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Colorimetric and fluorometric dual-modal probes for cyanide detection based on the doubly activated Michael acceptor and their bioimaging applications

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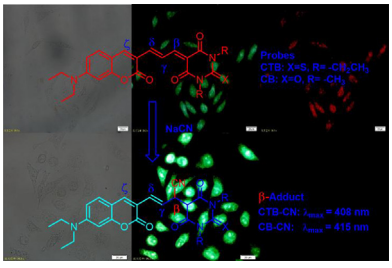
HIGHLIGHTS

- Two colorimetric and fluorometric dual-modal probes specific for cyanide has been developed.
- The probes can be applied to analyze CN^--contaminated water and biomimetic samples.
- The probes were cell-permeable and can be used to monitor CN^- level in living cell and subcellular imaging.
- The highly reactive nature of the β-site were verified by DFT/TDDFT calculations.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, we synthesized **CTB** and **CB** probes based on doubly activated Michael acceptors to selectively detect cyanide (CN^-) anions through a one-step condensation reaction of coumarinyl acrylaldehyde with the corresponding derivatives of malonyl urea (thiourea). Through the conjugated addition of CN^- to the β-site of the Michael acceptor, both probes displayed colorimetric and fluorometric dual-modal responses that were highly reactive and selective. **CTB** generates an active fluorescent response, whereas **CB** displays a ratiometric fluorescent response. The fluorescent signal of the probes reached its maximum given only 1 CN^- equivalent and the signal change was linearly proportional to CN^- concentrations ranging from 0 to 5 μM with the detection limits 18 and 23 nM, respectively. The reaction rate of the probes is highly dependent on the methylene acidity of malonyl urea derivatives. Thus, the response rate of **CTB** to CN^- is 1.2-fold faster than that of **CB**, and the response rate of **CB** to CN^- is 1.2-fold faster than that of the previously examined **CM**. We then verified the highly reactive nature of the β-site of the probes through density functional reactivity theory calculations. In addition, according to proof-of-concept experiments, these probes may be applied to analyze CN^- contaminated water and biomimetic samples. Finally, cell cytotoxicity and bioimaging studies revealed that the probes were cell-permeable and could be used to detect CN^- with low cytotoxicity.

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1. Introduction

Fluorescent probes have been developed to identify biologically and environmentally important anions. These anions are basic components in various chemical, environmental, and biological processes [1,2]. Among the numerous important anions, highly toxic cyanide (CN^-) has attracted much interest because it is extremely harmful to the environment and to human health [3]. CN^- ions can efficiently bind hemes to deactivate cytochrome *c* and inhibit the electron-transport chains in mitochondria. As a result, living organisms are lethally poisoned [4]. Moreover, many fire victims are killed by CN^- poisoning when the CN^- concentrations in blood reach the range between 23 and 26 μM [5,6]. Hence, CN^- concentrations in drinking water must be lower than 1.9 μM [7]. Nonetheless, CN^- is extensively used in fields such as gold mining, electroplating, metallurgy, synthetic fiber, and resin, as well as in the synthesis of nylon and acrylic polymers [8]. Therefore, accidental releases of CN^- salts can seriously damage the environment. Therefore, we must develop sensitive and selective chemical probes to detect CN^-.

Various chemical probes have been developed specifically for this purpose. CN^- selective colorimetric or fluorescent probes have been generated based on CN^- coordination and nucleophilic reactivity [9,10]. These probes integrate metal complexes [11–13] through the displacement method [14–18] and consider the bond-forming reaction between CN^- and either an electrophilic carbon [19,20] or a boron center [21,22].

