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Nitroolefin-based BODIPY as a novel water-soluble ratiometric fluorescent probe for detection of endogenous thiols

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ABSTRACT

Small molecule biothiols, including cysteine (Cys), homocysteine (Hcy), and glutathione (GSH), play many crucial roles in physiological processes. In this work, we have prepared a nitroolefin-based BODIPY fluorescent probe with excellent water solubility for detection thiols, which displayed ratiometric fluorescent signal for thiols. Incorporation of a nitroolefin unit to the BODIPY dye would transform it into a strong Michael acceptor, which would be highly susceptible to sulfhydryl nucleophiles. This probe shows an obvious ratio change upon response with thiols, an increase of the emission at 517 nm along with a concomitant decrease of fluorescence peak at 573 nm. Moreover, these successes of intracellular imaging experiments in A549 cells indicated that this probe is suitable for imaging of ex-/endogenous thiols in living cells.

1. Introduction

It is becoming increasingly clear that the small molecular thiols such as cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) play very critical role in physiological and pathological processes [1–3]. For instances, Cysteine (Cys) is one of the biothiols which possesses important biochemical functions including protein synthesis, detoxification, metal binding and metabolism. However, high/low level of Cys involved in diseases, such as metabolic disorders, slow growth in children, a loss of muscle and fat, skin lesions and AIDS [4–6]. Hcy is a risk factor for cardiovascular, plasma total Hcy (tHcy) concentration is related to birth defects and cognitive impairment in the elderly such as Alzheimer diseases [7–9]. As one of critical cellular antioxidant, GSH plays various roles in reversible redox reactions and has vital cellular functions in living cells. GSH deficiency can lead to diabetes mellitus, Parkinson's disease, atherosclerosis, and liver disease. However, excess GSH are detected in a variety of cancer cells, which will hamper the radiation therapy or chemotherapy [10–12]. Therefore, the design and synthesis of thiols probes are of great significant.

A variety of fluorescent probes for thiols was designed based on the high nucleophilic reactivity of the thiol group [13], cleavage reactions by thiols [14], and others [15–17]. Several excellent Michael acceptors have been exploited [18–22]. Among the various Michael acceptors, nitroolefin is very attractive because of the strong electron deficiency of the nitro group [23–29]. Such as, in 2012, Hao and coworkers developed a turn-on type nitroolefin-based probe BODIPY-1 can highly selective detection of thiols in CH_3CN-HEPES buffer (0.1 M, 1:1, v/v, pH = 7.4) [24]; in 2014, Akkaya and coworkers reported a turn-on type nitroolefin-based probe can selective detect GSH in pH 7.4 (6:4, 30 mM, MES buffer/acetonitrile) [25]. However, ratiometric type nitroolefin-based thiols probe which can applicable in aqueous solution has not been reported previously (Table S2). To address these points, we design a novel nitroolefin-based thiols probe (BDP-NO_2) based on the addition reaction of thiols to nitroolefin. This probe can realize the quantitative detection of thiols in aqueous solution.

Herein, we subtly connected a methoxy-BODIPY fluorophore with nitroolefin to prepare the probe (BDP-NO_2). The BDP-NO_2 was synthesized from the reaction between methoxy-BODIPY-aldehyde and nitromethane depicted in Scheme 1.

2. Material and Methods

2.1. Materials

All chemical regents were purchased from commercial sources, and used without further purification. Phosphate-buffered saline (PBS) was purchased from Solarbio and prepared using deionized water.

2.2. Instruments

All NMR spectra (δ values, ppm) were recorded with Bruker AVANCE-600 MHz spectrometers. The crystal structure of BDP-NO_2 was confirmed by D8 Venture X-ray crystal diffractometer. The

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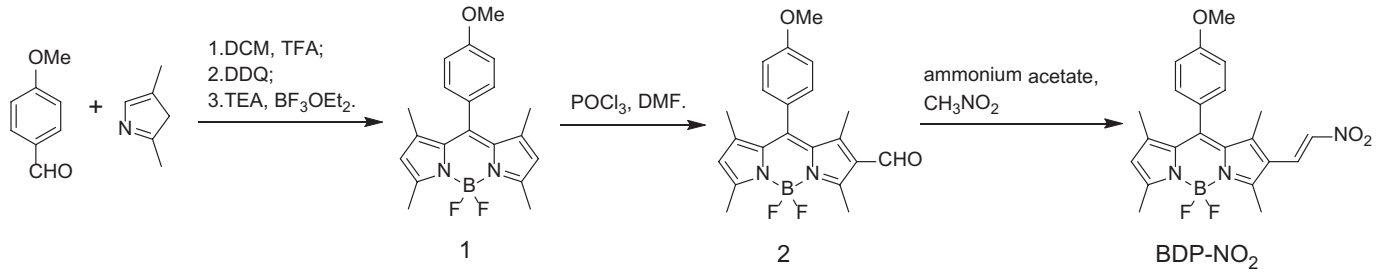

Scheme 1. The synthesis of the probe BDP-NO_2.

cytotoxicity experiments were evaluated on BioTek ELX 808 microplate reader. The cell imaging experiments were used Zeiss LSM-880 CLSM.

2.3. Synthesis of BDP-NO_2

The synthetic route outlined in Scheme 1 and the compound 1 and 2 were synthesized use the same methods as previous literature [30]. All related compounds were characterized by NMR spectroscopy. The probe was characterized by ESI-MS mass spectrometry and the crystal structure of probe was confirmed by X-ray crystal diffractometer.

