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Communication

Coumarinocoumarin-based fluorescent probe for the sensitive and selective detection of hydrazine in living cells and zebra fish

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ABSTRACT

A coumarinocoumarin-based fluorescent probe, **JCCA**, was developed for the detection of N_2H_4. **JCCA** exhibited a fast turn-on fluorescence enhancement in response to N_2H_4 with good selectivity, sensitivity and a detection limit of 7.4 nmol/L. Significantly, **JCCA** displayed a good capability for visualizing N_2H_4 in living cells and zebra fish.

Hydrazine (N_2H_4), a potentially versatile inorganic diamine with an ammonia-like smell, is widely used in many fields, including polymerization inhibitors, blowing agents, agricultural chemicals, rocket propellants, and anti-corrosion agents in water treatment [1–4]. Despite of its wide range of applications, N_2H_4 is highly toxic and dangerously unstable. Exogenous N_2H_4 can be readily absorbed and accumulated into human body through oral, dermal or breathing contact routes. In-depth studies on laboratory animals have revealed that endogenous N_2H_4 is mutagenic and carcinogenic, which can cause critical damages to kidneys, lungs, liver, and central nervous system [5–9]. As a result, the World Health Organization and US Environmental Protect Agency have set a recommended threshold limit value (TLV) of N_2H_4 to be as low as 10 ppb (0.32 μmol/L) [4,10]. Therefore, it is urgent and necessary to explore efficient analytical methods for sensitive and selective detection of hydrazine.

Conventional techniques for N_2H_4 detection such as flow detection [11], titrimetry [12], chromatography [13], potentiometry [14], electrochemical methods [15], chromatography-mass spectrometer [16], are usually subjected to expensive instrument, time-consuming, complicated sample preparation or relatively less sensitivity [17,18]. Fluorescent probes were proved to be useful tools for qualitative and quantitative detection of various species both *in vitro* and *in vivo* with the advantages such as high sensitivity, good selectivity, non-invasion, easy operation and real-time imaging [19–23]. Currently, various fluorescent probes for the detection of N_2H_4 have been reported; however, most of them suffer from slow response time and insufficient sensitivity (Table S1 in Supporting information) [24–35]. Moreover, the concentration of N_2H_4 *in vivo* is low and rapidly fluctuates during the production and catabolism [36]. Therefore, a robust fluorescent probe with a quick response and a low detection limit is needed for the real-time detection of N_2H_4 *in vivo*.

Coumarinocoumarin dyes are highly fluorescent and have a long-wavelength emission [37], which makes them a robust scaffold for the design of fluorescent probes. Herein, by incorporation of an acetate group to hydroxy julolidine-coumarinocoumarin (**JCC**), we have developed a novel fluorescent probe, **JCCA**, for the selective and sensitive detection of N_2H_4 (Scheme 1). We anticipated probe **JCCA** would be weakly fluorescent due to the photoinduced electron transfer effect from acetate group. Upon the treatment with N_2H_4, the ester bond in probe **JCCA** would be selectively cleaved to release a highly emissive dye **JCC**. The synthetic route of probe **JCCA** was shown in Scheme 1.

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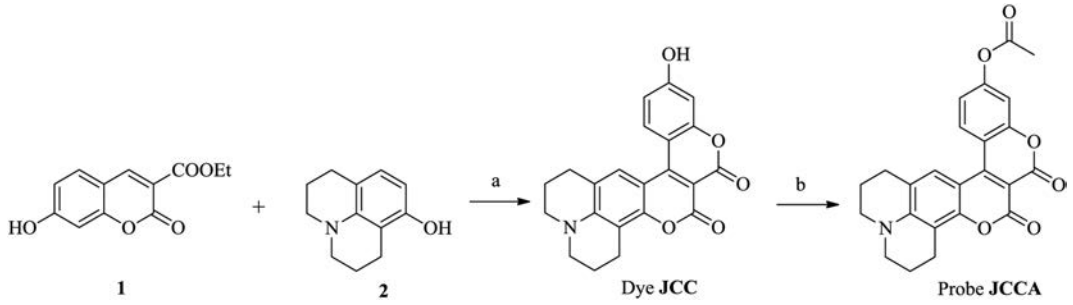


Scheme 1. Synthetic route of probe **JCCA**. (a) No solvent, 120 °C for 2 h, yield 42%. (b) Acetic anhydride, 60 °C for 2 h, yield 54%.

