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Thiol–chromene click chemistry: A coumarin-based derivative and its use as regenerable thiol probe and in bioimaging applications

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ABSTRACT

The synthesis and characterization of a coumarin–chromene (8, 9-dihydro-2H-cyclopenta[b]pyrano[2,3-f]chromene-2,10(7aH)-dione) (**1**) derivative and its use for thiol chemosensing in water was reported. Experimental details showed **1** acts as a probe for the detection of thiols including cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), whereas amino acids which do not contain thiols induced no changes in UV–vis spectra and fluorescence emission properties of **1**. A possible detection mechanism is a nucleophilic attack of thiols to the α,β-unsaturated ketone in **1** that resulted in a fluorescent coumarin derivative. Further studies showed that **1**-thiol derivatives can be applied to the design of regenerative chemodosimeters for Cu^{2+}, Hg^{2+} and Cd^{2+} in water based on M^{n+}-promoted desulfurization and recovery of **1**. Furthermore, the optical properties of the probe and its Cys-addition product were theoretically studied. The ability of probe **1** to detect thiols in living cells (HepG2 cells) via an enhancement of the fluorescence was proved. Moreover, the applicability of **1** for the direct determination of biorelevant thiols in a complex matrix such as human plasma was also demonstrated.

1. Introduction

Three mercapto biomolecules—cysteine (Cys), homocysteine (Hcy) and glutathione (GSH)—have similar structures and play many crucial roles in physiological matrices (Wu et al., 2012; Shao et al., 2011; Chen et al., 2010; Schulz et al., 2000; Seshadri et al., 2002; Ball et al., 2006; Hong et al., 2006). For example, Cys and Hcy are essential biological molecules required for the growth of cells and tissues in living systems. A deficiency of Cys causes various health problems such as retarded growth, hair depigmentation, lethargy, liver damage, muscle and fat loss, and skin lesions (Kannana and John, 2011; Shang and Dong, 2009; Shahrokhian, 2001; Deng et al., 2011; Zhou and Yong, 2012; Yang et al., 2011). An elevated level of Hcy in human plasma is a risk factor for Alzheimer's disease, cardiovascular disease (Chand et al., 2013; Refsum et al., 1998; Zhang et al., 2007), neural tube defect, inflammatory bowel disease, and osteoporosis (Xiao et al., 2011; Van Meurs et al., 2004). GSH, which is the most abundant intracellular non-proteinogenic thiol, plays a pivotal role in maintaining the reducing environment in cells and acts as a redox regulator (Sezgintürk and Dinçkaya, 2004; Miao et al., 2009; Chen et al., 2012; Hassan and Rechnitz, 1982; Hwang et al., 1992; Tietze, 1969; Meister and Anderson, 1983; Rahman and MacNee, 2000; Pullela et al., 2006.; Lee et al., 2012). Thus, it is important to develop efficient methods for the detection and quantification of biothiols in physiological media for academic research and clinical applications (Yao et al., 2011). Owing to their simplicity, cheapness, sensitivity and selectivity, optical approaches based on synthetic colorimetric and fluorescent probes have attracted increasing interest during the last decade (Moragues et al., 2011). There are various thiol probes, namely magnetic FRET nanoprobes, ratiometric fluorescent probe, FRET-based luminescent iridium(III)

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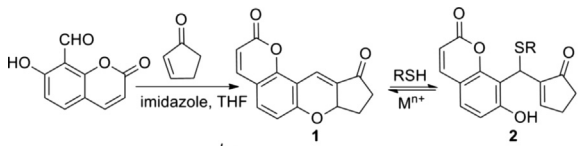
probe, two-photon fluorescent probes, bis-spiropyran ligands as dipolar molecule receptors, coumarin-based thiol chemosensor, etc. (P. Yang et al., 2012; Z. Yang et al., 2012; Yuan et al., 2011; Cao et al., 2011; Deng et al., 2011; Shiu et al., 2011; Shao et al., 2010; Lee et al., 2010; Jung et al., 2011). Thiols' strong nucleophilicity or their high binding affinity toward metal ions differentiates them from other species. Accordingly, most of the optical probes for thiols are in fact chemodosimeters, which involve specific reactions between probes and thiols, using addition reactions, displacement of coordinated ligands from metal complexes and others (Hu et al., 2011; Huo et al., 2011; Shao et al., 2010). One of the most attractive approaches involves the construction of receptors of thiol-based reaction through a powerful 'click' chemistry (Hoyle et al., 2010; Finn and Fokin, 2010; Kolb et al., 2001; Iha et al., 2009). A number of excellent Michael acceptors have been exploited for the detection of thiols such as maleimide (Girouard et al., 2005; Guy et al., 2007), squaraine (Sreejith et al., 2008), 7-oxanorbornadiene (OND) (Hong et al., 2009), quinone (Zeng et al., 2008a, b), propiolate (Owen, 2008), and acrylic acid (Lee et al., 2011), as well as an α,β-unsaturated aldehyde (Wang et al., 2005; Lim et al., 2010; Yuan et al., 2011), a ketone (Lin et al., 2009; Kim et al., 2011; Lim and Kim, 2011), diesters (Zuo et al., 2010) and malonitrile (Kwon et al., 2011). In a recent work, Chen and we have employed chromene, an α,β-unsaturated ketone, for the detection of thiols (Huo et al., 2009; X. Chen et al., 2010; X.Q. Chen et al., 2010; Huo et al., 2010). However these and other reported thiol reagents sometimes show certain limitations typically involving reactivity with other nucleophiles such as amines (including amino acids with amine residues), an on–off behavior in the presence of thiols, instability (for instance to light or via hydrolysis of the linkage to the fluorophore), limited applicability in water, low detection limits, complex synthetic procedures, etc.

