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# A new dual fluorogenic and chromogenic "turn-on" chemosensor for $Cu^{2+}/F^-$ ions

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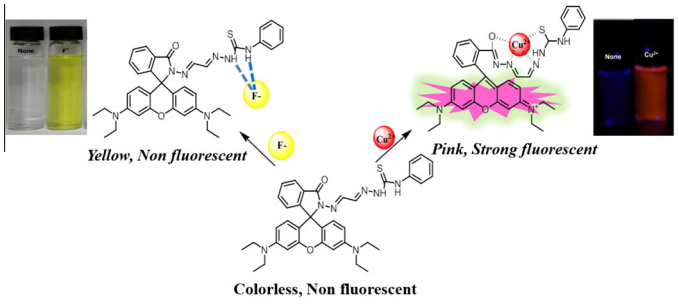
## HIGHLIGHTS

- We report a rhodamine appended thiourea based chemosensor for detection of biologically important $Cu^{2+}$ and $F^-$ ions.
- RBS shows fluorescence activity towards $Cu^{2+}$ ion.
- RBS sensing mode with $Cu^{2+}$ and $F^-$ ion was confirmed by $^1H$ NMR titration experiment.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Turn "off–on" chemosensor 2-(-2-((3′,6′-bis(diethylamino)-3-oxospiro[isoindoline-1,9′-xanthen]-2-yl)imino)ethylidene)-N-phenylhydrazine-1-carbothioamide (RBS) was designed and synthesized. Using the naked eye, RBS showed favorable observation characteristics with both $Cu^{2+}$ and $F^-$ ions. The various modes of sensitivity shown by RBS toward the $Cu^{2+}$ and $F^-$ ions were investigated by spectral techniques, including UV–Vis, fluorescence and $^1H$ NMR spectroscopy. The Job's plot indicated the formation of 1:1 complex between RBS and $Cu^{2+}/F^-$. The binding constant of the RBS-guest$^-$ complexes were found to be $1.3 \times 10^4$ and $6.2 \times 10^3$ $M^{-1}$ for the RBS–$Cu^{2+}$ and RBS–$F^-$, respectively.



## Introduction

The invention of chemosensors for recognizing cations, anion and neutral species has been attracted much dynamic in molecular recognition study and supramolecular chemistry [1] due to their main role in biological systems and high toxicity nature. Currently, single chemosensors for multiple analytes have been paid much attention by analysts because of its faster response and potential cost reduction [2–4]. Up to date, enormous amount of works have been employed for the design of fluorescent

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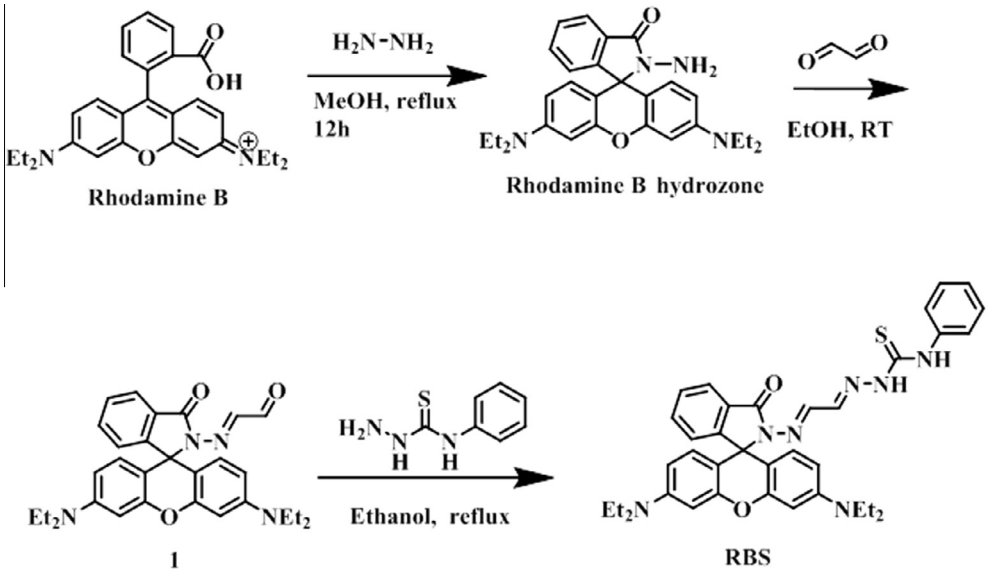
chemosensor for ions and neutral analytes [5–8]. Rhodamine derivatives are excellent chromophores/fluorophores and have attracted considerable interest due to their very good photophysical properties [9], such as long absorption and emission wavelengths, large extinction coefficients and high fluorescence quantum yields. Rhodamine derivatives with spirolactam structures are non-fluorescent; however, opening of the spirolactam ring results in a strong fluorescence emission [10–12]. The rhodamine framework is particularly suited for constructing OFF–ON fluorescent or colorimetric chemosensors [13,14] due to its special structural property. Several rhodamine-modified chemosensors have recently been developed for the detection of heavy and transition metal ions because of the widespread use of these metal ions and because of their harmful impacts on the environment and human health [15].