Before conducting fluorescence experiments, the solubility of probe **JCCA** in water (containing 5% DMSO) was measured to be 39.6 mg/L. The optical properties of probe **JCCA** in the absence/presence of N_2H_4 was investigated in PBS buffer (10.0 mmol/L, pH 7.4, containing 30% acetonitrile). Probe **JCCA** (10.0 μmol/L) exhibited an absorption maximum at 492 nm. Upon the treatment with excessive N_2H_4, the absorption at 492 nm disappeared and a new absorption peak at 473 nm emerged with a color change from pink to yellow (Fig. S1 in Supporting information). As expected, probe **JCCA** exhibited very weak fluorescence with λ_{max}^{em} = 582 nm (Fig. 1). After the addition of N_2H_4, the solution of probe **JCCA** produced a strong yellow fluorescence with λ_{max}^{em} = 542 nm. The intensities of the fluorescence signals at 542 nm gradually boosted up with increasing the concentration of N_2H_4 ranging from 0.0 μmol/L to 300.0 μmol/L. The maximal fluorescence enhancement was up to 21-fold. There was a good linearity (R = 0.996) between fluorescence intensity at 542 nm and the concentration of N_2H_4 in the range of 0.0–225.0 μmol/L (Fig. S2 in Supporting information). The detection limit was calculated to be 7.4 nmol/L (signal/noise = 3). Therefore, probe **JCCA** could be applied to qualitatively and quantitatively analyze N_2H_4 with excellent sensitivity. Dye **JCC** displayed an intensive absorption with an λ_{max}^{abs} at 474 nm and was strongly fluorescent with an λ_{max}^{em} at 542 nm. The similarity between the optical properties of dye **JCC** and the reaction mixture of probe **JCCA** with N_2H_4 evidently suggested that the addition of N_2H_4 to the solution of probe **JCCA** resulted in the formation of dye **JCC** (Scheme 2).

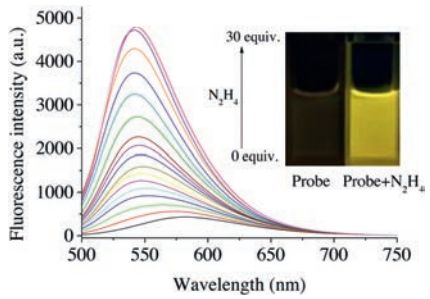
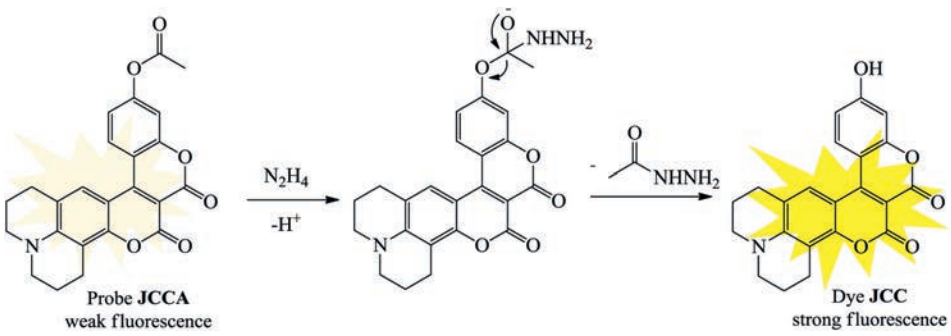


Fig. 1. Fluorescence spectra of probe **JCCA** (10.0 μmol/L) in response to N_2H_4 (0.0–300.0 μmol/L) in PBS buffer (10.0 mmol/L, pH 7.4, containing 30% acetonitrile). Excitation wavelength: 480 nm. Excitation and emission slits (nm): 5.0/5.0. Inset: photos of the solution of probe **JCCA** in the absence/presence of N_2H_4.

