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Double Functional Group Transformations for Fluorescent Probe Construction: A Fluorescence Turn-On Probe for Thioureas

Weiying Lin,* Xiaowei Cao, Lin Yuan, and Yundi Ding[a]

The development of fluorescent probes has attracted continuing attention due to the simplicity and high sensitivity of fluorescence detection. Recently, analyte-mediated organic reactions have been extensively employed to design a wide variety of fluorescent probes. In many reaction-based fluorescent probes, the fluorescence properties of the dyes are regulated by a single functional group. Thus, only a single functional group transformation is involved in the analyte-mediated reactions for the fluorescence response (Figure 1, top). However, the development of fluorescent probes based on a single functional group transformation with high selectivity is still very challenging, since structurally and chemically related analytes may compete with the same key functional group in the probes. For instance, a fluorescent probe with an electrophilic carbonyl group is known to react with strong nucleophiles, such as Cys and CN^-.[1] Consequently, the probe has poor selectivity for these distinct analytes. On the other hand, fluorescence turn-on probes may be designed based on the double functional group transformation strategy provided that the fluorescence intensity of a dye is suppressed by two different functional groups (Figure 1, bottom). Obviously, the intense fluorescence is restored only after both functional groups are transformed by the target analyte. However, if neither, or only one, functional group is transformed by other analytes, the fluorescence of the dye may remain very dim. Thus, a double functional group transformation strategy may allow the probe to show selectivity for the target analyte over other potentially competing species that can incite no, or only single, functional group transformation on the probe. This design approach, in principle, may be favorable for improvement of selectivity, as double and single functional group transformation products may have discrete emission profiles.

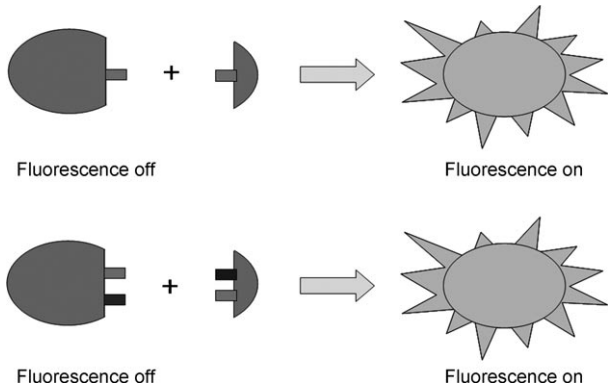

Figure 1. Top: development of reaction-based fluorescence turn-on probes by the single functional group transformation approach. Bottom: development of reaction-based fluorescence turn-on probes by the double functional group transformation strategy.

Thioureas have been widely used as fungicides[2] or accelerators of sprouting in dormant tubers[2a] in agriculture and as vulcanization accelerators in industry.[3] However, the toxicity of thioureas is associated with diseases, such as dermatitis, pulmonary edema, chronic goitrogenic difficulties, and thyroid and liver tumors.[4] Thus, it is critical to detect thioureas. To the best of our knowledge, fluorescence turn-on probes for thioureas that can operate in aqueous solution have not been previously developed.[5]

In this contribution, we report the development of the first fluorescence turn-on thiourea probe **1** (Scheme 1) by the double functional group transformation strategy. Probe **1** is composed of a coumarin dye, a carbonyl group, and a bromide group. The choice of the carbonyl and bromide groups is based on the following considerations: 1) The carbonyl group may significantly decrease the fluorescence of the coumarin dye due to the intersystem crossing from singlet (n, π^*) to triplet state (π, π^*).[6] In addition, the bromide moiety is also a well-known fluorescence quencher in light of its heavy atom effect. Thus, these two fluorescence

[a] Prof. W. Lin, X. Cao, L. Yuan, Y. Ding
State Key Laboratory of Chemo/Biosensing and Chemometrics
College of Chemistry and Chemical Engineering, Hunan University
Changsha, Hunan 410082 (P.R. China)
Fax: (+86) 731-88821464
E-mail: weiyinglin@hnu.cn

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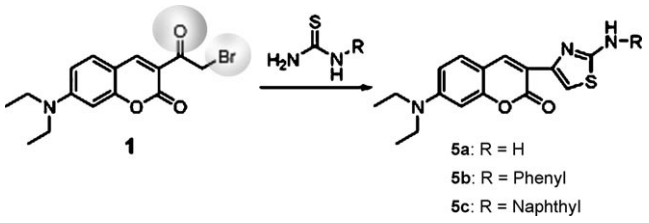
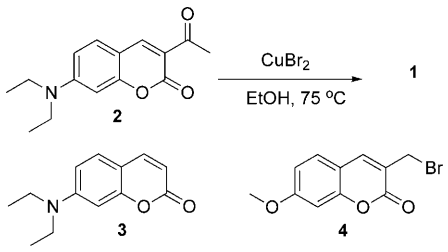