In human body, $Cu^{2+}$ is the third most abundance essential element (behind $Fe^{2+}$ and $Zn^{2+}$) and also $Cu^{2+}$ plays a critical role as a catalytic co-factor for a variety of metalloenzymes, including superoxide dismutase, cytochrome c oxidase, tyrosinase and so on [16]. However, excess intake of $Cu^{2+}$ can produce oxidative stress and disorders associated with neurodegenerative diseases, such as Alzheimer's disease, Wilson's disease, and Menke's disease, most likely because of its involvement in the production of reactive oxygen species [17]. Especially, the average concentration of blood copper in the normal group is 100–150 μg/L [18]. Notably, $Cu^{2+}$ can be toxic to biological systems when levels of $Cu^{2+}$ ions exceed cellular needs, due to its capability of displacing other metal ions which act as cofactor in enzyme-catalyzed reactions [19]. Due to the diverse nature of $Cu^{2+}$, the various methods have been developed to detecting the $Cu^{2+}$, including atomic absorption spectrometry [20], inductively coupled plasma mass spectroscopy (ICPMS) [21], inductively coupled plasma-atomic emission spectrometry (ICP-AES) [22], and voltammetry [23]. However, these methods require expensive equipment and involve time-consuming and laborious procedures that can be carried out only by trained professionals. Consequently, research has devoted to the development of new colorimetric/fluorescent chemosensors for the detection of $Cu^{2+}$ ions with enough selectivity [24,25]. To date, fluorescent probes for copper ion have been extensively explored owing to its biological significance. Even though great achievements have been obtained in the field of colorimetric and/or fluorescent chemosensors for $Cu^{2+}$ [26–29], most of these sensors cause quenching of the fluorescence emission upon the addition of the metal due to its paramagnetic nature [30–33]. Only a few chemosensors in which the binding of $Cu^{2+}$ leads to an increase in the fluorescence intensity have been found, which are desirable for analytical purposes by the fluorescence enhancement [34,35]. On the other hand, chemosensors for anions have received continuous attention as anions play important role in many fields. Among the anions, the detection of fluoride in chemical and physiological systems are of great interest because of their role in dental health, treatment of osteoporosis, association with hydrolysis of the nerve gas sarin [36–40]. However, excess fluoride can lead to fluorosis, which is a type of fluoride toxicity that generally manifests itself clinically in terms of increases in bone density. The diversity of its function makes the detection of fluoride anion important. The receptors containing pyrrole, amide, urea, imine, urea and thiourea groups act as both cation and anion sensors [41–45].

The anion can be recognized either by H-bonding or by the deprotonation of protons on the receptor–NH in organic solvents. Very few authors have been developed single chemosensor for multi analyte, for example, Kim and co-workers [46] reported bifunctional fluorescent Calix [4] arene chemosensor for both $Pb^{2+}$ and $F^-$. Yang et al. [47] designed N-butyl-1,8-naphthalimide derivative for $Cu^{2+}$ and $F^-$ ion. The results of this study indicated that a hydrazine NH was involved in hydrogen bonding and was subsequently deprotonated. Udhayakumari et al. [48] have reported that thiourea based dual-mode chemosensor for both $Cu^{2+}$ and $F^-$ ion. A survey of literature reveals that the thiourea/imine appended chemosensors were act as dual sensor for both $Cu^{2+}$ and $F^-$. However, those chemosensors are still rarely applied in neutral systems due to the strong hydration ability of $Cu^{2+}$ in aqueous solution. Most of these chemosensors only function well in organic solutions and show fluorescence quenching in the presence of an analyte due to the absence of a strong fluorophore. These limitations reduce the sensitivity and restrict the application of these chemosensors to environmental and biological samples. Therefore, it is important to develop some rapid and "simple-to-use" fluorescent chemosensors with $Cu^{2+}$ induced "turn-on" fluorescence signal, in neutral aqueous buffer containing organic cosolvent or in neutral pure water. To keep this point in our mind, herein a new thiourea appended rhodamine based dual chemosensor (RBS) was successfully designed and synthesized (Scheme 1), for sensing of both $Cu^{2+}$ and $F^-$ ion.




**Scheme 1.** Synthetic procedure for chemosensor RBS.

## Experimental section

### *Materials and instruments*

All reagents and rhodamine B dye were purchased from Aldrich Chemical Co. Ltd. and Alfa Aesar Chemical Co. Ltd. The commercially available Tris–HCl buffer solution (pH = 7.2) were purchased from Aldrich. Cations $Cu^{2+}$, $Hg^{2+}$, $Ag^{+}$, $Co^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Fe^{3+}$, $Fe^{2+}$, and $Mg^{2+}$ (as perchlorate salts) were also purchased from Aldrich. Anion stock solutions were prepared from tetrabutyl ammonium salts, all obtained from Aldrich. All solvents were analytical grade and used without further purification. Nuclear magnetic resonance ($^1$H-NMR) spectra were recorded in DMSO-$d_6$ on Brucker-300 MHz spectrometer with TMS as internal standard. Agilent 8453 UV–Vis spectrophotometer was used to record UV–Visible spectra using quartz cell with 1 cm path length. Fluorescence spectra were measured on a Shimadzu RF-5301 PC fluorescence spectrophotometer equipped with a xenon discharge lamp, 1 cm quartz cell at slit width Ex: 10 nm; Em: 10 nm. Elemental analyses were recorded on a Flash EA 1112 analyzer. Mass spectra were recorded on a JEOL MStation [JMS-700].

### *Synthesis*

Rhodamine B hydrazone and compound (**1**) were synthesized according to previously reported methods [49,50] and confirmed by $^1$H NMR, elemental analysis (EA) and mass spectrometry.

