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Novel pyrazoline-based fluorescent probe for detecting glutathione and its application in cells

Sheng-Qing Wang [a], Qing-Hua Wu [b], Hao-Yan Wang [a], Xiao-Xin Zheng [a], Shi-Li Shen [a], Yan-Ru Zhang [a], Jun-Ying Miao [b,*], Bao-Xiang Zhao [a,*]

[a] Institute of Organic Chemistry, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, PR China
[b] Institute of Developmental Biology, School of Life Science, Shandong University, Jinan 250100, PR China

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ABSTRACT

A novel compound, 2-(1,5-diphenyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenyl acrylate (probe **L**), was designed and synthesized as a highly sensitive and selective fluorescent probe for recognizing and detecting glutathione among cysteine, homocysteine and other amino acids. The structures of related compounds were characterized using IR, NMR and HRMS spectroscopy analysis. The probe is a non-fluorescent compound. On being mixed with glutathione in buffered EtOH:PBS=3:7 solution at pH 7.4, the probe exhibited the blue emission of the pyrazoline at 474 nm and a 83-fold enhancement in fluorescence intensity. This probe is very sensitive and displayed a linear fluorescence off–on response to glutathione with fluorometric detection limit of 8.2×10^{-8} M. The emission of the probe is pH independent in the physiological pH range. Live-cell imaging of HeLa cells confirmed the cell permeability of the probe and its ability to selectively discriminate GSH from Cys and Hcy in cells. The toxicity of the probe was low in cultured HeLa cells.

1. Introduction

Intracellular thiols, including cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), play crucial roles in maintaining biological systems. GSH is the most abundant intracellular thiol and serves many cellular functions; for instance, GSH maintains the reduced state of proteins to protect cells against the activity of reactive oxygen species (ROS), drugs or heavy metal ions. Abnormal levels of GSH can cause cancer, abnormal aging, heart problems, and other ailments (Caccamo et al., 2013; Morgenstern et al., 2003; Vreuls et al., 2013; Watanabe et al., 2013). Therefore, developing small molecule probes for selective and sensitive detection of GSH under physiological conditions is important (Niu et al., 2012).

In last decade, fluorescence detection of molecules has been developed because of its simplicity, low cost, high selectivity and sensitivity. Therefore, fluorescent probes capable of detecting small-molecular-weight biological thiols (bio-thiols) have received intense attention (Bouffard et al., 2008; Chan et al., 2012; Chen et al., 2010; Y.S. Guo et al., 2012; Jia et al., 2012; Kwon et al., 2011; Li et al., 2013; Peng et al., 2012; Shao et al., 2012; Shiu et al., 2010; H.L. Wang et al., 2012; Yang et al., 2011, 2013; Zhang et al., 2007; H.T. Zhang et al., 2012; W.Z. Zhang et al., 2012; Yao et al., 2011; Zhou and Yoon, 2012). Among them, various fluorescent probes for bio-thiols based on different mechanisms have been reported, including Michael addition (Z.Q. Guo et al., 2012; Jung et al., 2012a, 2012b; Kand et al., 2012; P. Wang et al., 2012; Xiong et al., 2013; Yang et al., 2011; X. Yang et al., 2012; Zhou et al., 2012; H.T. Zhang et al., 2012), cyclization reaction with aldehyde (Hu et al., 2011; Kong et al., 2013; Mei et al., 2013; P. Wang et al., 2012; Z.G. Yang et al., 2012; Yuan et al., 2011), cleavage reaction by thiols, and others (Shao et al., 2011; Tang et al., 2013; Wang et al., 2009; Li et al., 2010). However, distinguishing Cys, Hcy and GSH is still difficult because of their structural similarity (Kand et al., 2012; Shao et al., 2011; Wang et al., 2009). To date, only a few ratiometric fluorescent probes based on monochlorinated BODIPY, water-soluble cationic-conjugated polyelectrolytes, disulfide-bound molecular beacon and hydroxycoumarin can discriminate GSH (Y.S. Guo et al., 2012; Yao et al., 2009; Niu et al., 2012; Na et al., 2012; Zhai et al., 2013). In addition, a latent probe was found to detect cellular GSH, but it interferes with Cys or Hcy (Na et al., 2012), an alkaline environment (Yao et al., 2009) and toxic solvents (Niu et al., 2012). Developing fluorescent probes for discriminating GSH without interference from Cys or Hcy and constructing simple and effective fluorescent probes for selective and sensitive detection of GSH under physiological conditions is difficult.

* Corresponding authors. Tel.: +86 531 8836 6425; fax: +86 531 8856 4464.
E-mail addresses: miaojy@sdu.edu.cn (J.-Y. Miao), bxzhao@sdu.edu.cn, sduzhao@hotmail.com (B.-X. Zhao).

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In this paper, we designed and synthesized a new fluorescent probe, 2-(1,5-diphenyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenyl acrylate (**L**), for selective detection of GSH. Cys can react with acrylate derivatives in a Michael addition manner, followed by ammonolysis to form a ring (Yang et al., 2011; Z.G. Yang et al., 2012; W.Z. Zhang et al., 2012; P. Wang et al., 2012; Z.Q. Guo et al., 2012). GSH can also react with acrylate derivatives in a Michael addition manner; however, the adduct cannot decompose by ammonolysis for steric hindrance. Therefore, we attempted to design a probe that can react with Cys or Hcy to give a non-fluorescent compound but with GSH to give a fluorescent adduct. We found that probe **L** reacts with GSH to form a fluorescent compound but reacts with Cys to form a non-fluorescent compound, 2-(1,5-diphenyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenol (**3**). So we can distinguish GSH from Cys or Hcy. Chalcone, 1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one (**1**), was prepared according to the literature (Budakoti et al., 2009). The 1,3,5-triarylpyrazoline derivative **3** was obtained by reacting chalcone **1** with phenylhydrazine **2** under a reflux condition in 45.8% yield. The probe was synthesized by the reaction of compound **3** and acryloyl chloride at room temperature in 59.5% yield (Scheme 1). The structure of probe **L** was characterized by IR, NMR, and HRMS. Experimental details and characterization data for **L** are given as Supplementary information.