Scheme 1. Design of fluorescence turn-on thiourea probe **1**.

quenching factors should render probe **1** essentially nonfluorescent. 2) When treated with thioureas, these two functional groups may undergo double functional group transformations to become the thiazole compounds **5** by the Hantzsch reaction.[7] Thus, we envisioned that the chemical transformation of the α-bromoketone to thiazole structure should inhibit both the fluorescence quenching processes and, thus, restore the intense fluorescence of the coumarin dye. In other words, we should observe a pronounced fluorescence turn-on signal, if probe **1** can be converted to thiazole compounds **5** by thioureas. Notably, the Hantzsch reaction has not been previously exploited to construct fluorescent probes for thioureas.

Compound **1** was readily synthesized in one step by reaction of ketocoumarin **2** with $CuBr_2$ in EtOH (Scheme 2). ^1H and ^{13}C NMR spectroscopy, and ESI-MS were employed to



Scheme 2. Synthesis of compound **1** and the structures of reference compounds **3** and **4**.

characterize the structure of the product. Reference coumarin **3** is highly fluorescent ($\Phi_f = 0.556$, see the Supporting Information)[8] in DMF. By contrast, ketocoumarin **2** has much weaker fluorescence ($\Phi_f = 0.035$, see the Supporting Information) attributed to the aforementioned intersystem crossing. Indeed, introduction of a bromide group on the ketocoumarin scaffold further depresses the fluorescence of α-bromoketo coumarin **1** ($\Phi_f = 0.004$). Thereby, compound **1** seems promising as a fluorescence turn-on probe for thioureas provided that the fluorescence quenching groups, carbonyl and bromide, can be transformed by thioureas.

Although free probe **1** is almost nonfluorescent, introduction of thiourea elicited a dramatic increase in the emission around 494 nm in 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) buffer pH 7.4/DMF (1:1) (Figure 2). Furthermore, the addition of thiourea immediately turned the visual emission color of the solution of probe **1** from dark to bright green (Figure S1 see the Supporting Information), which further supports the fluorescence amplified response. The fluorescent intensities at 494 nm were plotted as a function of the thiourea concentration to obtain a linear calibration graph ranging from 5×10^{-7} to 1.5×10^{-4} M (Figure S2, see the Supporting Information), indicating that probe **1** is potentially useful for the quantitative determination of thiourea concentrations with a large dynamic range. We examined the kinetic profiles of the reaction under the pseudo-first-order conditions[9] with a large excess of thiourea (1.5 mM) over probe **1** (10 μM) in HEPES buffer pH 7.4/DMF (1:1) at 25 °C. The pseudo-first-order rate constant for the reaction was obtained, $k' = 4.92 \times 10^{-2}\ s^{-1}$ (Figure S3a and its inset, see the Supporting Information).By varying the concentrations of thiourea (from 0.1 to 1.5 mM), the second-order rate constant for the reaction of probe **1** (10 μM) and thiourea was calculated, $k = 3.35\ M^{-1}s^{-1}$ (Figure S3b, see the Supporting Information.). In addition, the probe showed high sensitivity to thiourea with a detection limit of 2.8×10^{-7} M under the experimental conditions (Figure S4, see the Supporting Information).

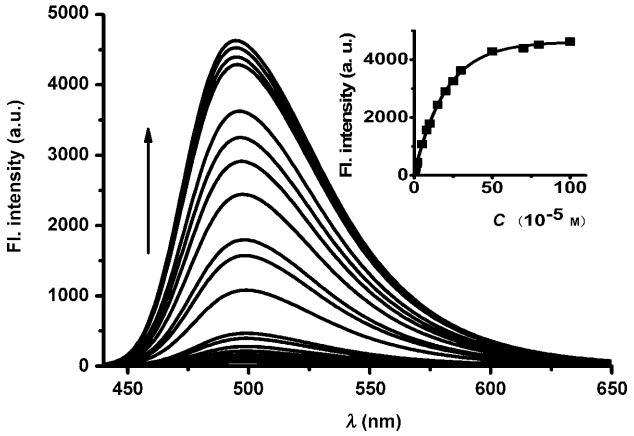


Figure 2. Emission spectra of probe **1** (10 μM) in HEPES buffer pH 7.4/DMF (1:1) in the presence of increasing concentrations of thiourea (0–1.0×10^{-3} M). The inset shows the changes in the fluorescence intensity of probe **1** (10 μM) at 494 nm in the presence of increasing concentrations of thiourea (0–1.0×10^{-3} M).