2.3.1. BDP-NO_2

BODIPY- aldehyde (100 mg, 0.26 mmol) was dissolved in nitromethane (5 mL) solution, then NH_4OAc (80 mg, 1 mmol) was added, the solution has been stirred at 90 °C for 5 days. The nnitromethane was removed in vacuo. The crude was subjected to silica gel using DCM (1% MeOH) as the eluent. BDP-NO_2 was obtained as an orange-red solid (96 mg, 87% yield). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.05 (d, J = 12 Hz, 1H), 7.36 (d, J = 12 Hz, 1H), 7.19 (d, J = 12 Hz 2H), 7.07 (d, J = 12 Hz, 2H), 6.16 (s, 1H), 3.91 (s, 3H), 2.71 (s, 3H), 2.62 (s, 3H), 1.53 (s, 3H), 1.48 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 161.3, 160.6, 154.1, 147.3, 142.9, 140.4, 134.5, 130.9, 129.1, 126.1, 123.9, 120.0, 114.9, 55.4, 15.1, 13.9, 13.0. ESI-MS m/z: $[M + H]^+$ calcd for 426.18; Found 426.18. Crystal size: 0.180 × 0.090 × 0.050 mm, space group Triclinic P-1 a = 6.530(3) Å, b = 10.714(5) Å, c = 15.418(8) Å, α = 75.138 (16) °, β = 89.886(18) °, γ = 83.179(17) °, V = 1034.8(9) Å^3, Z = 2, T = 291(2) K, 15,523 refections measured, 5105 unique (R_{int} = 0.0330). Final residual for 287 parameters and 5105 refections with $I > 2\sigma(I)$: R_1 = 0.0735, wR_2 = 0.2013 and GOF = 1.039 (Fig. 1).

2.4. Imaging Experiments

2.4.1. Cell Culture and Imaging

A549 cells were prepared in Dulbecco's modified Eagle's medium in the atmosphere of 5% CO_2 and 95% air at 37 °C. The excitation wavelength at 480 nm, the orange channel was set at 573 ± 20 nm and the green channel was set at 517 ± 20 nm for BDP-NO_2.

2.5. Cytotoxicity Experiments

A549 cells were prepared in 96-well plates for one day, then washed with PBS, a series of concentrations of BDP-NO_2 (0, 2.5, 5, 10, 30, and 50 μM) were added to the wells and incubated for 5 or 10 h. CCK-8 was added to each well, the plate was incubated for 1 h. Optical densities at 450 nm were measured. These results display BDP-NO_2 is little low toxic to A549 cells (Fig. 2).

3. Results and Discussion

3.1. Selectivity of BDP-NO_2 Toward Thiols Over Various Analytes

The selectivity is a very important index for probe design. BDP-NO_2 was treated with various relevant analytes including Ala, Asn, Arg, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, Thr and Val in PBS buffer (10 mM, pH 7.4), then their fluorescence emission was determinated on fluorescence spectrophotometer. As shown in Fig. 3, fluorescent intensity changes only observed with the GSH, Hcy, Cys added in the solution. Other amino acid samples exhibited no obvious changes in fluorescence signal. These experiments showed BDP-NO_2 is highly selective toward thiols.

3.2. Spectral Titrations for Thiols

As the most abundant biological thiol, GSH was used to further examine the UV-absorption and fluorescence response of probe BDP-NO_2. The absorption spectra of BDP-NO_2 with the GSH was carried out in solution PBS (10 mM, pH = 7.4). As the Fig. 4 showed that the BDP-NO_2 exhibited a maximum absorption at 547 nm. The maximum absorption peak presented a decline upon the addition of thiols, which due to the conjugate structure has been broken by the addition reaction. In addition, the UV-absorption spectra of BDP-NO_2 upon addition of Cys and Hcy were showed in Fig. S11.

The fluorescence spectra of probe BDP-NO_2 (5 μM) with the GSH (0–300 μM) in PBS buffer was displayed in Fig. 5. The BDP-NO_2 is orange fluorescent (573 nm) in the absence of thiols. Upon the addition of GSH, a strong emission peak at 517 nm appeared in the fluorescence spectra, which attribute to the rapid addition of unsaturated olefins, accompanying with the ICT process was interrupted. However, it is unexpected, as the nucleophilic reactivity in the order Cys > Hcy > GSH, but

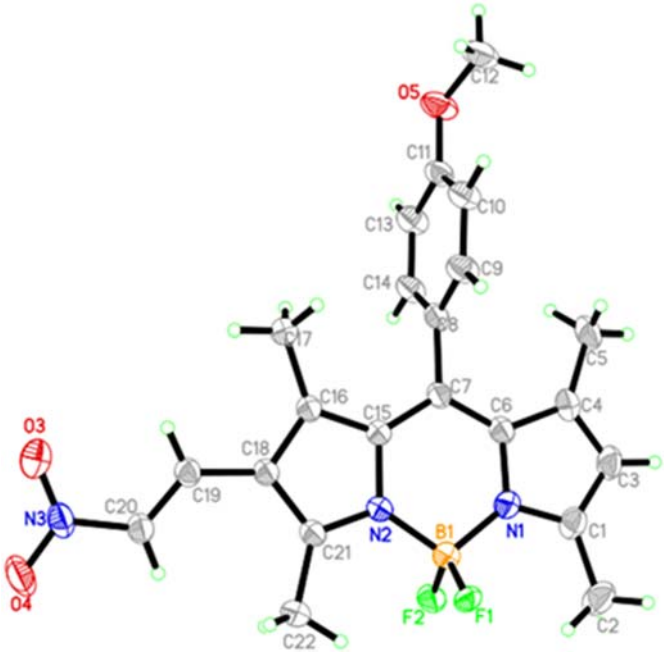

Fig. 1. The thermal ellipsoids of the BDP-NO_2 probe drawn at the 50% probability level.