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PII:	S1386-1425(17)30441-9
DOI:	doi: 10.1016/j.saa.2017.05.058
Reference:	SAA 15200
To appear in:	*Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*
Received date:	10 February 2017
Revised date:	16 May 2017
Accepted date:	28 May 2017

Please cite this article as: Khomsan Tiensomjitr, Rattha Noorat, Kanokorn Wechakorn, Samran Prabpai, Kanoknetr Suksen, Phongthon Kanjanasirirat, Yongyut Pewkliang, Suparerk Borwornpinyo, Palangpon Kongsaeree , A rhodol-based fluorescent chemosensor for hydrazine and its application in live cell bioimaging, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* (2017), doi: 10.1016/j.saa.2017.05.058

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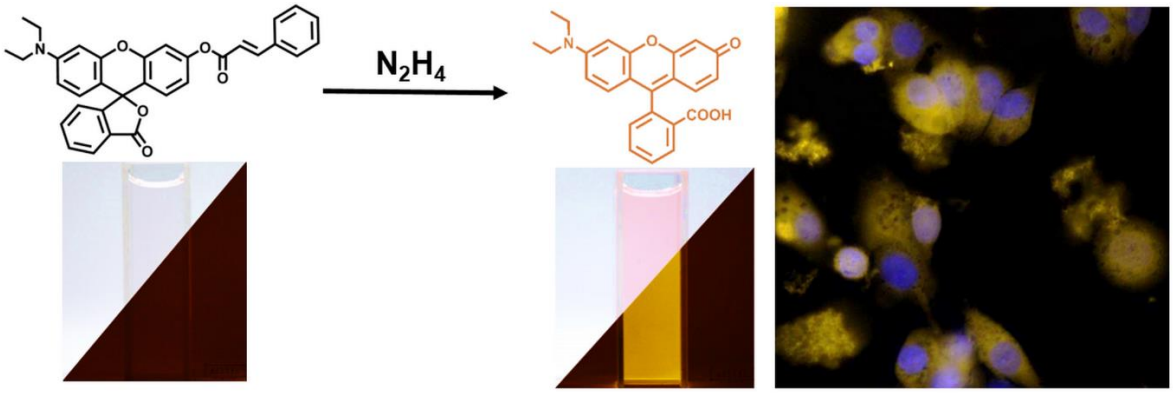
Graphical abstract



Research highlights

- A rhodol-based fluorogenic probe with a cinnamate moiety was synthesized and characterized by ^{1}H NMR, ^{13}C NMR and HRMS.
- The chemical probe is highly selective to hydrazine with a 48-fold fluorescence enhancement and a detection limit of 9.6 ppb.
- The cinnamoyl rhodol chemosensor is membrane permeable and used in bioimaging of hydrazine in HepG2 cells.

A rhodol-based fluorescent chemosensor for hydrazine and its application in live cell bioimaging

Abstract

A rhodol cinnamate fluorescent chemosensor (**RC**) has been developed for selective detection of hydrazine (N_2H_4). In aqueous medium, the rhodol-based probe exhibited high selectivity for hydrazine among other molecules. The addition of hydrazine triggered a fluorescence emission with 48-fold enhancement based on hydrazinolysis and a subsequent ring-opening process. The chemical probe also displayed a selective colorimetric response toward N_2H_4 from colorless solution to pink, readily observed by the naked eye. The detection limit of **RC** for hydrazine was calculated to be 300 nM (9.6 ppb). **RC** is membrane permeable and was successfully demonstrated to detect hydrazine in live HepG2 cells by confocal fluorescence microscopy.

Keywords: Hydrazine-selective chemosensor; Fluorescent detection; Rhodol; Bioimaging

1. Introduction

Hydrazine (N_2H_4), a colorless flammable inorganic liquid, has been used as a high energy fuel in missile propulsion systems [1,2]. Due to its basic and reducing properties, hydrazine is also an essential reagent in chemical, pharmaceutical, and agricultural industries [3-5]. Because of its high degree of toxicity to human health and the environment, hydrazine has been classified as a probable human carcinogen by the U.S. Environmental Protection Agency (EPA) with a threshold limit value (TLV) of 10 ppb [6,7]. Hydrazine causes neurotoxicity and mutagenic effects to liver, lung, and kidney by absorption through skin and lung [8,9]. Several methods can be used to efficiently determine hydrazine such as HPLC [10], gas chromatography [11], and capillary electrophoresis [12]. However, these methods are not applicable for intracellular detection of hydrazine due to its incompatible sample preparation. Therefore, development of chemosensors for selective recognition of hydrazine has attracted a great deal of scientific attention in recent years. Fluorescence-based methods are very practical in chemical and biological studies because of their advantageous high sensitivity and selectivity as well as biocompatibility [13-15]. With a reaction-based strategy, several naked eye and fluorescent chemosensors have been developed for hydrazine detection using various dyes including BODIPY [16,17], coumarin [18-20], naphthalimide [7,21], resorufin [22], benzothiazole [23], pyrazoline [24,25], and xanthene analogs [26-28]. Among these reported hydrazine-selective chemosensors, several prosthetic groups have been explored including malononitrile [17,24,29-31], cyanoacrylate [16], bromoacrylate [22,23,26], acrylate [19,21,27], acetylacetonate [32,33], and trifluoroacetylacetonate [7,18] but not cinnamate. Rhodol (or rhodafluor), a hybrid structure of rhodamine and fluorescein, is a useful organic dye with excellent photophysical properties such as high extinction coefficient, quantum yields, photostability, and strong fluorescence with low excitation

wavelength [34,35]. It has been applied for fluorescence detection of various cations [36-42], anions [36,39], NAD(P)H [43], glutathione and cysteine [44,45] but not for hydrazine.