2. Material and methods

2.1. Materials

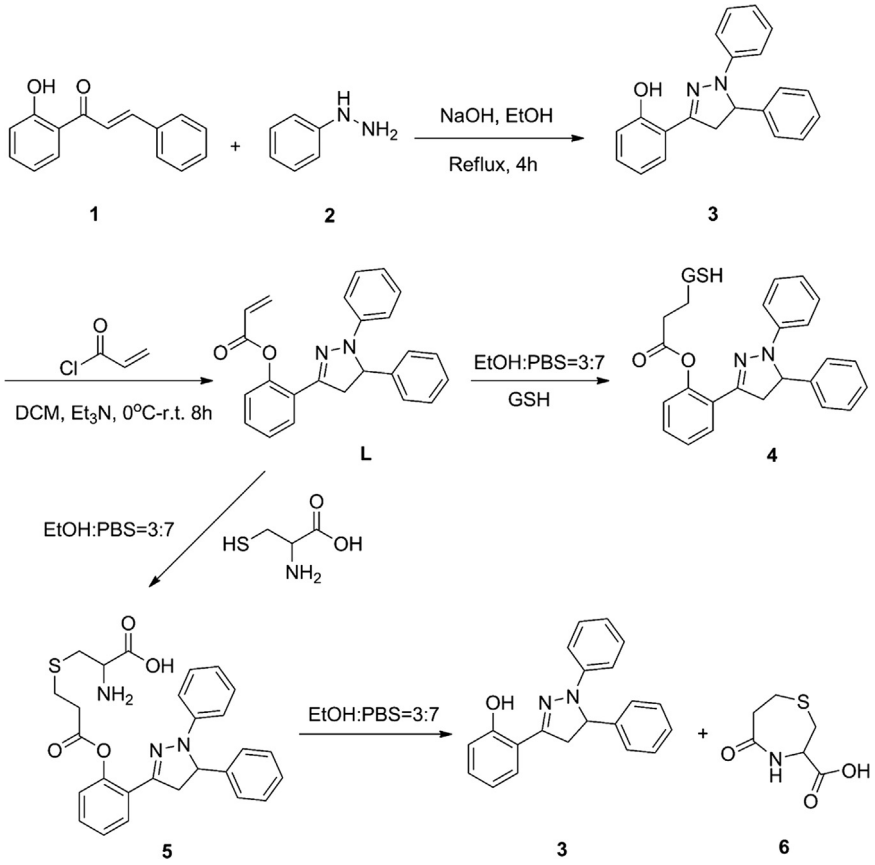
Thin-layer chromatography (TLC) was conducted on silica gel $60F_{254}$ plates (Merck KGaA). ^{1}H NMR spectra were recorded on a Bruker Avance 300 (300 MHz) spectrometer, with DMSO-d_6 or $DCCl_3$ used as a solvent and tetramethylsilane (TMS) as an internal standard. Melting points were determined on an XD-4 digital micro-melting-point apparatus. IR spectra were recorded with an IR spectrophotometer VERTEX 70 FT-IR (Bruker Optics). HRMS spectra were recorded with a Q-TOF6510 spectrographer (Agilent). UV–vis spectra were recorded on a U-4100 (Hitachi). Fluorescent measurements were recorded on a Perkin-Elmer LS-55 luminescence spectrophotometer. All pH measurements were made with a Model PHS-3C pH meter (Shanghai) at room temperature of about 298 K. Deionized water was used throughout the experiment. All reagents were purchased from commercial suppliers and used without further purification. The salts used in stock aqueous solutions of metal ions were KNO_3, $Ca(NO_3)_2 \cdot 4H_2O$, $NaNO_3$, $Mg(NO_3)_2 \cdot 6H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$, $Fe(NO_3)_3 \cdot 9H_2O$.

2.2. Spectroscopy data

A 1.0×10^{-3} M of stock solution of probe **L** was prepared in ethanol. The amino acids (Cys, Hcy, GSH, Arg, Asp, Glu, Gly, His, Lys, Ser, Thr, Trp, Tyr, Val), cationic (K^+, Ca^{2+}, Na^+, Mg^{2+}, Zn^{2+}, Fe^{3+}), peroxide and glucose stocks were all in deionized water with a concentration of 10^{-2} M for UV–vis absorption and fluorescence spectra analysis. For all measurements of fluorescence spectra, excitation was at 360 nm with 10.0 nm of excitation slit width and scan speed was 600 nm min^{-1}. All UV–vis and fluorescence titration experiments involved use of 10 μM probe **L** in 30% ethanol aqueous solution (pH 7.4, 10 mM PBS buffer) with varying concentrations of analytes at room temperature after 12 h.

2.3. Fluorescence quantum yield

Quantum yield (Φ_F) was determined by the relative comparison procedure, with quinine sulfate dehydrate (≥ 99.0%) in 0.1 N H_2SO_4



Scheme 1. Synthesis of 2-(1,5-diphenyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenyl acrylate.