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# Synthesis of a fluorogenic probe for thiols based on a coumarin schiff base copper complex and its use for the detection of glutathione

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ABSTRACT

Glutathione is the most abundant non-protein thiols compound in cells and plays important metabolic roles. Changes in the amount of glutathione or its metabolic dysregulation can lead to a series of diseases. The determination of glutathione levels is very helpful to the diagnosis and treatment of the related diseases. A coumarin schiff base (compound **1**) was synthesized from coumarin hydrazide and 2,6-pyridine dicarboxaldehyde and the fluorogenic probe for thiols (compound **1-Cu$^{2+}$**) was prepared by coordinating compound **1** with copper ions. Compound **1** showed strong fluorescence, while compound **1-Cu$^{2+}$** hardly had fluorescence due to the paramagnetism and/or photoinduced electron transfer of $Cu^{2+}$. However, after the addition of thiols-containing compounds, the fluorescence of compound **1** was restored. The UV–vis absorption and fluorescence spectra indicated that the fluorogenic probe had good thiols selectivity and sensitivity, particularly for glutathione in $CH_3CN$:HEPES (3:2, v/v) buffer. It was successfully applied to the fluorescence imaging detection of glutathione in human cervical squamous cancer cells (SiHa cells).



## 1. Introduction

The thiols in cells, such as cysteine, homocysteine, and glutathione, play important roles in the regulation of various physiological and pathological processes in humans as vital bioactive compounds. The endogenous concentrations of these thiols suggest the functional state of the corresponding enzymes and proteins and their abnormal levels correlate with diseases.[1] Glutathione is the most abundant non-protein thiols compound in cells, with concentration ranges from 1 to 10 mmol/L.[2] Glutathione can remove toxic and hazardous compounds and metabolites, maintain the completeness of the erythrocyte membrane, reduce DNA damage and mutation by reducing radical attacks to DNA, participate in the reduction of methemoglobin, and promote iron absorption.[3] Metabolic dysregulation or the change in glutathione levels in organisms can lead to various diseases including cancers, Alzheimer's disease, cardiovascular diseases, leukocyte loss, psoriasis, liver damage and AIDS.[4] Measuring the level of glutathione in living organisms is helpful to diagnose relevant diseases.[5] Therefore, the development of fast, convenient, accurate and sensitive methods to measure physiological and clinical glutathione levels has attracted wide attention.

Current methods to detect thiols include high performance liquid chromatography, capillary electrophoresis, UV–vis spectrometry, mass spectrometry, electrochemical analysis, and surface enhanced Raman scattering. Fluorescence detection was considered to be a convenient method due to its simplicity, sensitivity, and high efficiency.[6] To date, researches on thiols fluorogenic probe s have shown significant development. Many organic reactions have been applied to the synthesis of such probes, namely: Michael addition,[7] nucleophilic substitution,[8] disulfide exchange reactions,[9] cyclization reactions between aldehydes and aminothiols,[10] and demetallation.[11] However, due to the fact that most thiols in vivo have very weak or no fluorescence, a high sensitivity fluorophore is required for this application. Because of the similarities in the structures and properties of various thiols compounds, design and preparation of a probe with high selectivity and sensitivity is still a great challenge.

Coumarins are often used as chromophores in the preparation of highly effective fluorogenic probes due to their highly intense fluorescence, good solubility, ease of preparation, relatively high

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molar absorption coefficient and fluorescence quantum yield.[12] The excitation wavelength of coumarins falls within the visible range, and its derivatization and modification are simple.[13] Herein, in this paper we report a new fluorescence probe **1-Cu$^{2+}$**, which was produced by coordination between a coumarin derivate **1** and $Cu^{2+}$ ions. The precursor compound **1** was prepared by a simple schiff base condensation reaction of 7-(diethylamino)-2-oxo-2H-chromen-e-3-carbo- hydrazide (**2**) and pyridine-2,6-dicarbaldehyde. This probe could be used for rapid, highly selective and sensitive detection of thiols. The process and mechanism of detection by this probe were investigated by UV–vis, fluorescence spectra titration and mass spectrometry.

## 2. Experiment section

### 2.1. Instruments

$^1$HNMR and $^{13}$CNMR spectra were measured on a Bruker Ascend™ 400 spectrometer with chemical shifts reported as ppm with TMS as internal standard. Mass spectrometric data were obtained with a Bruker Microtof-QIII spectrometry. UV–vis absorption spectra were recorded with Shimadzu UV2550 spectrophotometer. Fluorescence spectra were recorded with Edinburgh Instruments FS-5 fluorescence spectrophotometer. Cell imaging was recorded with Nikon Eclipse TE2000-S.