### *Synthesis of chemosensor 2-(-2-((3',6'-bis(diethylamino)-3-oxospiro[isoindoline-1,9'-xanthen]-2-yl)imino)ethylidene)-N-phenylhydrazine-1-carbothioamide (RBS) dye*

Compound **1** (248 mg, 0.5 mmol) in 5 mL ethanol was added to a stirred solution of 4-phenylthiosemicarbazide (84 mg, 0.5 mmol) in 5 mL ethanol at room temperature. The stirred reaction mixture was heated to reflux for 5 h and monitored by TLC. After cooling to room temperature, the formed precipitate was washed with cold ethanol and dried under vacuum to obtain crude RBS. The crude product was purified by recrystallization from ethanol to give chemosensor RBS (160 mg, yield: 50.0%). $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ (ppm): 1.10 (*t*, 12H, *J* = 6.9 Hz), 3.35–3.33 (*q*, 8H, —CH2), 6.45–6.34 (*m*, 6H), 7.07 (*t*, 1H, *J* = 7.2 Hz), 7.17 (*t*, 1H, *J* = 7.5 Hz), 7.35 (*t*, 2H, *J* = 7.5 Hz), 7.61–7.48 (*m*, 5H), 7.90 (*d*, 1H, *J* = 7.2 Hz), 8.77 (*d*, 1H, *J* = 8.4 Hz), 10.09 (*s*, 1H), 11.92 (*s*, 1H); FAB mass: (m/z) Calc. 645.0 Found: 646 [M + H$^+$]; EA: Found: C, 68.54; H, 6.04; N, 15.01; S, 4.63. Calc. For $C_{37}H_{39}N_7O_2S$: C, 68.81; H, 6.09; N, 15.18; O, 4.95; S, 4.97.

## Results and discussion

### *Naked eye detection*

The colorimetric/fluorescent recognition property of chemosensor RBS ($1 \times 10^{-5}$ M in $CH_3CN$) toward various cations ($1 \times 10^{-5}$ M in $CH_3CN$:10 mM Tris–HCl, (1:1) pH = 7.2, as perchlorate salts) and anions ($1 \times 10^{-5}$ M in $CH_3CN$ as tetra butyl ammonium salt) were investigated using daylight and irradiation with a UV lamp (at 360 nm). As shown in Fig. 1, the color of RBS solution changed from colorless to dark pink in the presence of $Cu^{2+}$. Besides, the addition of $Co^{2+}$ influenced the tiny color changes in RBS solution. The fluorescence color of RBS changed from blue to bright yellow after the addition of $Cu^{2+}$ ions. However, the color remained unchanged after the addition of the other investigated cations such as $Mg^{2+}$, $Fe^{2+}$, $Hg^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Pb^{2+}$, $Ag^{+}$ and $Fe^{3+}$. In naked-eye experiments, RBS turned from colorless to yellow in the presence of 1 equivalent of tetrabutyl ammonium fluoride (Fig. 2). The visual color change implies the deprotonation of the receptor unit (thiourea). However, the addition of other anions such as $Cl^-$, $Br^-$, $I^-$, $AcO^-$, $H_2PO_4^-$, $HSO_4^-$ and $ClO_4^-$ even in excess was found to be insensitive. This observation indicated that RBS was act as a dual chemosensor for both $Cu^{2+}$ and $F^-$ ion.

### *Spectra recognition of $Cu^{2+}$ by RBS*

The sensing ability of RBS ($1 \times 10^{-5}$ M) towards relevance metal cations such as $Mg^{2+}$, $Fe^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Pb^{2+}$, $Hg^{2+}$, $Ag^{+}$, $Fe^{3+}$ and $Cu^{2+}$ as its perchlorate salts was monitored by the UV–Vis and fluorescence spectroscopic techniques. As shown in Fig. 3a, UV–Vis

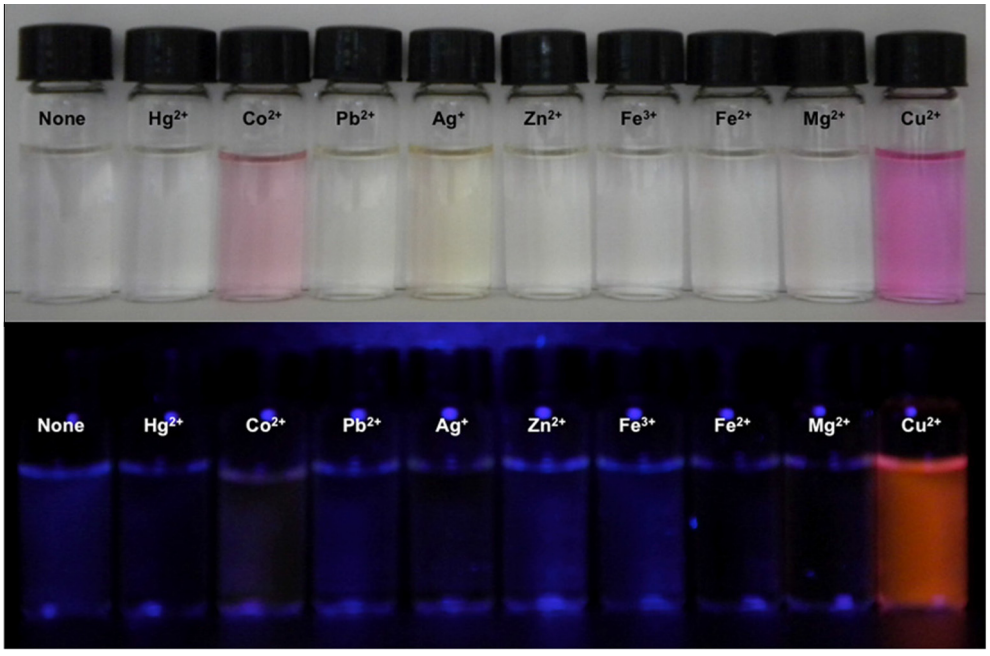



**Fig. 1.** Photographs of chemosensor RBS ($1 \times 10^{-5}$) in $CH_3CN$ at 25 °C, in the presence of different metal ions (in $CH_3CN$:Tris–HCl (10 mM, pH = 7.2) $1 \times 10^{-5}$ M) under (TOP) visible light and (Bottom) UV light.

