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A lysosome-targetable turn-on fluorescent probe for the detection of thiols in living cells based on a 1,8-naphthalimide derivative

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ABSTRACT

Biological thiols, like cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), play crucial roles in biological systems and in lysosomal processes. Highly selective probes for detecting biological thiols in lysomes of living cells are rare. In this work, a lysosome-targetable turn-on fluorescent probe for the detection of thiols in living cells was designed and synthesized based on a 1,8-naphthalimide derivative. The probe has a 4-(2-aminoethyl)morpholine unit as a lysosome-targetable group and an acrylate group as the thiol recognition unit as well as a fluorescence quencher. In the absence of biothiols, the probe displayed weak fluorescence due to the photoinduced electron transfer (PET) process. Upon the addition of biothiols, the probe exhibited an enhanced fluorescence emission centered at 550 nm due to cleavage of the acrylate moiety. The probe had high selectivity toward biothiols. Moreover, the probe features fast response time, excitation in the visible region and ability of working in a wide pH range. The linear response range covers a concentration range of Cys from 1.5×10^{-7} to 1.0×10^{-5} mol·L^{-1} and the detection limit is 6.9×10^{-8} mol·L^{-1} for Cys. The probe has been successfully applied to the confocal imaging of biothiols in lysosomes of A549 cells with low cell toxicity. Furthermore, the method was successfully applied to the determination of thiols in a complex multicomponent mixture such as human serum, which suggests our proposed method has great potential for diagnostic purposes. All of such good properties prove it can be used to monitor biothiols in lysosomes of living cells and to be a good fluorescent probe for the selective detection of thiols.

1. Introduction

Low molecular weight biothiols, such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), are crucial cellular components that play essential roles in many physiological and pathological events, including redox hemeostasis, biocatalysis, metal biding, signal transduction and cellular growth [1–4]. However, abnormal levels of biothiols are thought to be implicated with the formation of a variety of serious diseases. Cys is a precursor for the production of protein and its deficiency can cause a number of syndromes, such as edema, slow growth in children, skin lesions, hair depigmentation, liver damage and weakness [5,6]. An elevated level of Hcy is known as a risk factor for cardiovascular disease, neural tube defects, dementia and Alzheimer's disease [7–10]. GSH is the most prevalent intracellular thiol and its abnormal level is directly linked with cancer, aging, heart problems, and other ailments [11–13]. Therefore, it is of growing importance to develop sensitive and selective methods to detect these biological thiols for the early diagnosis and therapy of some related diseases.

Up to now, several analytical techniques have been devoted to detecting the biothiols, including high-performance liquid chromatography (HPLC) [14,15], capillary elctrophoresis (CE) [16,17], electrochemical assays [18,19], UV–vis absorption spectrophotometry [20,21], Fourier transform infrared (FTIR) spectroscopy [22], fluorimetric sensing [23–26], and mass spectrometry [27,28]. Among all the methods developed, fluorescence technique is a frequently used method due to its many advantages including high sensitivity, inherent simplicity, easy operation, in vivo and in vitro bioimaging. Till date, a wide variety of fluorescent probes have been designed based on the strong nucleophilic reactivity or high transition metal affinity of thiol group. The fluorescence sensing mechanisms include Michael addition [29,30], cyclization with aldehydes [31,32], cleavage of sulfonamide and sulfonate ester [33–35], cleavage of disulfide [36,37], cleavage of Se—N bond [38], metal complexes-displace coordination [39,40] and others [41,42]. Most of the fluorescence probes for thiols can image thiols in blood samples and living cells but without location specificity, in particular subcellular localization [29–42].