Time-dependent fluorescence experiments were carried out by treatment of the solution of probe **JCCA** (10.0 μmol/L) with N_2H_4 (30 equiv.) in PBS buffer (10.0 mmol/L, pH 7.4, containing 30% acetonitrile). As seen in Fig. 2, the addition of N_2H_4 instantaneously induced strong fluorescent signals, which were maximized within 3 min. In contrast, little fluorescence change was observed when the solution of probe **JCCA** was in the absence of N_2H_4 during the same interval. These results suggested probe **JCCA** was stable and exhibited a quick response toward to N_2H_4.

To evaluate the selectivity of probe **JCCA** for N_2H_4, the fluorescence responses of probe **JCCA** towards common relevant species (30.0 equiv.) were determined, including biothiols (Cys, Hcy, GSH), cations (Na^+, K^+, Mg^{2+}, Al^{3+}, Zn^{2+}, Cu^{2+}, Ca^{2+}, Hg^{2+}, Fe^{2+}), anions (N_3^-, F^-, Cl^-, I^-, Br^-, CO_3^{2-}, PO_4^{2-}, SO_4^{2-}, S^{2-}, SCN^-, NO_3^-, AcO^-) and nitrogen-containing compounds (aniline, thiourea, urea, NH_2OH). As shown in Fig. 3, the fluorescence spectra of probe **JCCA** exhibited negligible changes in the presence of these relevant analytes. Meanwhile, the co-existence of these relevant species caused no interference on the performance of probe **JCCA** for the detection of N_2H_4 (Fig. S3 in Supporting information). All the above results clearly showed that probe **JCCA** had a good selectivity toward N_2H_4.

In order to evaluate whether probe **JCCA** can function well in biological samples, we investigated its fluorescence property in the absence/present of N_2H_4 in the media with different pH values (Fig. S4 in Supporting information). In the absence of N_2H_4, the fluorescence intensity hardly changed in a pH range between 2.0-10.0. In the presence of N_2H_4 (30.0 equiv.), the solution of probe **JCCA** displayed strong fluorescent signals with a maximum at 542 nm in a pH range of 7.0-10.0. Therefore, it could be concluded



Scheme 2. Proposed sensing mechanism of probe **JCCA** towards N_2H_4.

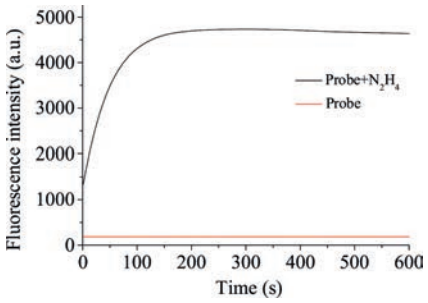


Fig. 2. Time-dependent fluorescence experiment of probe **JCCA** (10.0 μmol/L) in the absence and presence of N_2H_4 (300.0 μmol/L) in PBS buffer (10 mmol/L, pH 7.4, containing 30% acetonitrile).

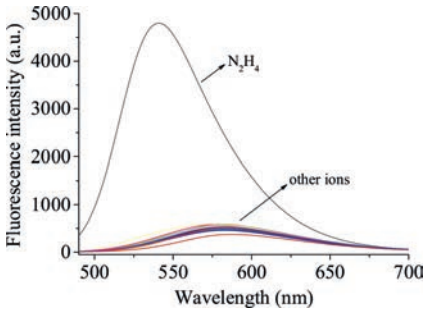


Fig. 3. Fluorescence spectra of probe **JCCA** (10.0 μmol/L) upon the addition of N_2H_4 and other analytes (300.0 μmol/L) in PBS buffer (10 mmol/L, pH 7.4, containing 30% acetonitrile).

that probe **JCCA** had a good performance in the detection of N_2H_4 under physiological condition.