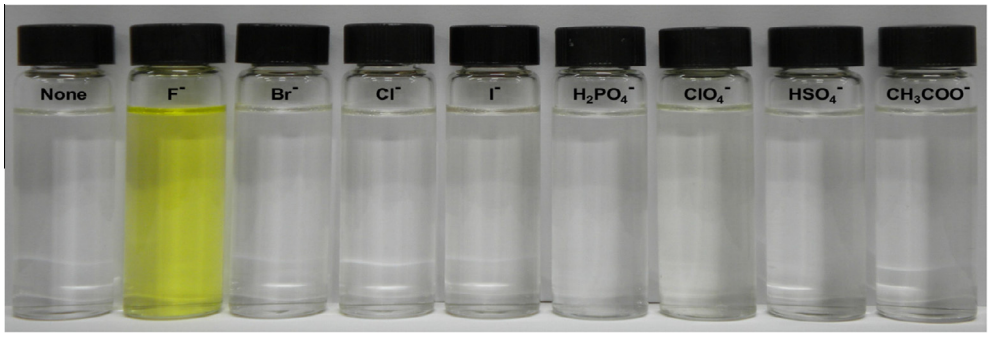



**Fig. 2.** Photograph of chemosensor RBS ($1 \times 10^{-5}$) in $CH_3CN$ at 25 °C, in the presence of different anions ($1 \times 10^{-5}$ M) in $CH_3CN$.

absorbance spectrum of RBS ($1.0 \times 10^{-5}$ M) exhibited no absorbance over 500 nm. Addition of 2.0 equivalent $Cu^{2+}$ into solution immediately resulted in a significant enhancement of absorbance at about 560 nm as well as the color of solution changes from colorless to red. In contrast, under the same condition other cations lead to much smaller spectral changes ($Co^{2+}$), or almost no spectral changes ($Mg^{2+}$, $Fe^{2+}$, $Zn^{2+}$, $Pb^{2+}$, $Hg^{2+}$, $Ag^{+}$ and $Fe^{3+}$). This result was clearly consistent with Irving–William series. $Cu^{2+}$ has a specific high thermodynamic affinity for typical N-donor ligands and fast metal-to ligand binding kinetics. On the other hand, fluorescence spectra (Fig. 3b) also show a similar result, which is consistent with that of UV–Vis spectra. Addition of 2.0 equivalent of $Cu^{2+}$ ion results in an obviously enhanced fluorescence at 582 nm (OFF–ON), while other ions induce much smaller ($Co^{2+}$) or no fluorescence enhancement ($Mg^{2+}$, $Fe^{2+}$, $Zn^{2+}$, $Pb^{2+}$, $Hg^{2+}$, $Ag^{+}$, $Fe^{3+}$ and Ag+).

The UV–Vis titration of the $Cu^{2+}$ was conducted by using $1 \times 10^{-5}$ M of RBS in $CH_3CN$ solution. As depicted in Fig. 4a, upon the addition of incremental amount of $Cu^{2+}$ (in $CH_3CN$-Tris–HCl) to RBS solution, the new absorption peak evidently increased at 560 nm, which induced a clear and gradually color change from colorless to pink (Fig. S1). This indicated the opening of the rhodamine-spirolactam ring [51]. To determine the stoichiometry of the RBS–$Cu^{2+}$ complex, Job's method for absorbance measurement was applied [52]. Keeping the sum of the initial concentration of $Cu^{2+}$ and RBS at $5 \times 10^{-5}$ M, the molar ratio of $Cu^{2+}$ was varied from 0.1 to 0.9. A plot of absorbance versus the mole fraction of $Cu^{2+}$ was provided in Fig. S2. It showed that the maximum

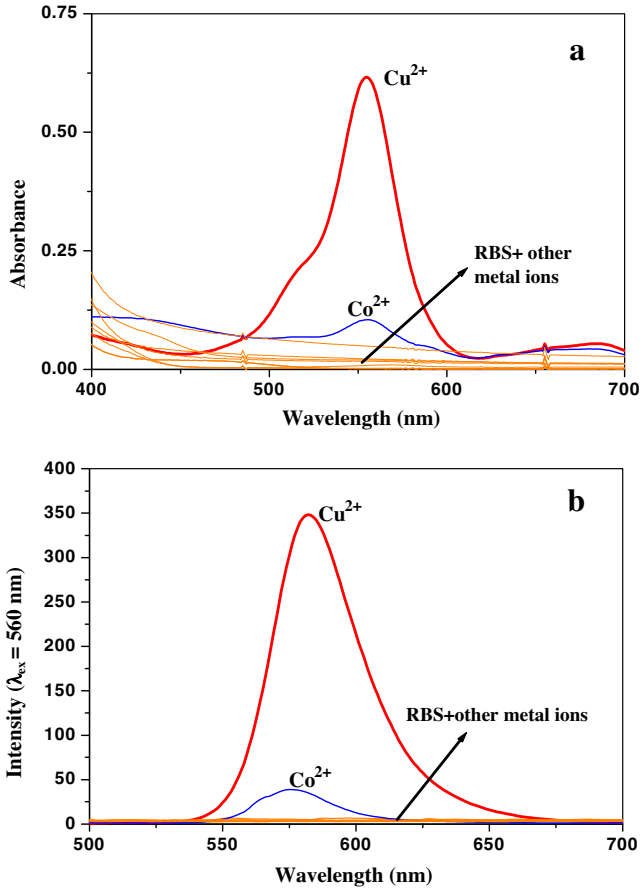



**Fig. 3.** (a) Absorbance spectra of RBS ($1 \times 10^{-5}$ M) in $CH_3CN$ in the presence of different metal ions (2 equiv.) in $CH_3CN$-Tris–HCl (10 mM, pH = 7.2); (b) Fluorescence spectra of RBS ($1 \times 10^{-5}$ M) in the presence of different metal ions (2 equiv.) in $CH_3CN$-Tris–HCl, (10 mM, pH = 7.0) excited at 560 nm.