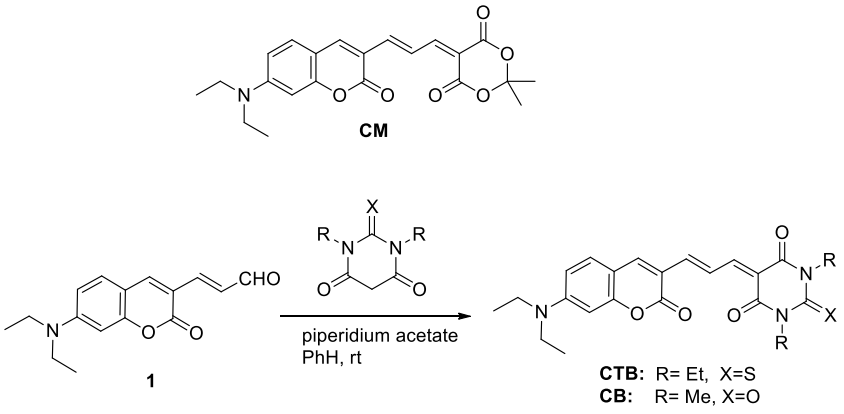
In the design of colorimetric and fluorescent CN^- probes, the Michael addition reaction has been successfully applied [23–37]. Michael addition-type probes can generally be constructed by covalently linking the Michael acceptor to a signaling group. The reaction of this acceptor with nucleophiles modifies cause either the change in color or fluorescence of the signaling group. In doubly activated Michael acceptors, two electron-withdrawing groups are connected to the C=C group [23–29]. These acceptors are more reactive [30–37] than general Michael acceptors, and produce the Michael addition reaction under mild conditions. However, most of the proposed CN^- probes based on doubly activated Michael acceptors can operate only in pure organic solutions. Moreover, some probes respond belatedly and require much CN^- (100 or higher) to maximize the fluorescent signal. Cheng et al. [23] developed a colorimetric and ratiometric fluorescent CN^- probe based on the coumarin–malononitrile conjugate. The probe was sensitive and highly selective with regard to CN^-, displayed a good ratiometric response, and was successfully applied in bioimaging. However, its reaction rate was unsatisfactory (ca. 30 min) in aqueous tetrahydrofuran (THF) (1:1). This slow response time may be attributed to malononitrile acidity ($pK_a = 11.0$). Zhou et al. [28] presented a colorimetric and ratiometric fluorescent CN^- probe based on the coumarin–indanedione conjugate. This probe was highly reactive (less than 60 s) to CN^- ions in acetonitrile given the strong acidity of indanedione ($pK_a = 7.2$). Encouraged by these studies, we had constructed a highly reactive conjugate (**CM**) composed of coumarin and Meldrum's acid ($pK_a = 4.97$), which is linked with a allylidene groups [25]. The **CM** conjugate was highly selective and sensitive toward NaCN, with a response time of less of 10 min in aqueous dimethyl sulfoxide (DMSO; 9:1). Moreover, the fluorescent signal is maximized given only 2 CN^- equivalents.

As a continuation of these previous works, we designed and synthesized two colorimetric and fluorometric dual-modal CN^- fluorescent probes (**CTB** and **CB**) based on a coumarin and a malonyl urea derivatives. Two units were covalently linked by allylidene linking groups (Scheme 1). Coumarin acted as the fluorophore in the probes, and either an allylidene thiobarbituric or allylidene barbituric acid groups served as the new putative CN^- dependent reactive subunit. Allylidene barbituric and allylidene thiobarbituric acid groups were selected as novel reaction sites because of the strong acidity of thiobarbituric ($pK_a = 3.96$) and barbituric acid ($pK_a = 4.01$), which would enhances the electrophilicity of either the β-, δ-, or ζ-carbon in the probes to provoke a CN^- attack on the strongly activated Michael acceptor (Scheme 2). This design also assures that breaking the conjugation in probes by addition of CN^- should elicit colorimetric and fluorometric dual-modal responses [23–37]. In this paper, we report the synthesis and crystal structures of **CTB** and **CB** and their application for detection of CN^- in Tris–HCl buffer, the CN^- contaminated water, biomimetic samples and living cell, together with DFRT calculations for the high reactivity of β-site in probes.

2. Experimental

2.1. General information and materials

Commercially available compounds were used without further purification. Solvents were dried according to standard procedures. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) using QingDao GF254 silica gel coated plates. Fluorescence spectra were carried out on a Shimadzu RF-5301PC fluorescence spectrophotometer. UV–vis spectra were recorded with a Shimadzu UV-2550 spectrophotometer. NMR spectra were recorded on a Bruker AV-300 Spetrometer (300 MHz for ^1H and 75 MHz for ^{13}C), and chemical shifts were referenced relative to tetramethylsilane (δH/δC = 0). Mass data



Scheme 1. Synthesis and structures of **CM**,**CTB**, and **CB**.

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