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Original article

Colorimetric and fluorimetric detection of cysteine: Unexpected Michael addition–elimination reaction

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ABSTRACT

The synthesis of three isomers based on Michael addition mechanism for the detection of sulfur-containing species in aqueous solution is described. These compounds are constructed by conjugating an enone to a coumarin fluorophore. A substituted-phenyl (*o*, *m*, or *p*-) was appended at the carbonyl carbon to adjust the reactivity. The experimental results showed that (*E*)-7-(diethylamino)-3-(3-(3-hydroxyphenyl)-3-oxoprop-1-en-1-yl)-2H-chromen-2-one (*m*-QPS) and (*E*)-7-(diethylamino)-3-(3-(4-hydroxyphenyl)-3-oxoprop-1-en-1-yl)-2H-chromen-2-one (*p*-QPS) barely react with sulfur-containing nucleophiles, while (*E*)-7-(diethylamino)-3-(3-(2-hydroxyphenyl)-3-oxoprop-1-en-1-yl)-2H-chromen-2-one (*o*-QPS) exhibited a fast response toward sulfite, sulfide and thiols in DMSO/phosphate buffer (2:1). The above results are probably due to the intramolecular H-bonding activated Michael addition. More interestingly, cysteine triggered unusual photophysical responses of *o*-QPS: the original absorption (488 nm) and emission peaks (573 nm) underwent significant blue shifts initially and then recovered, which might be caused by the Michael addition and elimination reaction, respectively.

1. Introduction

Sulfur-containing compounds play vital roles in environment, industry and biological systems [1–5]. Consequently, the detection of these species has received growing attention. Many fluorescent and/or colorimetric probes have been developed for the determination of sulfur-containing compounds utilizing the mechanisms of nucleophilic reaction [6–8], Michael addition [9–12], reduction [13–16], cleavage of 2,4-dinitrobenzenesulfonyl [17–22] and forming complexes with metal ions [23–25]. In our previous work, we designed several probes for sulfur-containing compounds by incorporating α,β-unsaturated ketone on a coumarin fluorophore [26–28]. The experimental results revealed that a strong electron-donating group suppressed the Michael reaction. Recently, Kim, et al., reported a coumarin-based probe with a strong electron-donating group (*o*-QPS) activated by an intramolecular H-bonding for the fast detection of thiols in DMSO/Hepes buffer [29]. The Michael reaction required more than one hour to be completed with a large excess of 2-mercaptoethanol (10 mmol/L). This result attracted our interest, and we repeated the experiments under the same conditions. However, we found that the spectral responses of *o*-QPS toward thiols at lower concentrations (less than 1 mmol/L) were quite slow (more than 10 hours for GSH) and believed that Hepes is not a good solvent for thiol detection. In addition, the spectral response of the probe toward other sulfur-containing compounds was not reported in the literature [29]. Our previous study found that sulfite and sulfide are more active nucleophilic reagents than thiols. Therefore, sulfite and sulfide are expected to form Michael addition products with *o*-QPS under the same experimental conditions.

Herein, we turned our attention to investigating the effects of the position of the substituent upon the Michael reaction and the nucleophilic reactivities of different sulfur-containing species. We synthesized three isomers (Scheme 1) and studied their spectral responses toward various species, including thiols, sulfite, sulfide and some other amino acids. The experimental conditions were optimized to shorten the detection time. The reaction is much faster in phosphate buffer solution than in Hepes solution taking

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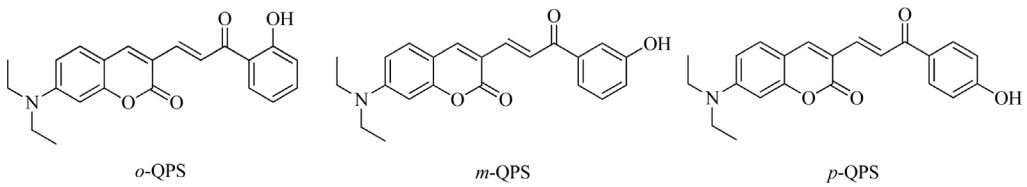


Scheme 1. The chemical structures of the compounds studied.

only 10 min for *o*-QPS to react with Cys. To our surprise, *o*-QPS showed quite a different response to Cys, which allowed us to distinguish Cys from other sulfur-containing compounds.

2. Experimental

2.1. Chemicals and materials

Unless otherwise specified, all the commercial reagents were of analytical grade and used without further purification. All the chemicals were purchased from Aladdin Corporation. Ultra-pure water was prepared through Sartorius Arium 611DI system.