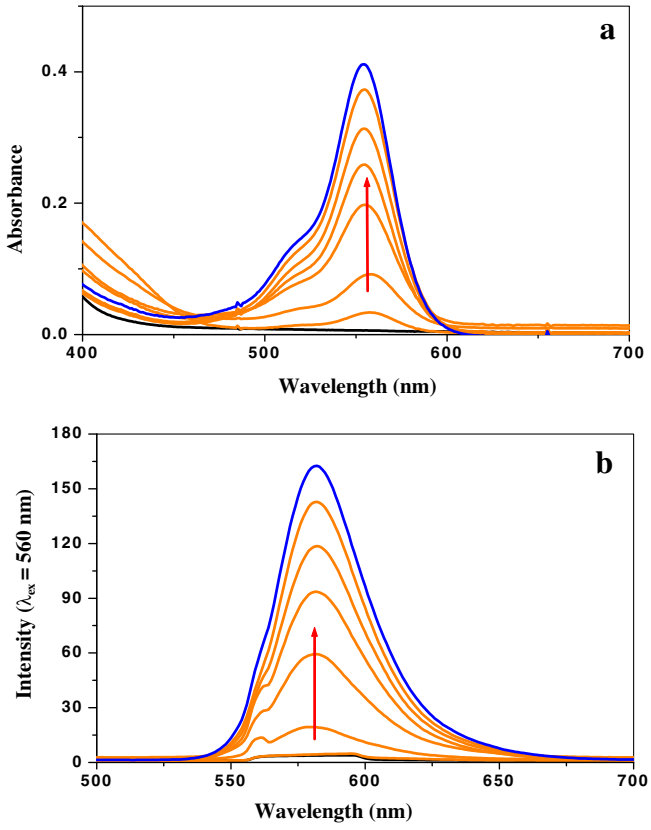



**Fig. 4.** (a) Absorption spectra of RBS ($1 \times 10^{-5}$ M) upon addition of increased amount of $Cu^{2+}$ (0–1 $\times 10^{-5}$ M) in $CH_3CN$-Tris–HCl media (10 mM, pH 7.2); (b) The fluorescent intensity of RBS ($1 \times 10^{-5}$ M) upon addition of increased amount of $Cu^{2+}$ in $CH_3CN$-Tris HCl media (10 mM, pH = 7.0).

of the curve was appear at of 0.5 mol fraction, indicating a 1:1 stoichiometry of the $Cu^{2+}$ to RBS in the complex [52]. From the increase in the absorption intensity at 560 nm the binding constant of the RBS–$Cu^{2+}$ complex was calculated using the following Benesi–Hildebrand equation [53].

$$1/(A - A_0) = 1/K_a(A_{max} - A_0)(1/[Cu^{2+}]) + 1/(A_{max} - A_0)$$

where, $A_0$ is absorption intensity in the absence of $Cu^{2+}$, $A$ is the absorption intensity at various $Cu^{2+}$ ion concentration, $A_{max}$ is the absorption intensity at maximum concentration of $Cu^{2+}$, $K_a$ is the binding constant for the RBS with $Cu^{2+}$ ion. In the present investigation, a plot of $1/(A - A_0)$ versus $1/[Cu^{2+}]$ is linear (Fig. S3). The association constants thus computed was $1.3 \times 10^4\ M^{-1}$ for the RBS–$Cu^{2+}$ complex.

To estimate the sensing ability of RBS, the fluorescence titration experiment has also been carried out. For free RBS there was no emission peak at 582 nm when excited at 560 nm. Whereas, the addition of incremental amount of $Cu^{2+}$ ion to RBS solution the new emission peak was slowly enhanced at 582 nm (Fig. 4b), which is attributed to the opening of the spirolactam ring of rhodamine to an amide form [51]. The solution showed an intense orange fluorescence (Fig. S4), with an approximately 150-fold enhancement in the fluorescence intensity at 582 nm.