The selectivity of probe **1** was then examined. As anticipated, the addition of thiourea and its derivatives, such as phenylthiourea (PTU) and α-naphthylthiourea (ANTU) caused a significant enhancement in the fluorescent intensity around 494 nm in HEPES buffer pH 7.4/DMF (1:1) (Figure 3). In contrast, only a minimal fluorescence response was noted upon introduction of the representative species including glucose, CS_2, urea, phenol, aniline, DMSO, and arginine. Remarkably, the probe exhibited a high selectivity for thiourea over structurally related ureas, since α-bromoketo compounds do not react with ureas and no functional group transformation on probe **1** occurs. By contrast, the reported fluorescence quenching thiourea probe has poor selectivity for thiourea over urea.[5] Furthermore, the visual response of probe **1** to the various species (Figure S1, see the Supporting Information) indicates that probe **1** can be employed conveniently for detection of thioureas by simple

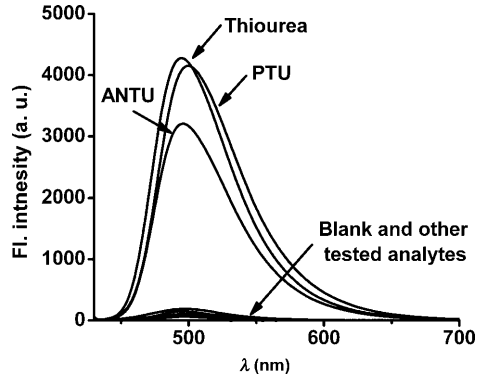


Figure 3. The fluorescence spectra of probe **1** (10 μM) in the presence of various relevant analytes (70 equiv) in HEPES buffer pH 7.4/DMF (1:1): Thiourea, phenylthiourea (PTU), α-naphthylthiourea (ANTU), glucose, CS_2, urea, phenol, aniline, DMSO, and arginine.

visual inspection. Additionally, other species have negligible interference with the fluorescence response of the probe to thiourea (Figure S5, see the Supporting Information), reinforcing the high selectivity of the probe.

To shed light on the functional role of the carbonyl and bromide groups of probe **1**, we investigated the fluorescence response of control compounds **2** and **4** to thiourea. As shown in Figure 4a, control compound **2**, which contains only a carbonyl group has almost no response to thiourea. Similarly, control compound **4**, which possesses only a bromide group showed a minimal response to thiourea (Figure 4b). Thus, these data clearly demonstrate that both the carbonyl and bromide functional groups of probe **1** are required for the fluorescence response to thioureas.

To verify that the fluorescence sensing response of the probe to thioureas is indeed due to the double functional group transformation of probe **1** to the corresponding thiazole compounds **5a**–**5c**, the reaction products of probe **1** with thioureas were isolated by column chromatography. Studies by ^{1}H NMR spectroscopy and mass spectrometry confirmed that probe **1** reacted with thioureas to form the corresponding thiazole compounds **5a**–**5c** (Figures S6–11 in the Supporting Information). This is further supported by the observation that the absorption and emission spectra of the isolated products were identical to those of the corresponding standard compounds **5a**–**5c** (Figures S12–14 in the Supporting Information). The photophysical data of the novel compounds **5a**–**5c** are compiled in Table 1.

When a strong nucleophile, such as thiophenol, was added to the solution of probe **1**, almost no fluorescence enhancement was observed (Figure 4c), although it was anticipated that thiophenol could react with probe **1** by a single functional group transformation. To confirm this, the reaction product of probe **1** with thiophenol was isolated and characterized as compound **6** (Scheme 3) by ^{1}H NMR spectroscopy and mass spectrometry (Figures S15 and 16 in the Supporting Information). Compound **6** still possesses the ketocoumarin scaffold and consequently exhibits very weak fluorescence (Table 1). Thereby, double functional group transfor-