Herein, we present a rhodol derivative appended with a cinnamate moiety as a highly selective and sensitive turn-on fluorescent probe for hydrazine with a lower detection limit and a faster detection time than some of previously reported probes. We envisioned that the rhodol cinnamate conjugate would be colorless and non-emissive. A loss of the cinnamate moiety, promoted by hydrazinolysis, would lead to a subsequent ring opening of the spirocyclic moiety and trigger chromogenic and fluorogenic changes.

2. Materials and methods

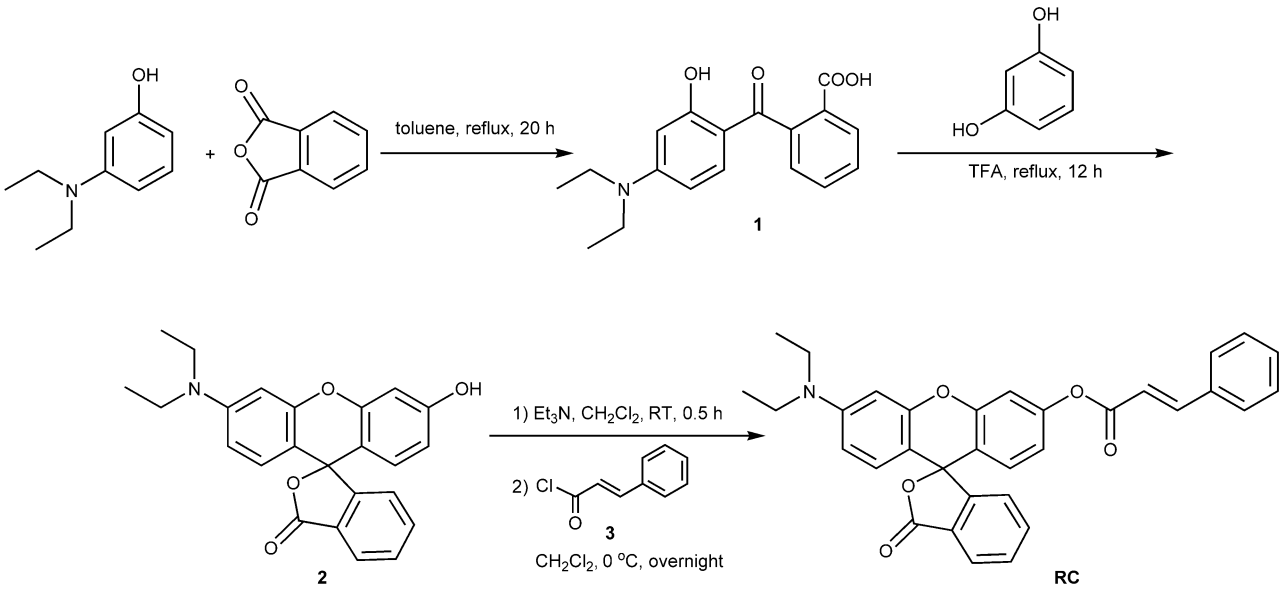
All chemicals and solvents used in this work were of analytical grade, obtained commercially and used without further purification. Column chromatography was performed using silica gel 60 (Merck). Other common solvents including CH_2Cl_2, EtOAc and hexane were distilled prior to use. UV-vis absorption spectra were recorded on an Agilent 89090A spectrophotometer at ambient temperature. Fluorescence measurements were performed on a Cary Eclipse fluorescence spectrophotometer with a 10-mm quartz cuvette. ^{1}H- and ^{13}C-NMR spectra were obtained from a Bruker AV-400 spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. ESI-HRMS mass spectra were acquired from a Bruker MicrOTOF mass spectrometer.

2.1 Synthesis of rhodol cinnamate (RC)

Preparation of 2-(4-(N,N-diethylamino)-2-hydroxybenzoyl) benzoic acid (1)

Compound **1** was synthesized according to the previously reported procedure (Scheme 1) [40]. A suspension of 3-diethylaminophenol (1.46 g, 10.6 mmol) and *o*-phthalic anhydride (1.57 g, 10.6 mmol) in toluene was refluxed for 20 h. The reaction solvent was removed under vacuo and the

crude product was dissolved in CH_2Cl_2. The organic phase was washed with 1 N HCl and then dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the crude product was purified by silica gel column chromatography to give **1** as a white solid (2.08 g, 55% yield).



Scheme 1. Synthesis of rhodol cinnamate (**RC**).

Preparation of rhodol (2)

Rhodol (**2**) was obtained in excellent yield according to the reported literature procedure [40]. To a suspended solution of **1** (0.94 g, 3 mmol) in trifluoroacetic acid (TFA, 25 mL) was added resorcinol (0.34 g, 3 mmol) and then the reaction mixture was stirred and refluxed for 12 h. The reaction mixture was cooled to room temperature and then the solvent was evaporated under vacuo to give the product precipitate. The crude product was further purified by recrystallization from EtOAc to obtain rhodol **2** as a white solid (1.12 g, 3 mmol, 99 %yield).