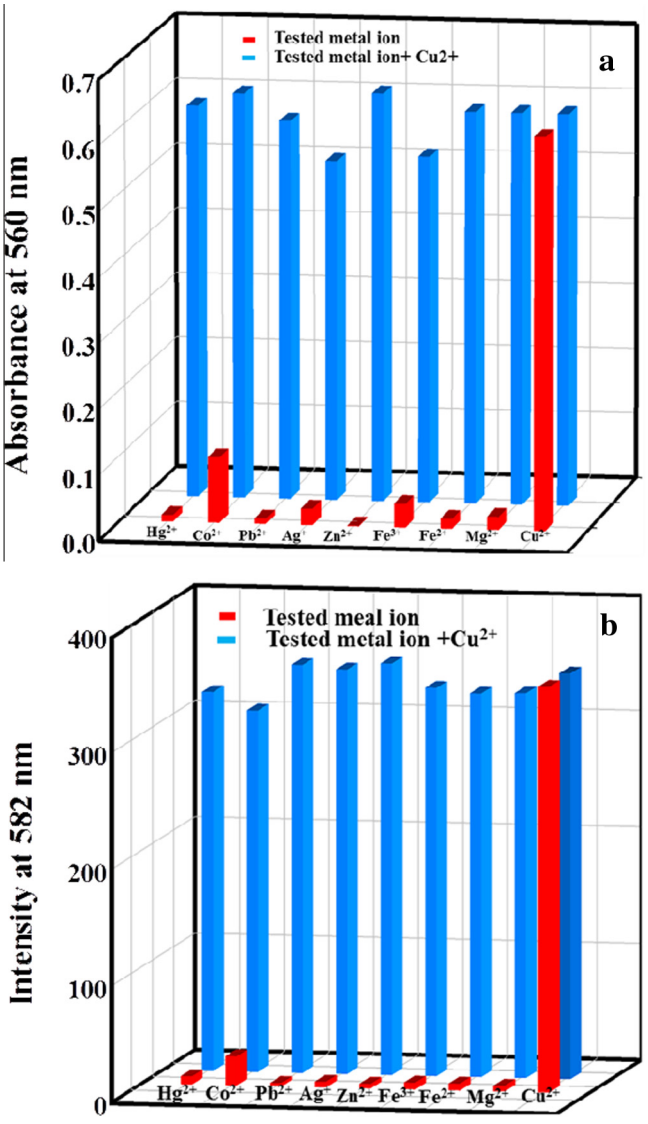



**Fig. 5.** (a) Absorbance intensity of RBS ($1 \times 10^{-5}$ M) in the presence of $Cu^{2+}$ (2.0 equiv.) and additional various ions (2.0 equiv.) in $CH_3CN$-TrisHCl media (10 mM, pH = 7.2) (b) Fluorescence response of RBS ($1 \times 10^{-5}$ M) in the presence of $Cu^{2+}$ (2.0 equiv.) and additional various metal ions (2.0 equiv) ($CH_3CN$-Tris–HCl, 10 mM, pH = 7.0).

*Competitive experiments*

Possible interference by other metal ions was evaluated through competition experiments. The changes in the RBS absorbance were measured after treatment with 2.0 equivalents of $Cu^{2+}$ in the presence of potentially interfering metal ions, including $Mg^{2+}$, $Fe^{2+}$, $Hg^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Pb^{2+}$, $Ag^{+}$ and $Fe^{3+}$ (Fig. 5). As part of the competition experiments, the fluorescence properties of RBS along with other metal ions (above-mentioned) were measured, showing that the increase of fluorescence intensity resulting from the addition of the $Cu^{2+}$ was not influenced by the addition of excess metal ions. It is gratifying to note that all the tested cations have no obvious influence on the chemosensor RBS function.

*Spectra recognition of $F^-$ by RBS*

The interaction of chemosensor RBS with the anions was investigated through UV–Vis spectra in $CH_3CN$ solution. In the absence

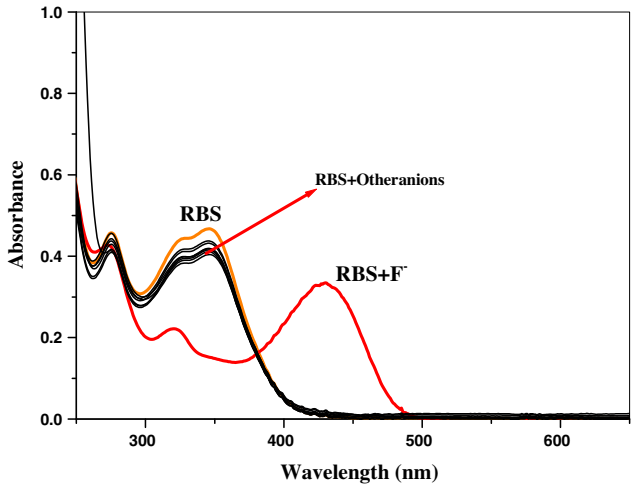



**Fig. 6.** Absorbance spectra of RBS ($1 \times 10^{-5}$ M) in $CH_3CN$ in the presence of different anions (1.0 equiv.).

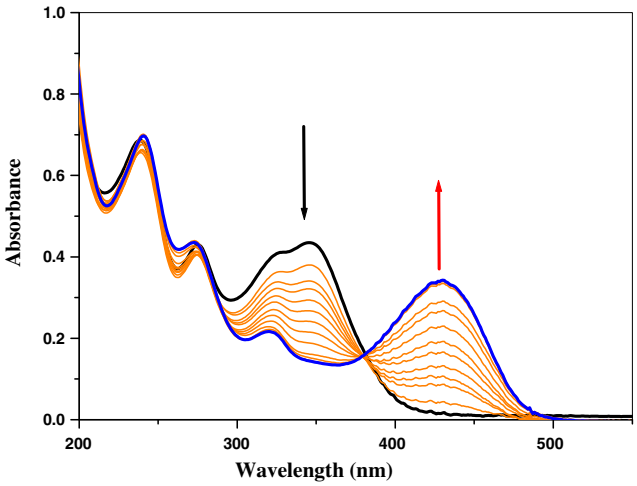



**Fig. 7.** Change in UV–Vis spectra for RBS ($1.0 \times 10^{-5}$ M) in $CH_3CN$ upon the addition of ($0–1 \times 10^{-5}$ M) of fluoride ion.

of anions, the maximum absorption wavelength of RBS is at about 346 nm. As shown in Fig. 6, when 1.0 equivalent of TBAF was added to the solution of RBS ($1 \times 10^{-5}$ M), the peak at 346 nm disappeared and new peak was appear at 430 nm. In the same conditions, no significant changes happened when 1.0 equivalent of tetrabutylammonium $Cl^-$, $Br^-$, $I^-$, $AcO^-$, $H_2PO_4^-$, $HSO_4^-$ and $ClO_4^-$ were added. The results suggest that RBS has a high selectivity for the fluoride anion over other common anions. To further get the binding ability of RBS with the fluoride was investigated through UV–Vis spectrophotometric titration by adding standard solution of TBAF with different concentration (Fig. 7). As the concentration of fluoride ion increased, the absorbance intensity decreased at 346 nm, and a new band at 430 nm increased. The isosbestic point at about 380 nm observed in both cases was attributed to the equilibrium between two species (RBS and RBS–F) throughout the titration process [54]. The stoichiometry of RBS–$F^-$ complex was calculated as 1:1 ratio by jobs continuous variation method (Fig. S2). From the spectrometric titration curve the association constant ($K_a$) of RBS to $F^-$ was obtained as $6.2 \times 10^3$ $M^{-1}$ by Benesi–Hildebrand equation (Fig. S5).