### 2.2. Reagents

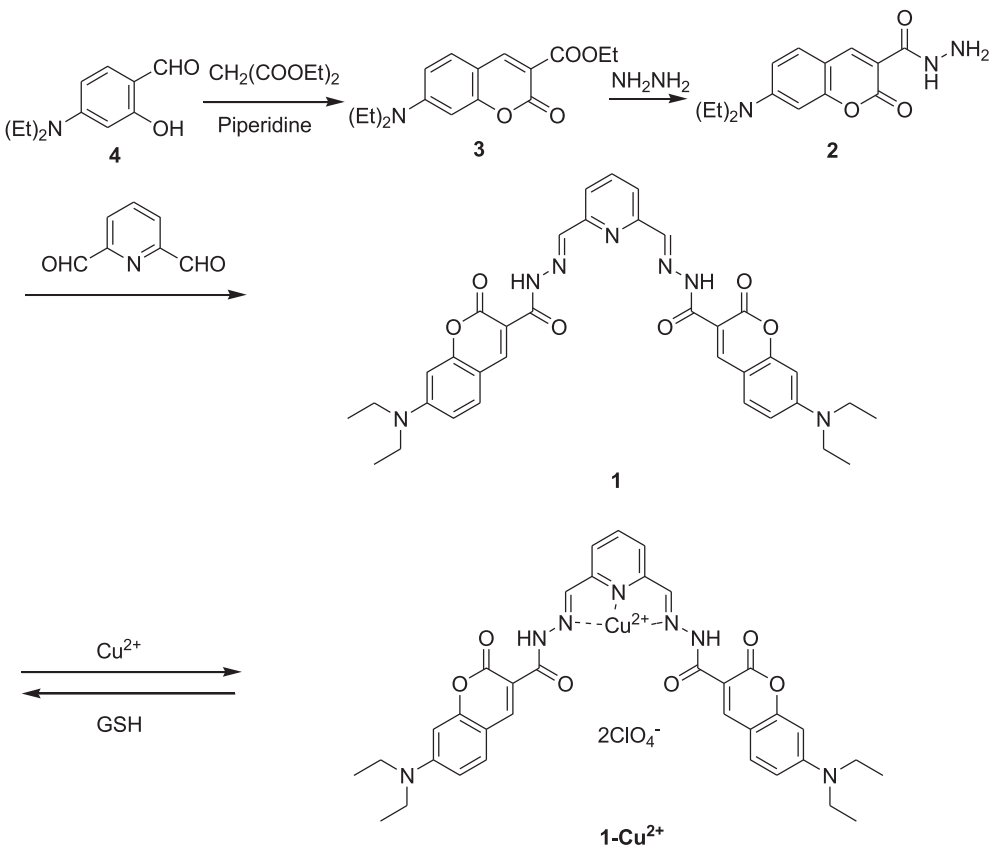
All the chemicals were of analytical grade and used as received. 2,6-pyridine dicarboxaldehyde was purchased from Tianjin Heowns Biochemical Technology Co., Ltd. 4-(Diethylamino)-2-hydroxybenzaldehyde, diethyl malonate, piperidine, hydrazine hydrate (80%) and the other reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. Stock solutions ($2.0 \times 10^{-2}$ M) of the perchlorate $Na^+$, $Mg^{2+}$, $K^+$, $Ca^{2+}$, $Cr^{3+}$, $Mn^{2+}$, $Fe^{3+}$, $Fe^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Ag^+$, $Cd^{2+}$, $Hg^{2+}$, $Pb^{2+}$ and the amino acids plus GSH were prepared in aqueous solutions. Stock solutions of compound **1** and compound **1-Cu$^{2+}$** (10 μM) for spectral measurement were prepared in $CH_3CN$:HEPES (3:2, v/v) solution. Stock solutions of compound **1** and compound **1-Cu$^{2+}$** for fluorescence imaging in cells were prepared in DMSO solution. DMSO has a better solubility for compound **1** and compound **1-Cu$^{2+}$** and the lower cytotoxicity. Each time a 3 mL compound **1** or compound **1-Cu$^{2+}$** was filled in a quartz cell of 1 cm optical path length, and different stock solutions of metal ions or amino acids were added into the quartz cell gradually by using a micro-syringe.

### 2.3. Synthetic procedure

The synthetic pathway for compound **1** was shown in Scheme 1. 7-N,N-dimethylamino-2-oxo-2H-3-coumarate (compound **3**) and coumarin hydrazine (compound **2**) were prepared according to previous reports.[14] Compound **1** was synthesized from compound **2** and 2,6-pyridine dicarboxaldehyde, and characterized by $^1$H NMR, $^{13}$C NMR, ESI-MS and IR spectra (Figs. S1–S4). Compound **1-Cu$^{2+}$** was prepared from compound **1** and $Cu(ClO_4)_2$ in ethanol and characterized by ESI-MS and IR spectra (Figs S5–S7, S11). ESI-MS showed a 1:1 M ratio between compound **1** and $Cu(ClO_4)_2$.

#### 2.3.1. Synthesis of ethyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate[14a]

Compound **4** (3.8648 g, 0.02 mol), diethyl malonate (6.4067 g,




**Scheme 1.** Syntheses of compound **1** and compound **1-Cu$^{2+}$**.

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