2.2. Characterization and measurement

NMR spectra were acquired on a Bruker AV-400 spectrometer (400 MHz). Mass spectra were recorded on a MA 1212 Instrument under standard conditions (ESI, 70 eV). Absorption spectra were measured with an Evolution 220 UV-vis spectrophotometer (Thermo Scientific). Fluorescence spectra were recorded on a Lumina Fluorescence Spectrometer (Thermo Scientific), all the fluorescence spectra were uncorrected. The experiments were performed at 25 °C using non-degassed samples.

2.3. Absorbance and fluorescence titration

Accurately weighted amounts of the dyes were dissolved in DMF to obtain 3×10^{-3} mol/L stock solutions. The stock solution was diluted with the corresponding medium to acquired 10 μmol/L dye solutions.

Sulfur-containing species and other amino acids were freshly prepared by dissolving in water/DMF to obtain stock solutions (20 mmol/L). A 45 μL aliquot of the above solution was added to 3 mL of 1×10^{-5} mol/L dye in 2:1 DMSO/PBS (10 mmol/L, pH 7.4) to make [substrate] = 300 μmol/L.

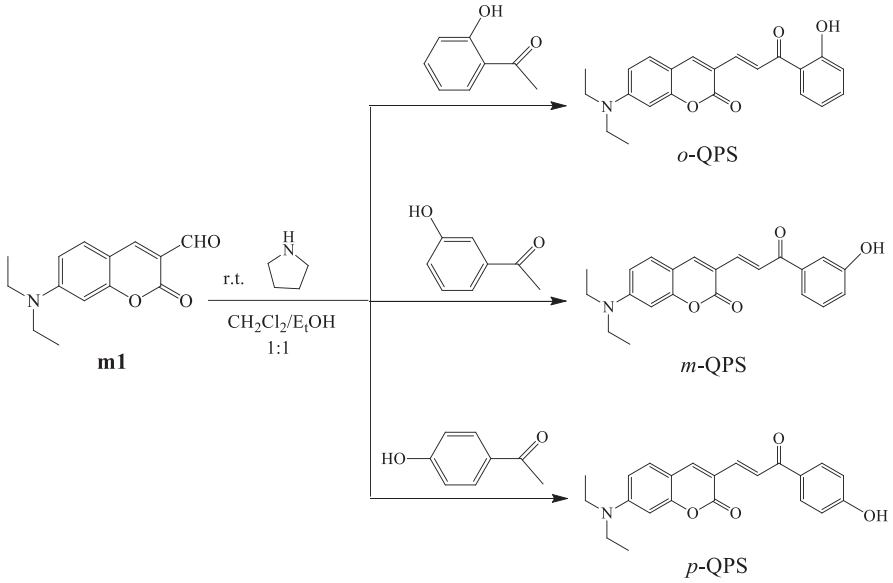
2.4. HPLC traces

HPLC analyses were performed on an Elliot 1203 system and a Zobax C18 reversed-phase column (4.6 mm × 10 cm). The mobile phases were degassed with an ultrasonic apparatus for 10 min. Injection volume: 25 μL; mobile phase: A-0.1% TFA/water, B-acetonitrile; gradient elution: 3–15 min 5–90% B; isocratic elution: 0–3 and 18–20 min, 5% B; 15–18 min, 90% B; flow rate: 1.0 mL/min; detection wavelength = 430 nm.

2.5. Synthesis

Three isomers were synthesized according to the following procedures (Scheme 2). Compound **m1** was prepared according to Refs. [26–29]. *o*-, *m*-, or *p*-QPS: 100 mg (0.4 mmol) of compound **m1**, 0.72 mmol of *o*-, *m*-, or *p*-hydroxy-acetophenone and five drops of pyrrolidine were added to 20 mL of EtOH/DCM (1:2, v/v). After stirring at room temperature for two days, the solvent was evaporated under vacuum. Compounds *o*-, *m*- or *p*-QPS were purified by column chromatography (PE:DCM, 5:1, v/v) to give a salmon colored solid.

o-QPS: (22.0%), ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.43 (s, 1H), 8.46 (s, 1H), 8.03 (d, 1H, J = 15.4 Hz), 7.96 (d, 1H, J = 8.6 Hz), 7.61 (d, 1H, J = 15.4 Hz), 7.50 (d, 1H, J = 8.9 Hz), 6.91 (d, 2H, J = 8.5 Hz), 6.80 (d, 1H, J = 9.0 Hz), 6.60 (s, 1H), 3.48 (t, 4H, J = 6.8 Hz), 1.15 (t, 6H, J = 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 198.456, 167.068, 165.196, 161.870, 157.414, 151.498, 145.618, 141.318, 136.137, 135.277, 126.017, 125.225, 124.451, 123.056, 118.085, 115.315, 113.664, 101.483, 49.617, 17.592. HR-MS m/z: 364.1535 $(M+H)^+$; calcd. molecular weight of $C_{22}H_{45}NO_4$: 364.1549 for $(M+H)^+$.



Scheme 2. The synthesis procedures of the three isomers.