### *$^1$H NMR titration of RBS with $Cu^{2+}$ and $F^-$*

To get the better insight into the binding mode of RBS–$Cu^{2+}$ and RBS–$F^-$ in solution, $^1$H NMR titrations were carried out. On addition of 1.0 equivalent $Cu^{2+}$ to the DMSO-$d_6$ solution of RBS, the peak assigned to the proton of the (CH=N) imines moieties were broadened and shifted downfield from $\delta$ 7.90 and 8.76 to 8.3 and 9.86 ppm. Further the peak consistent to thiourea NH has also been broadened with very tiny downfield shift from $\delta$ 10.09 and 11.92 ppm. The signal of the xanthene proton on rhodamine B protons completely shifted to up field (see Fig. 8), this is indicating the ring opening of rhodamine ring by $Cu^{2+}$ coordination (Fig. 8). It should be noted that the signal of the —NH moiety was well retained with broadening during the titrations. This suggested that $Cu^{2+}$ interacts with 'S' and develop the resonating bond between sulfur and NH group. The shifting of imine peak suggested that involvement of the imine nitrogen on complex formation. Thus, according to the obtained results, it is very likely due to the metal ion-induced ring opening of rhodamine spirolactam, rather than other possible reactions. The chemosensor RBS is the most likely to chelate with $Cu^{2+}$ via nitrogen on the two imine moieties and oxygen on the spirolactam, as well as sulfur on the thiourea group (Scheme 2).

Based on the optical and spectrophotometric experiment it is challenging to predict whether the noticeable changes include formation of complex between RBS and $F^-$ through hydrogen bonding or deprotonation of RBS by sufficient basic strength of $F^-$ molecules. Fig. 9 displays the $^1$H NMR spectra of RBS ($1 \times 10^{-5}$ M DMSO-$d_6$) titrated with [$Bu_4N$]F. Upon addition of 0–1.0 equivalent of TBAF, the signal at $\delta$ 10.09 and 11.92 ppm, assigned to the thiourea NH protons, was broadened gradually and shifted to downfield, indicating the formation of a RBS–$F^-$ hydrogen-bonding complex between RBS and $F^-$. On further addition of $F^-$ ions up to 1.5 equivalent, the signal of the —NH protons disappeared completely, which demonstrated the deprotonation of the —NH fragment [55,56]. While in the case of other aromatic and xanthene protons are not disturbed even in

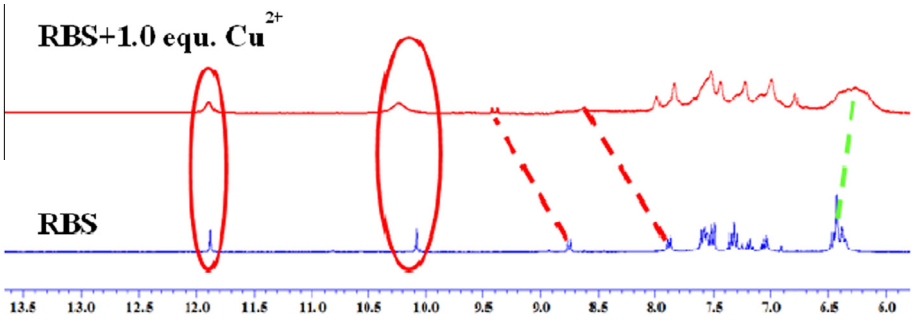
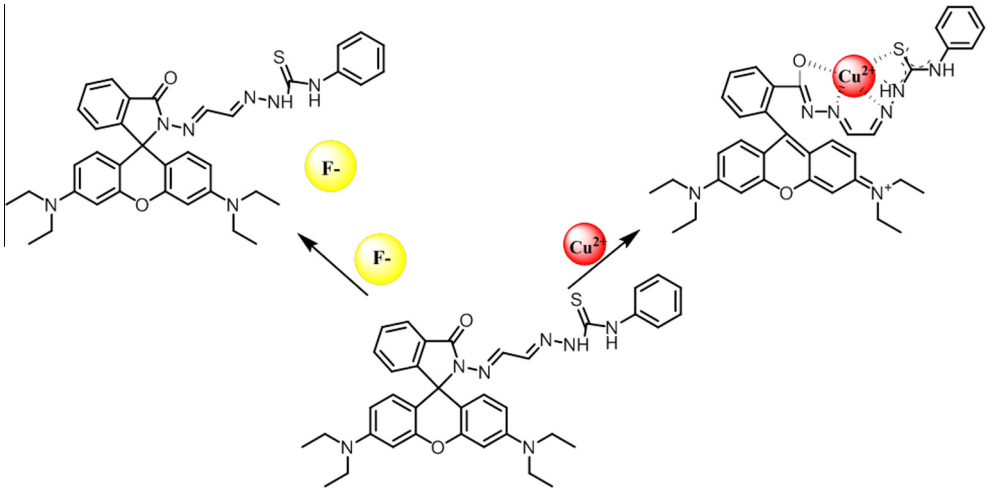