Preparation of rhodol cinnamate (RC)

Rhodol (**2**) (387 mg, 1.0 mmol) was stirred in CH_2Cl_2 (30 mL) and triethylamine (TEA) (5 mL) for 30 min (Scheme 1). The mixture was cooled to 0 °C and cinnamoyl chloride (330 mg, 2.2 mmol) in CH_2Cl_2 (10 mL) was slowly added and stirred overnight. The solvent was then removed under reduced pressure and the obtained residue was further purified by column chromatography, with EtOAc-hexane (40:60, v/v) as an eluent. The purified product was recrystallized in EtOH-hexane to obtain the desired **RC** probe as colorless prism-like crystals (390 mg, 76% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 7.5 Hz, 1H), 7.91 (d, J = 6 Hz, 1H), 7.68 (t, J = 7.4 Hz, 1H), 7.63 (t, J = 4 Hz, 1H), 7.62 (t, J = 3.5 Hz, 1H), 7.46 (t, J = 2.5 Hz, 3H), 7.26 (t, J = 9.3 Hz, 2H), 7.16 (d, J = 1.7 Hz, 1H), 6.87 (d, J = 8.6 Hz, 1H), 6.83 (d, J = 8.6 Hz, 1H), 6.65 (d, J = 16 Hz, 1H), 3.39 (q, J = 7.1 Hz, 4H), 1.25 (t, J = 7.1 Hz, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 164.9, 152.9, 151.9, 147.2, 134.93, 134.0, 130.9, 129.7, 129.1, 128.4, 124.99, 124.2, 118.9, 110.3, 77.2, 12.4; HRMS (ESI) *m*/*z* 518.1966 $[M+H]^+$, calcd for $C_{33}H_{28}NO_5$ 518.1962.

2.2. Spectroscopic experiments

A stock solution of **RC** (1×10^{-3} M) was prepared in EtOH. All stock solution (1×10^{-2} M) of additives including cations (Na^+, K^+, Ag^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Pd^{2+}, Ni^{2+}, and Hg^{2+}), anions (HCO_3^-, CO_3^{2-}, NO_3^-, PO_4^{3-}, Cl^-, I^-, Br^-, F^-, CN^-, N_3^-, HSO_4^-, SO_4^{2-}, HSO_3^-, SO_3^{2-}, SCN^-, CH_3COO^-, and HS^-), and other neutral species (NH_2OH, CH_3NH_2, urea, thiourea, cysteine, and diaminopropane) were prepared in HEPES buffer (10 mM, pH 7.4). All measurements were carried out in aqueous EtOH (1:1, v/v, 10 mM HEPES buffer, pH 7.4) at room temperature. The samples were excited at 520 nm with the 5-nm slit widths and the emission spectrum was scanned from 525 nm to 700 nm.

2.3. Cell culture and confocal fluorescence microscopy

HepG2 cells were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) with 10% FBS, 100 μg/ml streptomycin and 100 U/ml penicillin at 37 °C in an atmosphere of 5% CO_2. Cells were plated on a 96-well microtiter plate and allowed to adhere for 24 h. The cultured cells (3×10^4/well) were incubated with Hoechst 33342 (20 μM) at 37 °C for 10 min and then treated with hydrazine (900 μM) in DMEM/F-12 medium at 37 °C for 1 h. Treated cells were then washed with DMEM/F-12 medium to remove excess hydrazine prior to incubation with **RC** (30 μM, 0.1% DMSO) for 1 h. Cells were imaged in DMEM/F-12 medium at 37 °C under 5% CO_2 with an Operetta high-content imaging system (PerkinElmer) using a 40× objective lens and the Harmony® software. For the fluorescence imaging experiments, the emission wavelength was collected at 560-630 nm and the excitation wavelength was set at 520-550 nm. For Hoechst-stained nucleus, the emission wavelength was collected at 410-480 nm and the excitation wavelength was set at 360-400 nm.

3. Results and discussion

The rhodol cinnamate probe **(RC)** was successfully synthesized in high yield via a coupling reaction between rhodol and cinnamoyl chloride (Scheme 1). The structure of **RC** was established by analysis of ^{1}H-, ^{13}C-NMR, and HR-ESIMS data. Its ^{1}H-NMR spectral data with the doublet signals at δ 7.91 and δ 6.65 ppm (J = 16 Hz) supported a *trans*-cinnamate moiety. In the ^{13}C-NMR data, the characteristic spirocarbon signal at δ 77.2 ppm indicated the spirolactone ring in the closed form, and thus the compound was non-fluorescent due to the absence of the conjugation system. Furthermore, the structure of **RC** was investigated by a single-crystal X-ray crystallographic analysis and the results clearly confirmed the spirocyclic lactone form (Fig. 1).

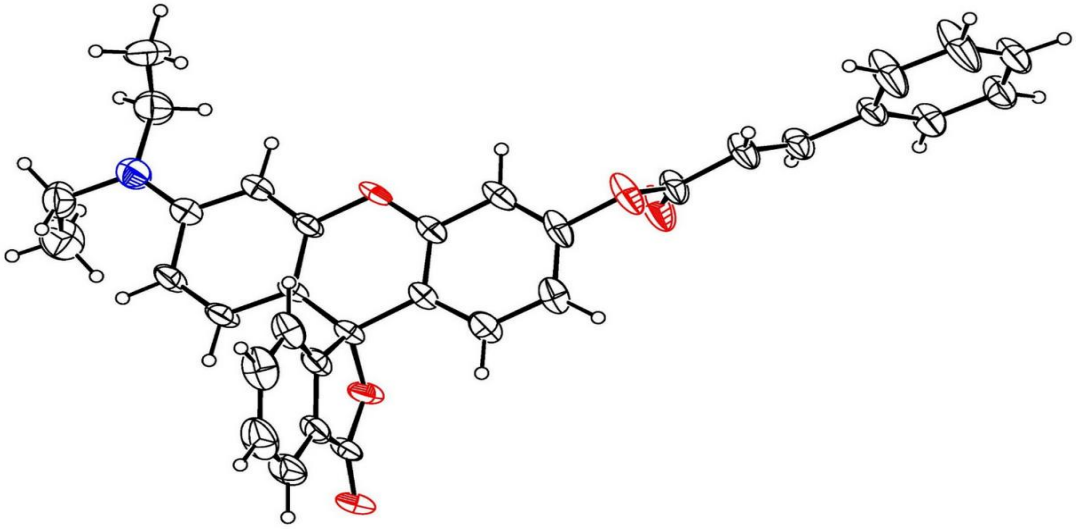


Fig. 1. ORTEP plot of rhodol cinnamate (**RC**).

