon fluorescent probe (probe **1**, Fig. 1) for thiophenols.

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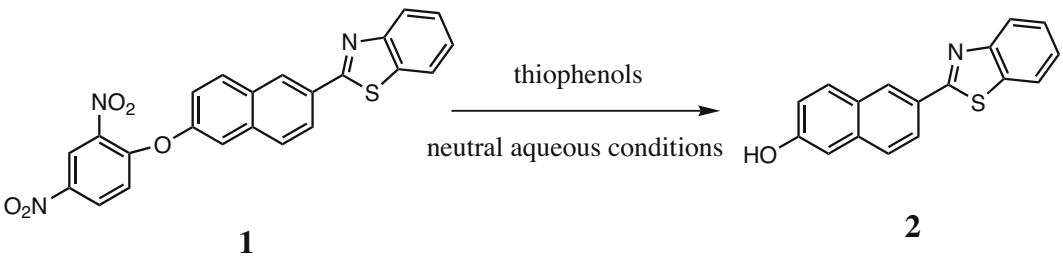

Fig. 1. The design of two-photon fluorescent probe **1** for thiophenols. Neutral aqueous conditions: 10 mM PBS buffer, pH 7.4, containing 60% DMF as a co-solvent.

The high nucleophilic reactivity [34,41–43] or transition metal-affinity [44,45] of the thiol group has been utilized to developed fluorescent thiol probes. Notably, fluorescent thiophenol probes have been constructed by exploiting the thiolysis of dinitrobenzenesulfonylamides [13–20] or dinitrophenyl ethers [22,23]. In the present research, we judiciously designed and synthesized a selective two-photon fluorescent probe **1** for thiophenols based on the thiolysis of dinitrophenyl ethers. Probe **1** includes a D-π-A-structured naphthalene derivative and a dinitrophenyl ether moiety. It could be anticipated that the introduction of an electron-withdrawing dinitrophenyl group into the naphthalene platform would break its D-π-A structure and significantly diminish the TP emission of compound **1**. If compound **1** is able to be converted into compound **2** with electron-donor hydroxyl moiety by thiophenols under the appropriate conditions, a substantial fluorescence turn-on response would be observed. To prove the feasibility of our design concept, probe **1** was synthesized and further evaluated for the quantitative detection of thiophenol in water samples and two-photon fluorescent thiophenol imaging in living cells and tissues.

2. Experimental

2.1. Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Doubly distilled water was used throughout all experiments. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer (Thermo Finnigan). NMR spectra were recorded on a Bruker DRX-500 spectrometer using TMS as an internal standard. All chemical shifts are reported in the standard δ notation of parts per million. Thin layer chromatography (TLC) was carried out using silica gel 60 F254, and column chromatography was conducted over silica gel (200–300 mesh), both of which were obtained from the Qingdao Ocean Chemicals (Qingdao, China). The pH was measured with a Mettler-Toledo Delta 320 pH meter. UV–vis absorption spectra were recorded on a UV-2600 spectrophotometer (Tokyo, Japan). The one-photon excited fluorescence measurements were conducted at room temperature on a Fluoromax-4 spectrofluorometer (HORIBA JobinYvon, Edison, NJ) with both excitation and emission slit set at 3.0 nm. Two-photon fluorescence images of live cells and living tissues were obtained using an Olympus FV1000-MPE multiphoton laser scanning confocal microscope (Japan). Data processing was performed on a Pentium IV computer with software of SigmaPlot.

2.2. Syntheses

Compound 1 was readily prepared from 2-aminothiophenol and 6-hydroxy-2-naphthaldehyde by a two-step procedure under mild conditions with a good yield (Scheme S1). The structures of **1** and **2** were well characterized using ^{1}H NMR, ^{13}C NMR, and MS (see ESI).

2.3. Measurement of two-photon cross section

The two-photon cross section (δ) was determined by using femtosecond fluorescence measurement technique as described [46]. The two-photon excited fluorescence was measured in DMF solvent. The test solutions of the compound **1** (5.0 μM) and compound **2** (5.0 μM) in DMF solvent were prepared, respectively. The two-photon induced fluorescence intensity was measured at 700–800 nm by using rhodamine B (5.0 μM) in methanol as the reference, whose two-photon property has been well characterized in the literature [46]. The intensities of the two-photon induced fluorescence spectra of the reference and sample emitted at the same excitation wavelength were determined. The two-photon absorption cross section was calculated by using $\delta = \delta_r(S_s\Phi_r\ \phi_r c_r)/(S_r\Phi_s\ \phi_s c_s)$: where the subscripts s and r stand for the sample and reference molecules. The intensity of the signal collected by a CCD detector was denoted as S. Φ is the fluorescence quantum yield. ϕ is the overall fluorescence collection efficiency of the experimental apparatus. The number density of the molecules in solution was denoted as c. δ_r is the two-photon absorption cross section of the reference molecule.

2.4. Cytotoxicity assay

MTT test was performed referred to the protocol [47] with minor change. HeLa cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) containing high glucose supplemented with 10% fetal bovine serum, 100 units mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. When in the proliferative period, HeLa cells ($\sim 3 \times 10^5$ cell/mL) were dispersed within replicate 96-well microliter plates to a total volume of 100 μL/well and maintained at 37 °C in a 5% CO_2/95% air incubator for 24 h. Then, the culture media was removed and the cells were incubated in culture medium containing the compound **1** and compound **2** with concentrations (0, 2, 4, 8, 16, 20 μM) for 48 h, respectively. Then the cells were washed with the culture medium. An amount of 100 μL of the new culture medium containing MTT (10 μL, 5 mg mL^{-1}) was then added, followed by incubating for 4 h to allow the formation of formazan dye. After removing the supernatant, 150 μL DMSO was added to each well to dissolve the formazan crystals. After shaking for 10 min, the absorbance at 570 nm was measured by microplate reader (Synergy 2, Bio Tek Instruments Inc.). The number of viable cells was determined by an MTT assay as described [48].

2.5. Confocal imaging in live cells

HeLa cells were cultured in DMEM containing high glucose supplemented with 10% fetal bovine serum, 100 units mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. One day before imaging studies *in vivo*, the cultured cells were passaged and plated on a Petridish.