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

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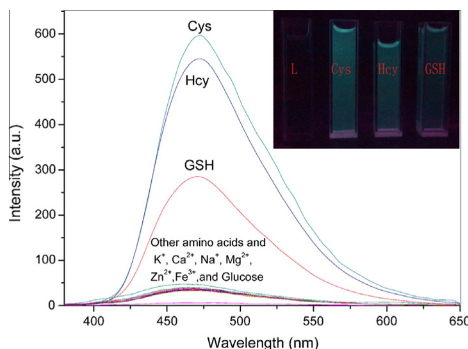
HIGHLIGHTS

- This probe can detect thiols in DMSO:HEPES = 1:1 solution at pH 7.4.
- The structure of the probe was characterized by IR, NMR and HRMS spectroscopy analysis.
- This probe can be used for living cell imaging.

GRAPHICAL ABSTRACT

A highly sensitive and selective fluorescent probe for recognizing and detecting thiol from other amino acids was developed.



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ABSTRACT

A new compound, *N*-(4-(1,5-diphenyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenyl)-acrylamide (probe **L**), was designed and synthesized as a highly sensitive and selective fluorescent probe for recognizing and detecting thiol from other amino acids. On being mixed with thiol in buffered DMSO:HEPES = 1:1 solution at pH 7.4, the probe exhibited the blue emission at 474 nm. This probe is very sensitive and displayed a linear fluorescence off–on response to thiol. The fluorescence emission of the probe is pH independent in the physiological pH range. Living cell imaging of HeLa cells confirmed its cell permeability and its ability to selectively detect thiol in cells. The structure of the probe was characterized by IR, NMR and HRMS spectroscopy analysis.

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Introduction

In the biological system, thiols (Cysteine, Homocysteine, Glutathione), play significant roles in many physiological processes, and they have produced wide-ranging and deep-going impact on the physiological processes [1,2]. Glutathione (GHS) maintains the appropriate redox status of proteins, cells, and organisms matters [3]. Homocysteine (Hcy) has been referred to various types of vascular and renal diseases. Increased Hcy in blood (>12 μM) is a risk factor for cardio-vascular and Alzheimers disease, neutral tube defects, complications during pregnancy, inflammatory bowel disease, and osteoporosis [4–7]. Elevated level

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of cysteine (Cys) has been connected with neurotoxicity and low level of Cys is related to slowed growth rate, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, skin lesions, and weakness [8–12]. Therefore, it is beyond dispute that design and synthesis of a probe for the detection and quantification of biothiols in physiological media is our top priority.

In recent years, a wide variety of methods for quantitative measurement of thiols have been developed, such as HPLC, capillary electrophoresis, colorimetric assays, electrochemical methods and mass spectrometry. Like the application of fluorescent probe in detecting metal ions and anions [13–29], fluorescence-based methods were widely used for detecting thiols because of their high sensitivity, selectivity, simplicity of operation and potential application in living cell imaging [30–36]. Many fluorescent probes for thiols have been developed according to various chemical mechanisms, including Michael addition [37–40], cyclization with aldehyde [41–43], disulfide bond cleavage reaction [44,45], binding with nanoparticles [46,47], cleavage of sulfonamide and sulfonate ester by thiols [48–51], conjugate addition-cyclization [52–54] and the displacement-rearrangement reaction [55,56].

Pyrazolines are vital nitrogen-containing 5-membered heterocyclic compounds which could have stronger fluorescence, good membrane permeability, low toxicity and high quantum yield [57–59]. Therefore, pyrazoline derivatives have widely been used as whitening or brightening reagents for synthetic fibers, fluorescent chemosensors for the recognition of metal ions and thiols [60–65]. In light of the fact that pyrazolines have so many advantages, we have developed a new and facile fluorescent probe for the selective detection of thiols in aqueous solution. The structure of the probe was characterized by IR, ^{1}H NMR and HRMS. The detection limit of the probe towards Cys was 2.48×10^{-6} M.

Material and methods

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 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. 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, $Ca(NO_3)_2 \cdot 4H_2O$, $NaNO_3$, $Mg(NO_3)_2 \cdot 6H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$, $Fe(NO_3)_3 \cdot 9H_2O$.