A colorless solution of **RC** in aqueous EtOH (1:1, v/v, 10 mM HEPES buffer, pH 7.4) was non-emissive and showed no absorption band in the visible range (400-700 nm). Upon the addition of hydrazine (10 equiv.), the solution of **RC** (10 μM) turned orange with a new absorption band centered at 520 nm and strongly emitted yellow-orange fluorescence with a maximum emission peak at 545 nm (Fig. S1). This optical change was due to the ring-opening process of the spirolactone that gave fluorescein-like spectroscopic properties [39,41,42,45]. To evaluate the selectivity of **RC** to various cations, anions and neutral species (10 equiv.) including Na^+, K^+, Ag^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Pd^{2+}, Ni^{2+}, Hg^{2+}, HCO^{3-}, NO^{3-}, PO_4^{3-}, Cl^-, I^-, Br^-, F^-, CN^-, N_3^-, CO_3^{2-}, HS^-, SCN^-, HSO_4^-, SO_4^{2-}, HSO_3^-, SO_3^{2-}, CH_3COO^-, NH_2OH, CH_3NH_2, urea, thiourea, cysteine, and diaminopropane, were investigated for their ability that may affect the fluorescence change of **RC** compared with hydrazine. These analytes did not trigger any fluorescence change of the probe indicating that **RC** could be used as a highly selective "off-on" fluorescent chemosensor for hydrazine (Fig. 2). Interference studies also suggested that the fluorescence response of **RC** to hydrazine

remained unaffected in the presence of the above-mentioned neutral and ionic additives (Fig. S2).

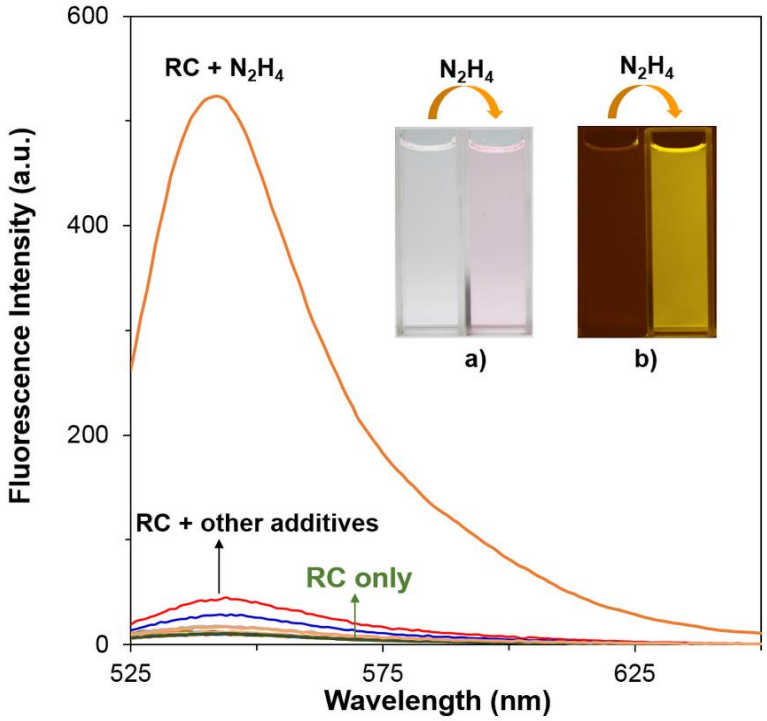


Fig. 2. Fluorescence spectra of **RC** (10 μM) in aqueous EtOH solution (1:1, v/v, 10 mM HEPES buffer pH 7.4) upon the addition of various additives (Na^+, K^+, Ag^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Pd^{2+}, Ni^{2+}, Hg^{2+}, HCO^{3-}, NO^{3-}, PO_4^{3-}, Cl^-, I^-, Br^-, CO_3^{2-}, CN^-, N_3^-, F^-, HS^-, SCN^-, HSO_4^-, HSO_3^-, SO_4^{2-}, SO_3^{2-}, CH_3COO^-, NH_2OH, CH_3NH_2, urea, thiourea, cysteine, and diaminopropane) and hydrazine (10 equiv.) for 30 min (λ_{ex} = 520 nM). Inset: a) color change observed in visible light and b) fluorescence emission under blue-LED illumination.

A quantitative "off-on" response of **RC** was further investigated by a fluorescence titration study. The emission spectra of **RC** (10 μM) solutions with increasing concentrations of hydrazine in aqueous EtOH (1:1, v/v, 10 mM HEPES buffer, pH 7.4) were recorded (Fig. 3). Upon the addition of hydrazine (0-300 equiv.), the fluorescence intensity at 545 nm of **RC** was responsive to the hydrazine concentrations with a linear response in the range of 0.25-1.5 mM. The limit of detection (3σ/slope) of **RC** to hydrazine, the lowest concentration of hydrazine that the probe can reliably detect [46], was calculated to be 300 nM (9.6 ppb), below the TLV recommended by the U.S. EPA, and lower than those of several other reported hydrazine-sensitive probes (Table S1) [16-18,22,24]. For the kinetic of the reaction, the fluorescence intensity of **RC** with hydrazine (10 and 20 equiv.) at 545 nm in aqueous EtOH