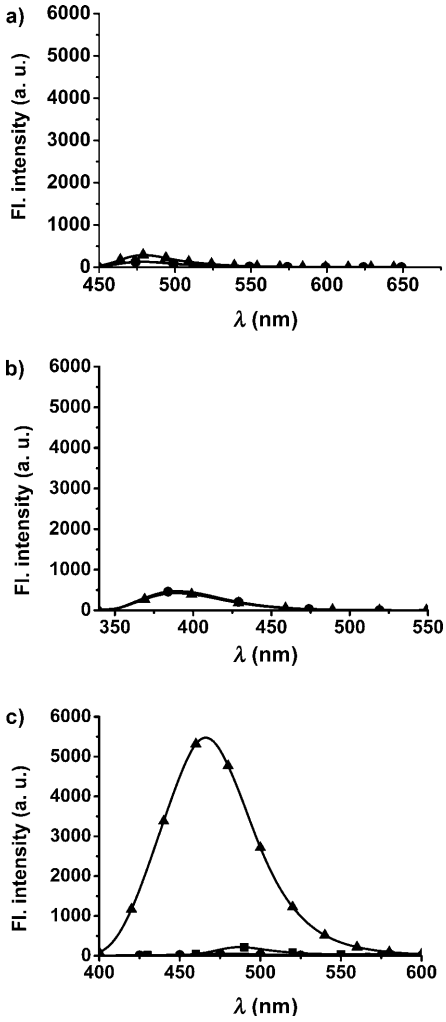
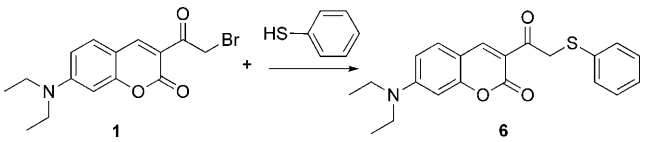


Figure 4. a) The emission spectra of control compound **2** (10 μM) in the absence (●) or presence of 70 equiv of thiourea (▲) in DMF; b) The emission spectra of control compound **4** (10 μM) in the absence (●) or presence of 70 equiv of thiourea (▲) in DMF; c) The emission spectra of probe **1** in the absence (●) or presence of 70 equiv of thiophenol (■) in DMF. For comparison, the emission spectrum of probe **1** in the presence of 70 equiv of thiourea (▲) in DMF was also shown.

Table 1. Photophysical data of compounds **5a**–**5c** and **6**.

	λ_{abs} [nm][a]	Log ε_{max}	λ_{em} [nm][b]	Φ_f[c]
5a	428	4.67	494	0.498
5b	432	4.60	498	0.490
5c	426	4.58	495	0.439
6	451	4.80	498	0.034

[a] The maximal absorption of the coumarins; [b] The maximal emission of the coumarins; [c] Fluorescence quantum yield was determined in spectroscopic DMF by using quinine sulfate (Φ_f=0.546) as a standard.[8]



Scheme 3. Reaction of probe **1** with thiophenol to form compound **6**.

mations between probe **1** and thiourea allow the probe to distinguish thiourea over thiophenol, which can only render single functional group transformation on the probe.

It is important to determine the level of thioureas in the water samples because thioureas are extensively used as fungicides in agriculture and may contaminate the water sources. Probe **1** was preliminarily employed to measure the concentrations of thiourea in the water samples of YueLu spring and Xiang River (see the Supporting Information). Added thiourea in the water samples could be accurately determined with good recovery (Table S1, see the Supporting Information), suggesting that probe **1** is effective for quantitative detection of thiourea in water samples.

The widespread use of thioureas in agriculture may also lead to contamination in soil. To examine whether probe **1** could be applied to detect thiourea in soil samples, we conducted a prototypic experiment. The soil sample containing different levels of thiourea could be readily detected with a large fluorescence signal (Figure 5a). This indicates that the

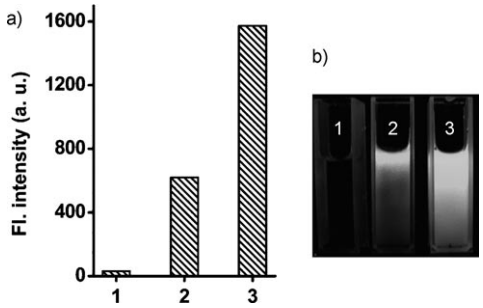


Figure 5. a) The fluorescence intensity and b) the fluorescence emission color changes of the solution of probe **1** (10 μM) treated with the soil samples. Sample 1: the soil blank; samples 2–3: the soil contaminated with 5 and 15 equiv of thiourea, respectively.

fluorescent probe operates well in crude soil samples. The detection of thiourea in soil samples can be conveniently visualized (Figure 5b). Furthermore, the probe could also be employed to quantitatively detect thiourea in soil samples (Table S2, see the Supporting Information), indicating the potential usefulness of the fluorescence detection system.

In conclusion, we have developed the first fluorescence turn-on probe for thioureas by the double functional group transformation strategy. The probe exhibited high sensitivity with a detection limit of 2.8×10^{-7} M. Remarkably, the probe is highly selective for thioureas over other structurally and chemically related species, including urea and thiophenol, demonstrating the advantage of the double functional group transformation approach, since urea and thiophenol can induce no or only single functional group transformations on the probe, respectively. Furthermore, we have shown that the probe could be employed to monitor thiourea in both water and soil samples. We believe that the double or multiple functional group transformation approach can be extended to construct other fluorescent probes.

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Keywords: coumarins · dyes/pigments · fluorescence spectroscopy · fluorescent probes · thioureas

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