Spectroscopy data

A 1.0×10^{-3} M of stock solution of probe **L** was prepared in DMSO. 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 1.0×10^{-1} M for UV–vis absorption and fluorescence spectra analysis. For all measurements of fluorescence spectra, excitation was at 360 nm with 12.0 nm slit width and scan speed was 600 nm min^{-1}. All UV–vis absorption and fluorescence titration experiments involved 10 μM probe **L** in 50% DMSO aqueous solution (pH 7.4, 20 mM HEPES buffer) with varying concentrations of analytes at room temperature after 12 h.

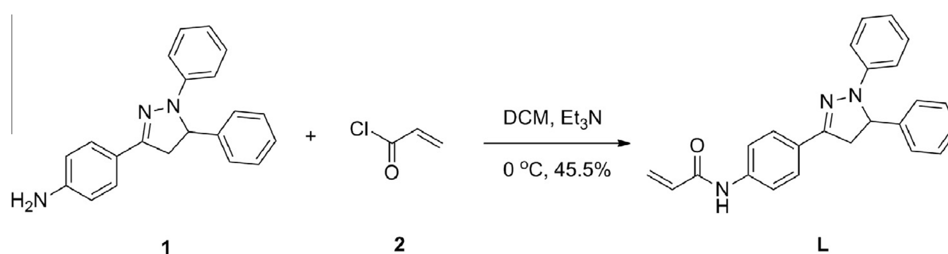
*General procedure for the synthesis of probe **L***

Acryloyl chloride (1.0 mmol) was added dropwise at 0 °C to a stirred solution of 4-(1,5-diphenyl-4,5-dihydro-1*H*-pyrazol-3-yl)aniline **1** (1.0 mmol) and Et_3N (2.0 mmol) in dichloromethane (10 mL). After completion of the reaction, the mixture was washed with brine (10 mL × 3) and the organic layer was dried over $MgSO_4$. After filtered, the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with 30:1 dichloromethane:ethyl acetate (v/v) to afford probe **L** (149 mg, 45.5%) as a pale yellow solid (Scheme 1). M.P. 262–264 °C; IR (KBr, cm^{-1}): 3306, 3026, 2896, 1668, 1599; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.08 (dd, 1H, *J* = 6.3, 17.4 Hz, CH_2), 3.90 (dd, 1H, *J* = 12.3, 17.4 Hz, CH_2), 5.45 (dd, 1H, *J* = 6.3, 12.3 Hz, 5-H of pyrazoline), 5.77 (dd, 1H, *J* = 2.1, 9.9 Hz, —CH=CH$_2$), 6.27 (dd, 1H, *J* = 2.1, 17.1 Hz, —CH=CH$_2$), 6.45 (dd, 1H, *J* = 9.9, 17.1 Hz, —CH=CH$_2$), 6.70 (t, 1H, *J* = 7.2 Hz, Ar—H), 6.98 (d, 2H, *J* = 7.8 Hz, Ar—H), 7.14 (dd, 2H, *J* = 7.5, 8.7 Hz, Ar—H), 7.69–7.76 (m, 4H, Ar—H), 7.22–7.37 (m, 5H, Ar—H), 10.29 (s, 1H, N—H); ^{13}C NMR (75 MHz, DMSO-d_6): 163.1, 146.9, 144.3, 142.6, 139.4, 131.7, 128.9, 128.8, 127.4, 127.3, 127.1, 126.4, 125.8, 119.2, 118.4, 112.8, 63.1, 43.1; HRMS: calcd for $[M + H]^+$ $C_{24}H_{22}N_3O$: 368.1763; found: 368.1766.

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 as the main standard. The corrected emission spectra were measured for the quinine sulfate dehydrate standard (λ_{ex} = 360 nm; *A* (absorbance) < 0.01; Φ_F = 0.560) [66]. For all measurements of fluorescence spectra, scan speed was 600 nm min^{-1} with a quartz cell of 1 cm optical path length. UV–vis absorption spectra were recorded in a standard 1-cm path-length quartz cell of range 250–600 nm with spectral resolution 1 nm. The general equation used in the determination of relative quantum yields from earlier research is Eq. (1) [67]

$$\Phi_F = (\Phi_{FS})(FAu)(As)(\eta_u^2)/(FAs)(Au)(\eta_s^2), \quad (1)$$



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

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