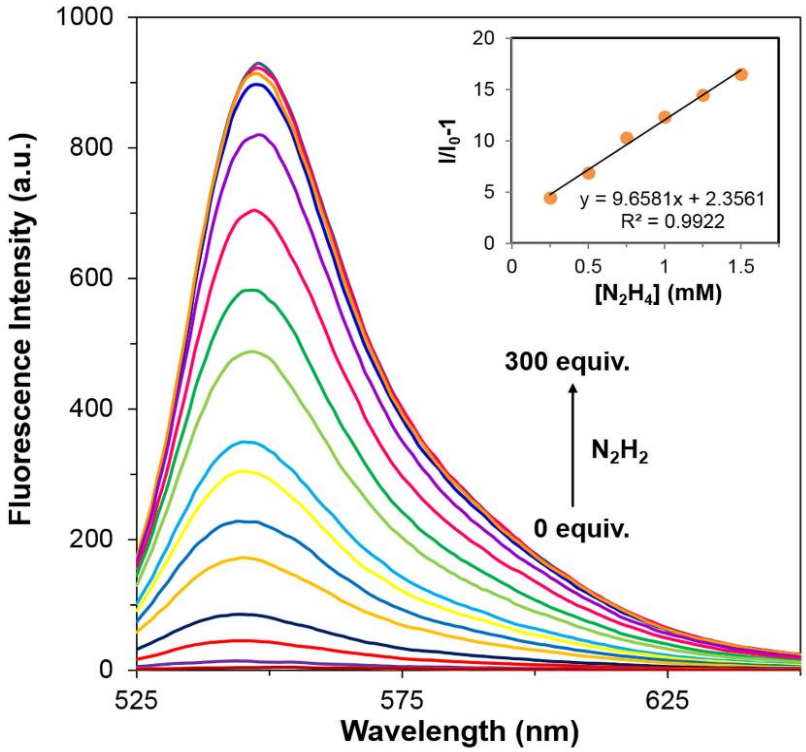
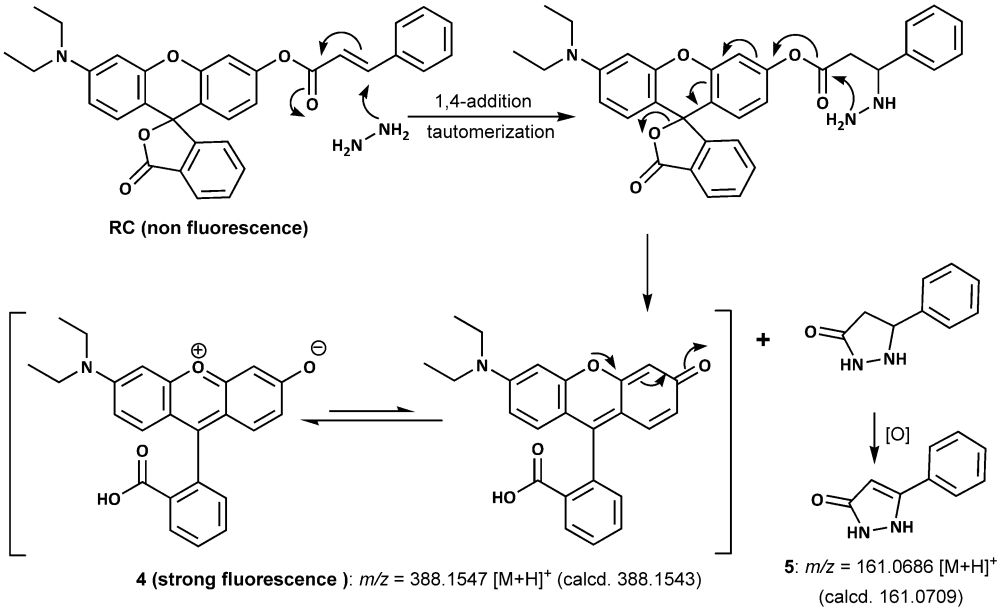


Fig. 3. Fluorescence titration of **RC** (10 μM) in aqueous EtOH solution (1:1, v/v, 10 mM HEPES buffer, pH 7.4) upon the addition hydrazine (0-3.0 mM) (λ_{ex} = 520 nM). Inset: a linear plot of [I/I_0-1] vs. the hydrazine concentration (0.25-1.5 mM).

(1:1, v/v, 10 mM HEPES buffer, pH 7.4) revealed that fluorescence intensity was increased to reach a plateau within 30 min (Fig. S3), faster than some reported hydrazine probes [7,19,22,47,48].

In order to understand the transformation of **RC** by hydrazine, the reaction between **RC** and hydrazine (1:1 molar ratio) in DMSO-d_6 (Scheme 2) was investigated with ^{1}H NMR and HR-ESIMS analyses. It is plausible that hydrazinolysis of **RC** occurred via a nucleophilic 1,4-addition of hydrazine at the β-carbon of the cinnamate moiety to give an enol which was then tautomerized to a carbonyl group. Subsequently, the nucleophilic amine attacked the carbonyl ester with the intramolecular cyclization then eliminated the rhodol unit and triggered a ring-opening of spirolactone and the fluorescence emission (**4**). A major molecular ion of rhodol $[M+H]^+$ *m/z* 388.1547 was detected (calcd 388.1543) along with a molecular ion *m/z* 161.0686 (calcd. 161.0709) for the corresponding cyclization product (**5**) (Fig. S10). The ring-closing reaction of hydrazine was similar to other reported systems [23,26]. In the ^{1}H



Scheme 2. A proposed mechanism of hydrazine detection by **RC**.

NMR data, the *trans*-H_a and H_b doublet signals of **RC** at δ 6.91 ppm and δ 7.90 ppm (Fig. 4a) were absent upon incubation with hydrazine (Fig. 4b). The transformation was also confirmed by a down-field shift of the doublet H_c peak of **RC** from δ 8.03 ppm (Fig. 4a) to δ 8.12 ppm due to the presence of more electrophilic carboxylic group in **4** (Fig. 4b) [40]. In addition, the reactivity of rhodol butanoate, a saturated analog of **RC**, was evaluated and it was found to be 20 times less reactive toward hydrazine than **RC**, supporting the significance of the cinnamate ester (Fig. S11).