Based on these concepts, and being aware of the importance of developing thiol chromo-fluorogenic chemosensors for the detection of bio-relevant thiols in complex real matrices we report herein the synthesis of a simple coumarin–chromene probe (8, 9-dihydro-2H-cyclopenta[b] pyrano [2,3-f]chromene-2,10(7aH)-dione) (**1**). The probe displays sensing features via a nucleophilic attack of thiols to the α,β-unsaturated ketone in **1** that resulted in the formation of a fluorescent coumarin derivative. Moreover the probe was successfully applied to the detection of thiols in living cells and to the quantification of bio-relevant thiols in blood. As an additional feature, **1**-thiol derivatives can be used for the chromo-fluorogenic detection in water of the metal cations Cu^{2+}, Hg^{2+} and Cd^{2+} via a metal-promoted desulfurization reaction and recovery of **1**.

Probe **1** was straightforwardly synthesized with 20% yield through a simple reaction of 7-hydroxycoumarinaldehyde, prepared as previously described (Huo et al., 2009, 2010; Chen et al., 2010; Lee et al., 2008), with 2-cyclopentenone in the presence of imidazole in tetrahydrofuran (THF) (Scheme 1). Experimental details and characterization data for **1** are given as Supplementary information (Fig. S1).



Scheme 1. Above: synthesis of probe 1 and thiol–chromene click chemistry; bottom: kinetics model.

2. Experimental

2.1. Materials

4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Sigma-Aldrich (St. Louis, MO). All chemicals and solvents were of analytical grade without further purification. Chromatography was carried out on silica gel (200–300 mesh). Thin layer chromatography (TLC) was carried out using silica gel GF254 plates with a thickness of 0.20–0.25 mm. Deionized water was used to prepare all aqueous solutions. The solutions of anions were prepared from their sodium salts and the solutions of metal ions were prepared from their chloride or nitrate salts.

2.2. Instruments

A pH meter (Mettler Toledo, Switzerland) was used to determine the pH. Ultraviolet–visible (UV–vis) spectra were recorded on a Cary 50 Bio UV–visible spectrophotometer. Fluorescence spectra were measured on a Cary Eclipse fluorescence spectrophotometer. A PO-120 quartz cuvette (10 mm) was purchased from Shanghai Huamei Experiment Instrument Plants, China. ^{1}H NMR, and ^{13}C NMR spectra were recorded on a Bruker AVANCE-300 MHz and 75 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Melting point (mp) was determined on WRS-2 digital melting point apparatus (Shanghai Physical Optical Instrument Factory). ESI-MS was measured with an UPLC-ESI-Q-TOF synapt G2 (Waters) instrument. The ability of probe **1** reacting to thiol in the living cells was also evaluated by laser confocal fluorescence imaging using a Leica TCS SP5 laser scanning microscope.

3. Results and discussion

3.1. The selectivity of probe for thiol

Fig. 1 shows the absorbance and fluorescence changes that **1** undergoes upon the addition of various amino acids, including Cys, Hcy, GSH, Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val and cystine in HEPES (10 mM, pH 7.4). The probe **1** displays UV–vis spectral changes and a fluorescence enhancement (λ_{ex}=365 nm, λ_{em}=455 nm) in the presence of the thiol-containing compounds Cys, Hcy and GSH. However, analytes without thiols induced no changes in UV–vis spectra or in the fluorescence emission properties under the same conditions. It was also confirmed that for other thiols, such as mercaptopropionic acid (MPA) and mercaptoethanol (ME), probe **1** showed UV–vis and fluorescence responses similar to Cys, Hcy and GSH (Fig. S2).

3.2. UV–vis and fluorescence spectra of detecting Cys

As a typical biological thiol, Cys was used to further examine the UV–vis response of probe **1**. Fig. 2a shows the change in the UV–vis spectra when Cys was added to HEPES (10 mM, pH 7.4) solution containing **1** (35 μM). With increasing Cys concentration (0–1 equiv.), the absorption peak at 320 nm gradually decreased and a new peak appeared at 372 nm (ε=9.29 × 10^3 M^{-1} cm^{-1}) (red shifted 52 nm). A well-defined isosbestic point was noted at 350 nm, which may indicate the formation of single new species. When changes at 320 nm and 372nm were monitored, the relative absorption values were linearly related to the Cys concentration between 0 and 35 μM, respectively (Fig. S3). The changes in the fluorescence spectra of probe **1** (Φ_f=0.1386) (10 μM) in the absence or presence of Cys (0–10 μM) in HEPES buffer are displayed in Fig. 2b. As it can be seen the addition of Cys caused changes in the fluorescence spectra, i.e. an enhancement of the