Finally, the potential application of probe **JCCA** in living cells was carried out (Fig. 4). First, the cytotoxicity of probe **JCCA** was evaluated using HeLa cells by the methyl thiazolyl tetrazolium (MTT) assay. A 96% cell viability was obtained when cells were incubated with 10.0 μmol/L of probe **JCCA** for 24 h, indicating probe **JCCA** was non-toxic (Fig. S5 in Supporting information). When HeLa cells were incubated with probe **JCCA** (10.0 μmol/L) for 30 min, very weak orange fluorescence (bandpass filter 560–600 nm) was observed (Fig. 4d). When HeLa cells were incubated

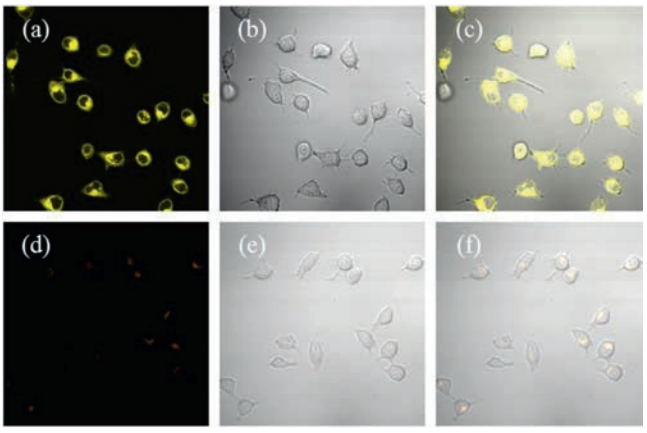


Fig. 4. Fluorescence (left column), bright field (middle column) and merged (right column) images of HeLa cells. Top row: cells incubated with probe **JCCA** (10.0 μmol/L) for 30 min and subsequently treated with N_2H_4 (300.0 μmol/L) for another 30 min. Bottom row: cells incubated with probe **JCCA** (10.0 μmol/L) for 30 min. Yellow channel (520–560 nm, excited at 488 nm); Orange channel (560–600 nm, excited at 488 nm).

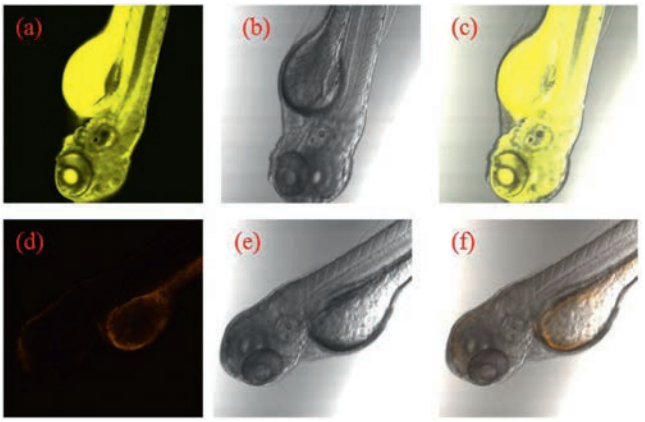


Fig. 5. Fluorescence (left column), bright field (middle column) and merged (right column) images of three-day old zebra fish. Top row: zebra fish incubated with probe **JCCA** (10.0 μmol/L) and then treated with N_2H_4 (300.0 μmol/L). Bottom row: zebra fish incubated with probe **JCCA** (10.0 μmol/L). Yellow channel (520–560 nm, excited at 488 nm); Orange channel (560–600 nm, excited at 488 nm).

with probe **JCCA** (10.0 μmol/L) for 30 min and then treated with N_2H_4 (300.0 μmol/L) for another 30 min, bright yellow fluorescent signals (bandpass filter 520–560 nm) occurred from cells (Fig. 4a).

Furthermore, we used three-day old zebra fish to investigate the capability of probe **JCCA** to detect N_2H_4 in living organisms (Fig. 5). When zebra fish was incubated with probe **JCCA** (10.0 μmol/L) for 30 min at 28 °C, and then treated with N_2H_4 (300.0 μmol/L) for another 30 min, strong yellow fluorescent signals (bandpass filter 560–600 nm) were seen from zebra fish (Fig. 5a). In contrast, the incubation of zebra fish with probe **JCCA** (10.0 μmol/L) only resulted in weak orange fluorescence (bandpass filter 560–600 nm) (Fig. 5d).

In conclusion, we developed a new fluorescent probe for the detection of N_2H_4 on the basis of a coumarinocoumarin derivative. This probe displayed a fast response time with high sensitivity, good selectivity, and a low detection limit (7.4 nmol/L). The application of this probe in living cells and organism demonstrated it had a good potential for the detection of N_2H_4 in biological systems.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2019.04.021.

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