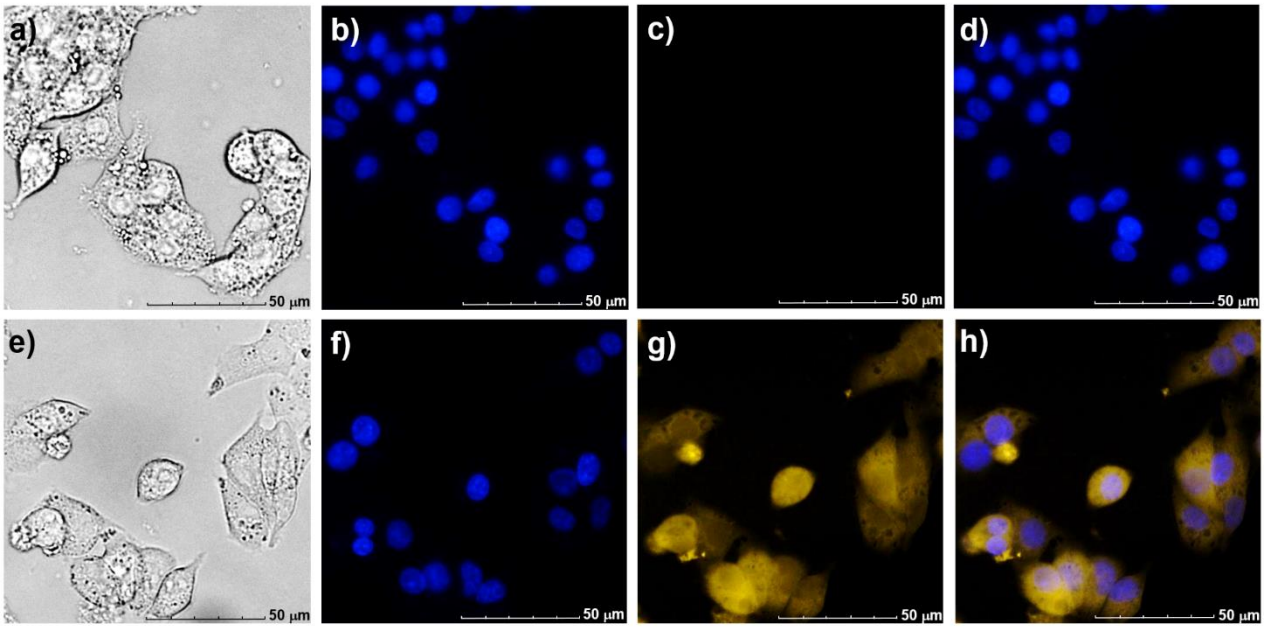


Fig. 5. Confocal fluorescence images of hydrazine detection by **RC** in live HepG2 cells. The cultured cells were incubated with hydrazine (900 μM) in DMEM/F-12 medium for 1 h (e-h) and then incubated with **RC** (30 μM) for 1 h (a-h). (a) and (e) Bright-field images; (b) and (f) fluorescence images of nuclei counterstained with Hoechst 33342; (c) and (g) fluorescence images; (d) and (h) merged fluorescence images of (b)-(c) and (f)-(g), respectively.

The ability of **RC** to detect hydrazine in cellular environments was further investigated in live HepG2 human liver cancer cells by confocal fluorescence microscopy (Fig. 5). HepG2 cells incubated with hydrazine (900 μM) for 1 h prior to incubation with **RC** (30 μM) for 1 h showed a distinct intracellular fluorescence emission (Fig. 5g). For the control experiment, the cells incubated with only **RC** were not emissive (Fig. 5c). The cytotoxicity was also evaluated for **RC** (30 μM) and for hydrazine (1500 μM), showing that there was no any adverse effect on cell viability (Fig. S12). These results suggested that **RC** was membrane permeable and could be utilized to detect hydrazine in living cells.

4. Conclusion

The rhodol-cinnamate chemosensor **RC** was successfully developed as a hydrazine-selective probe with a turn-on fluorescence response at 545 nm. It was demonstrated that the cinnamate group could be used as a hydrazine-sensing unit with rhodol as a reporter group. The chemosensor was successfully applied to monitor hydrazine in HepG2 cells by fluorescence microscopy.

Acknowledgments

We would like to acknowledge Mahidol University, National Research University Project, the Thailand Research Fund, and Center for Innovation in Chemistry (PERCH-CIC) for financial support. K.T. and R.N. thank the Development and Promotion of Science and Technology Talents Project (DPST) under the Royal Thai Government for scholarships.

References

[1] X. Chen, Y. Xiang, Z. Li, A. Tong, Sensitive and selective fluorescence determination of trace hydrazine in aqueous solution utilizing 5-chlorosalicylaldehyde, Anal. Chim. Acta 625 (2008) 41-46.

[2] S.D. Zelnick, D.R. Mattie, P.C. Stepaniak, Occupational exposure to hydrazines: treatment of acute central nervous system toxicity, Aviat. Space Environ. Med. 74 (2003) 1285-1291.

[3] U. Ragnarsson, Synthetic methodology for alkyl substituted hydrazines, Chem. Soc. Rev. 30 (2001) 205-213.

[4] K. Yamada, K. Yasuda, N. Fujiwara, Z. Siroma, H. Tanaka, Y. Miyazaki, T. Kobayashi, Potential application of anion-exchange membrane for hydrazine fuel cell electrolyte, Electrochem. Commun. 5 (2003) 892-896.

[5] S.S. Narayanan, F. Scholz, A comparative study of the electrocatalytic activities of some metal hexacyanoferrates for the oxidation of hydrazine, Electroanalysis 11 (1999) 465-469.

[6] A. Umar, M.M. Rahman, S.H. Kim, Y.-B. Hahn, Zinc oxide nanonail based chemical sensor for hydrazine detection, Chem. Commun. (2008) 166-168.