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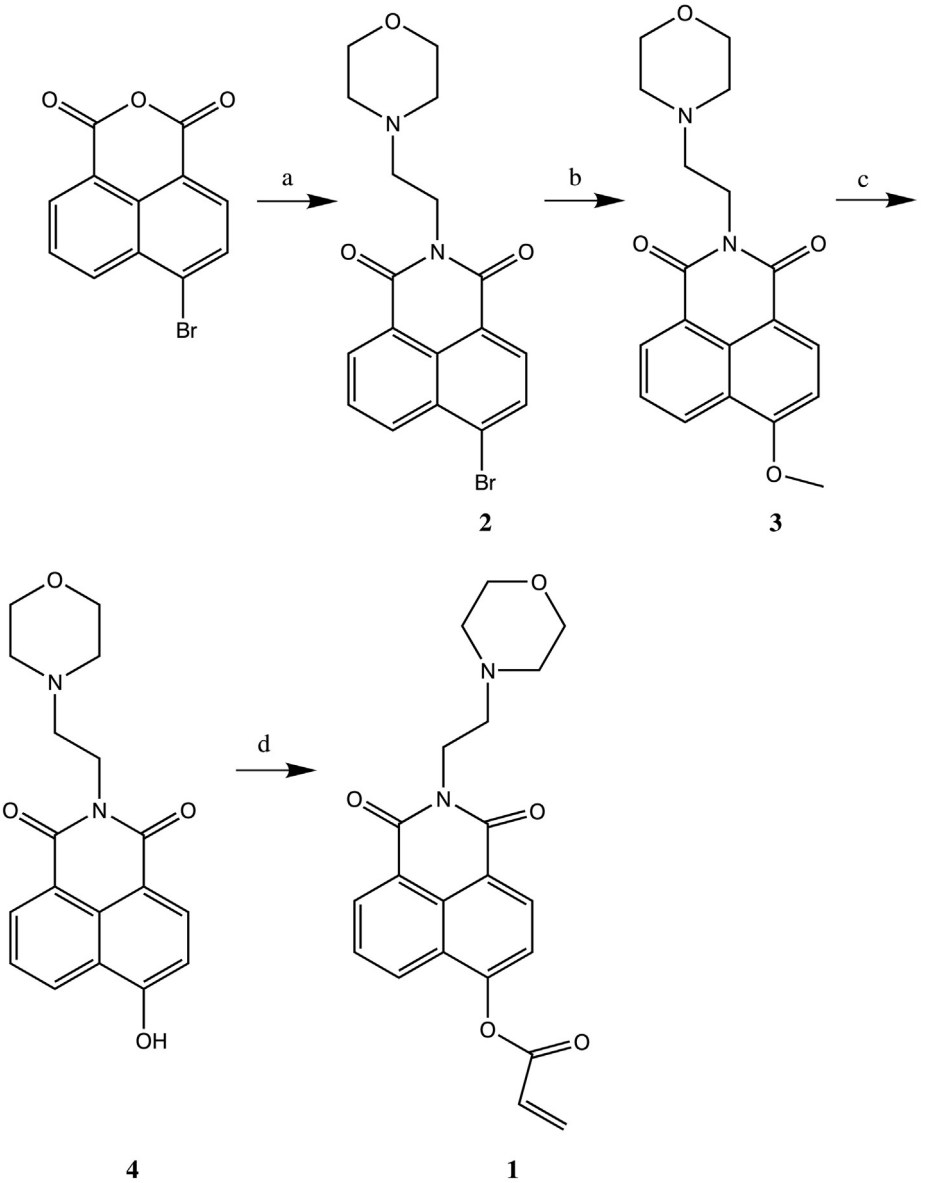
In living cells, lysosome is a major subcellular organelle that contains numerous enzymes and protein displaying a variety of activities and function at pH values (4.5–5.5) [43]. Thiols are closely associated with intralysosomal proteolysis by reducing disulfide bonds [44,45]. For example, GSH is an effective stimulant of albumin proteolysis in kidney lysosomes and Cys can effectively stimulate the degradation of albumin in liver lysosomes [44]. For better understanding the role of lysosomal thiols the efficient monitoring and detection of thiols in lysosomes is of great significance. However, only a few lysosome-targetable fluorescent probes for thiols have been reported [46–50]. Therefore, searching for lysosome-targetable fluorescent probes for thiols is still an active field as well as a challenge for the analytical chemistry research effort.

Naphthalimide and its derivatives were widely used in optical sensing because they exhibited good photophysical properties such as excellent stability, visible excitation and emission and a large Stokes shift that minimized the effects of the background fluorescence [51]. In this paper, we developed a lysosome-targeted fluorescent thiol probe which employed 1,8-naphthalimide as fluorescent chromophore and acrylate as the interaction site. The morpholine unit is incorporated into the probe as a lysosome-targeting group. The probe was constructed based on the Michael addition reaction mechanism. In the absence of biothiols, the probe showed weak fluorescence by a photoinduced electron transfer (PET) pathway. Upon the addition of biothiols, the PET pathway was suppressed and the probe exhibited an enhanced fluorescence emission. The probe displayed highly sensitivity and selectivity toward the biothiols. Moreover, the probe can be used effectively as an indicator to monitor the level of biothiols in lysosomes.

2. Experimental

2.1. Materials and Instruments

4-(2-Aminoethyl)morpholine and acryloyl chloride were purchased from Heowns Biochemical Technology Company. HI (55%) was obtained from Energy Chemical (Shanghai, China). Cysteine (Cys) and homocysteine (Hys) were purchased from TCI (Shanghai) Development Company. Glutathione (GSH) is purchased from Aladdin Reagent Company. Threonine (Thr), leucine (Leu), methionine (Met), valine (Val),



Scheme 1. Synthesis of fluorescent probe **1**: (a) absolute ethanol, 4-(2-aminoethyl)morpholine, reflux, 8 h, 72%; (b) CH_3OH, K_2CO_3, reflux, 24 h, 76%; (c) concentrated HI (57%), reflux, 6 h, 86%; (d) CH_2Cl_2, TEA, acryloyl chloride, room temperature, 12 h, 71%.