[7] M.H. Lee, B. Yoon, J.S. Kim, J.L. Sessler, Naphthalimide trifluoroacetyl acetonate: A hydrazine-selective chemodosimetric sensor, Chem. Sci. 4 (2013) 4121-4126.

[8] S. Garrod, M.E. Bollard, A.W. Nicholls, S.C. Connor, J. Connelly, J.K. Nicholson, E. Holmes, Integrated metabonomic analysis of the multiorgan effects of hydrazine toxicity in the rat, Chem. Res. Toxicol. 18 (2005) 115-122.

[9] C.A. Reilly, S.D. Aust, Peroxidase substrates stimulate the oxidation of hydralazine to metabolites which cause single-strand breaks in DNA, Chem. Res. Toxicol. 10 (1997) 328-334.

[10] H. Bhutani, S. Singh, S. Vir, K.K. Bhutani, R. Kumar, A.K. Chakraborti, K.C. Jindal, LC and LC-MS study of stress decomposition behaviour of isoniazid and establishment of validated stability-indicating assay method, J. Pharm. Biomed. Anal. 43 (2007) 1213-1220.

[11] M. Sun, L. Bai, D.Q. Liu, A generic approach for the determination of trace hydrazine in drug substances using in situ derivatization-headspace GC-MS, J. Pharm. Biomed. Anal. 49 (2009) 529-533.

[12] J. Liu, W. Zhou, T. You, F. Li, E. Wang, S. Dong, Detection of hydrazine, methylhydrazine, and isoniazid by capillary electrophoresis with a palladium-modified microdisk array electrode, Anal. Chem. 68 (1996) 3350-3353.

[13] J.F. Zhang, Y. Zhou, J. Yoon, J.S. Kim, Recent progress in fluorescent and colorimetric chemosensors for detection of precious metal ions (silver, gold and platinum ions), Chem. Soc. Rev. 40 (2011) 3416-3429.

[14] X. Chen, X. Tian, I. Shin, J. Yoon, Fluorescent and luminescent probes for detection of reactive oxygen and nitrogen species, Chem. Soc. Rev. 40 (2011) 4783-4804.

[15] X. Chen, T. Pradhan, F. Wang, J.S. Kim, J. Yoon, Fluorescent chemosensors based on spiroring-opening of xanthenes and related derivatives, Chem. Rev. 112 (2012) 1910-1956.

[16] Y.D. Lin, T.J. Chow, A pyridomethene-BF_2 complex-based chemosensor for detection of hydrazine, RSC Adv. 3 (2013) 17924-17929.

[17] S. Goswami, S. Paul, A. Manna, A highly reactive (<1 min) ratiometric chemodosimeter for selective "naked eye" and fluorogenic detection of hydrazine, RSC Adv. 3 (2013) 18872-18877.

[18] S. Goswami, S. Das, K. Aich, D. Sarkar, T.K. Mondal, A coumarin based chemodosimetric probe for ratiometric detection of hydrazine, Tetrahedron Lett. 55 (2014) 2695-2699.

[19] K. Li, H.-R. Xu, K.-K. Yu, J.-T. Hou, X.-Q. Yu, A coumarin-based chromogenic and ratiometric probe for hydrazine, Anal. Methods 5 (2013) 2653-2656.

[20] M.G. Choi, J. Hwang, J.O. Moon, J. Sung, S.-K. Chang, Hydrazine-selective chromogenic and fluorogenic probe based on levulinated coumarin, Org. Lett. 13 (2011) 5260-5263.

[21] Y. Sun, D. Zhao, S. Fan, L. Duan, A 4-hydroxynaphthalimide-derived ratiometric fluorescent probe for hydrazine and its in vivo applications, Sens. Actuators B: Chem. 208 (2015) 512-517.

[22] Y. Qian, J. Lin, L. Han, L. Lin, H. Zhu, A resorufin-based colorimetric and fluorescent probe for live-cell monitoring of hydrazine, Biosensors Bioelectron. 58 (2014) 282-286.

[23] J. Zhou, R. Shi, J. Liu, R. Wang, Y. Xu, X. Qian, An ESIPT-based fluorescent probe for sensitive detection of hydrazine in aqueous solution, Org. Biomol. Chem. 13 (2015) 5344-5348.

[24] X.X. Zheng, S.Q. Wang, H.Y. Wang, R.R. Zhang, J.T. Liu, B.X. Zhao, Novel pyrazoline-based selective fluorescent probe for the detection of hydrazine, Spectrochim. Acta A 138 (2015) 247-251.

[25] L. Wang, F.Y. Liu, H.Y. Liu, Y.S. Dong, T.Q. Liu, J.F. Liu, Y.W. Yao, X.J. Wan, A novel pyrazoline-based fluorescent probe for detection of hydrazine in aqueous solution and gas state and its imaging in living cells, Sens. Actuators B: Chem. 229 (2016) 441-452.

[26] S. Goswami, K. Aich, S. Das, S. Basu Roy, B. Pakhira, S. Sarkar, A reaction based colorimetric as well as fluorescence 'turn on' probe for the rapid detection of hydrazine, RSC Adv. 4 (2014) 14210-14214.

[27] D.Y. Qu, J.L. Chen, B. Di, A fluorescence "switch-on" approach to detect hydrazine in aqueous solution at neutral pH, Anal. Methods 6 (2014) 4705-4709.

[28] S. Nandi, A. Sahana, S. Mandal, A. Sengupta, A. Chatterjee, D.A. Safin, M.G. Babashkina, N.A. Tumanov, Y. Filinchuk, D. Das, Hydrazine selective dual signaling chemodosimetric probe in physiological conditions and its application in live cells, Anal. Chim. Acta 893 (2015) 84-90.

[29] J. Fan, W. Sun, M. Hu, J. Cao, G. Cheng, H. Dong, K. Song, Y. Liu, S. Sun, X. Peng, An ICT-based ratiometric probe for hydrazine and its application in live cells, Chem. Commun. 48 (2012) 8117-8119.

[30] M. Sun, J. Guo, Q. Yang, N. Xiao, Y. Li, A new fluorescent and colorimetric sensor for hydrazine and its application in biological systems, J. Mater. Chem. B 2 (2014) 1846-1851.

[31] Shweta, A. Kumar, Neeraj, S.K. Asthana, A. Prakash, J.K. Roy, I. Tiwari, K.K. Upadhyay, A highly sensitive naphthaoxazole-based cell-permeable ratiometric chemodosimeter for hydrazine, RSC Adv. 6 (2016) 94959-94966.

[32] L. Xiao, J. Tu, S. Sun, Z. Pei, Y. Pei, Y. Pang, Y. Xu, A fluorescent probe for hydrazine and its in vivo applications, RSC Adv. 4 (2014) 41807-41811.

[33] S. Goswami, A.K. Das, U. Saha, S. Maity, K. Khanra, N. Bhattacharyya, Rapid detection of hydrazine in a naphthol-fused chromenyl loop and its effectiveness in human lung cancer cells: tuning remarkable selectivity via the reaction altered pathway supported by theoretical studies, Org. Biomol. Chem. 13 (2015) 2134-2139.

[34] J.E. Whitaker, R.P. Haugland, D. Ryan, P.C. Hewitt, R.P. Haugland, F.G. Prendergast, Fluorescent rhodol derivatives: Versatile, photostable labels and tracers, Anal. Biochem. 207 (1992) 267-279.

[35] S. Kenmoku, Y. Urano, H. Kojima, T. Nagano, Development of a highly specific rhodamine-based fluorescence probe for hypochlorous acid and its application to real-time imaging of phagocytosis, J. Am. Chem. Soc. 129 (2007) 7313-7318.

[36] A. Manna, D. Sarkar, S. Goswami, C.K. Quah, H.K. Fun, Single excited state intramolecular proton transfer (ESIPT) chemodosimeter based on rhodol for both Hg^{2+} and OCl^{-}: Ratiometric detection with live-cell imaging, RSC Adv. 6 (2016) 57417-57423.

[37] E. Tomat, S.J. Lippard, Ratiometric and intensity-based zinc sensors built on rhodol and rhodamine platforms, Inorg. Chem. 49 (2010) 9113-9115.

[38] Y.M. Poronik, G. Clermont, M. Blanchard-Desce, D.T. Gryko, Nonlinear optical chemosensor for sodium ion based on rhodol chromophore, J. Org. Chem. 78 (2013) 11721-11732.

[39] J. Qin, H. Yao, S. He, X. Zeng, Design and synthesis of a rhodol isomer and its derivatives with high selectivity and sensitivity for sensing Hg^{2+} and F^- in aqueous media, RSC Adv. 6 (2016) 75570-75577.

[40] H.G. Im, H.Y. Kim, S.K. Chang, Dual signaling of Hg^{2+} ions by selective cleavage of thiophosphinated rhodol, Sens. Actuators B: Chem. 191 (2014) 854-859.

[41] M. Taki, K. Akaoka, K. Mitsui, Y. Yamamoto, A mitochondria-targeted turn-on fluorescent probe based on a rhodol platform for the detection of copper(I), Org. Biomol. Chem. 12 (2014) 4999-5005.

[42] K. Wechakorn, S. Prabpai, K. Suksen, P. Piyachaturawat, P. Kongsaeree, Rhodol-based fluorescent probe for Au^{3+} detection and its application in bioimaging, RSC Adv. 6 (2016) 24752-24755.

[43] H. Komatsu, Y. Shindo, K. Oka, J.P. Hill, K. Ariga, Ubiquinone-rhodol (UQ-Rh) for fluorescence imaging of NAD(P)H through intracellular activation, Angew. Chem. Int. Ed. 53 (2014) 3993-3995.

[44] X.F. Yang, Q. Huang, Y. Zhong, Z. Li, H. Li, M. Lowry, J.O. Escobedo, R.M. Strongin, A dual emission fluorescent probe enables simultaneous detection of glutathione and cysteine/homocysteine, Chem. Sci. 5 (2014) 2177-2183.

[45] Y. Liu, K. Xiang, B. Tian, J. Zhang, Rhodol-based far-red fluorescent probe for the detection of cysteine and homocysteine over glutathione, Luminescence 32 (2017) 78-85.

[46] D. MacDougall, W.B. Crummett, et al., Guidelines for data acquisition and data quality evaluation in environmental chemistry, Anal. Chem. 52 (1980) 2242-2249.

[47] S. Zhu, W. Lin, L. Yuan, Development of a near-infrared fluorescent probe for monitoring hydrazine in serum and living cells, Anal. Methods 5 (2013) 3450-3453.

[48] C. Hu, W. Sun, J. Cao, P. Gao, J. Wang, J. Fan, F. Song, S. Sun, X. Peng, A ratiometric near-infrared fluorescent probe for hydrazine and its in vivo applications, Org. Lett. 15 (2013) 4022-4025.

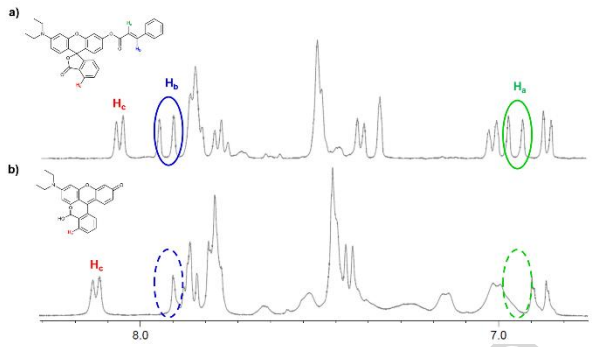


Fig. 4