**Fig. 8.** Change in partial $^1$H NMR (300 MHz) spectra of RBS with 1.0 equivalent metal ion in DMSO-$d_6$.




**Scheme 2.** Proposed binding modes for complex RBS–$Cu^{2+}$ and RBS–$F^-$.

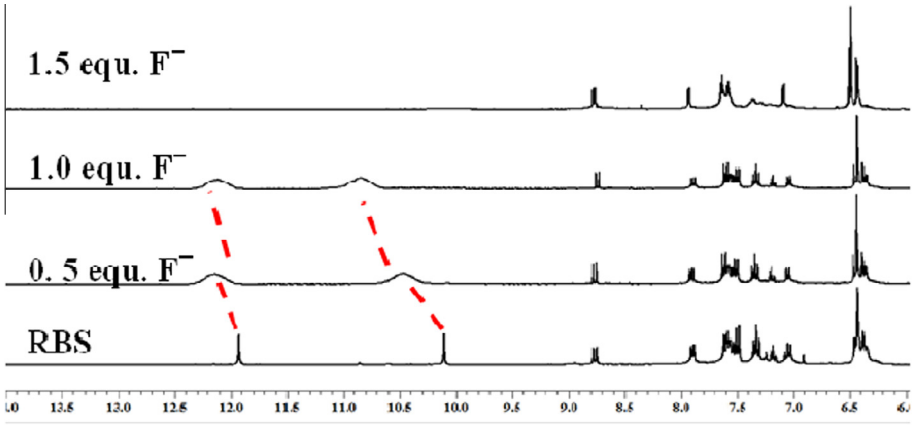



**Fig. 9.** Change in partial $^1$H NMR (300 MHz) spectra of RBS with various equivalent of $F^-$ ion in DMSO-$d_6$.

**Table 1**
Performances comparison of various colorimetric/ fluorescent chemosensors for $Cu^{2+}/F^-$ ion.

| Modes | Fluorophore | Testing media | Sensing ions | Remarks | Ref |
|---|---|---|---|---|---|
| UV–Vis, fluorescence, cyclic voltammetry | Ferrocene | $CH_3CN$ | $Cu^{2+}/F^-$ | Complex formation/hydrogen bonding | 56 |
| UV–Vis, fluorescence | Isoindoline | DMSO | $Cu^{2+}/F^-$ | Complex formation/hydrogen bonding | 57 |
| UV–Vis | Dipyrrole | $CH_3CN$ | $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Zn^{2+}/F^-$ | Complex formation/hydrogen bonding | 58 |
| UV–Vis, fluorescence | Azoaniline | DMSO:HEPES | $Cu^{2+}/F^-$ | Chemodosimeter | 59 |
| UV–Vis, fluorescence | Benzthiazole | Ethanol/Water | $F^-/Cu^{2+}$ | Chemodosimeter/sequential | 60 |
| UV–Vis, fluorescence | Rhodamine | Ethanol/Water Tris-$HNO_3$ | $Al^{3+}/F^-$ | Chemosensor/sequential | 61 |
| UV–Vis, fluorescence | BODIPY | $CH_3CN$ | $Cu^{2+}$/Cys | Complex formation/sequential | 62 |
| UV–Vis, fluorescence | Rhodamine | CH3CN:Tris HCl pH = 7.0 | $Cu^{2+}/F^-$ | Chemosensor/hydrogen bonding | This work |

excess amount of $F^-$ ion. These results clearly indicating the sensing mode of fluoride ion on RBS is quite different from the $Cu^{2+}$ ion. The likely binding modes between RBS and $Cu^{2+}$, as well as between RBS and $F^-$, are shown in Scheme 2.

*Method performance comparison*

The performance of the proposed dual chemosensor RBS toward $Cu^{2+}/F^-$ was compared with some reported colorimetric/fluorescent chemosensors based on different fluorophores. As shown in Table 1, rhodamine B structural motif dual sensor for $Cu^{2+}$ and $F^-$ is either very rare or nil. All the methods present good selectivity for $Cu^{2+}/F^-$ with signal enhancement, and most of them can adopt dual chromo- and fluorogenic changes toward $Cu^{2+}$ and $F^-$ [57–63], as well as our proposed RBS. A few of chemosensors possess wide metal ion sensing ability [59], but some of them need more rigorous testing media and the biological pH are not investigated [57–59,63]. Notably, as for the three types of sequential fluorescent chemosensors based on bezthiazole [61], rhodamine [62] and BODIPY derivatives [63] as sequential dual-function detection for $F^-/Cu^{2+}$ [61], $Al^{3+}/F^-$ [62], and $Cu^{2+}$/Cys [63], are realized, respectively, however, in these sensors, applications for second target analytes are not possible in the absence of first target ion. Our newly developed chemosensor presents a number of attractive analytical features such as high sensitivity, distinct naked eye color change and biological pH. The fluorescent chemosensor RBS based on thiourea modified rhodamine spirolactam derivative is easy to prepare with low cost and can be used for rapid analysis of $Cu^{2+}$ and $F^-$ in biological pH. To the best of our knowledge, this is the first attempt for the $Cu^{2+}$ and $F^-$ ions dual sensor based on rhodamine derivatives, which follow different mechanism.

## Conclusion

In conclusion, we have successfully designed and synthesized a new rhodamine based turn "off–on" fluorescent chemosensor bearing imines and thiourea moiety that shows dual response for $Cu^{2+}$ and $F^-$ ion through different mechanisms. A remarkable enhancement in the fluorescence intensity of RBS and a noticeable color change from colorless to pink were observed upon binding with $Cu^{2+}$. The response of the RBS to $Cu^{2+}$ was unaffected by the presence of other common coexistent metal ions. $^1$H NMR studies supported that the $Cu^{2+}$ binding to N atom of imine and S atom of thiourea moiety in RBS. The RBS was form 1:1 complexes with $F^-$ by multiple hydrogen-bonding interactions. Among the relevant anions, RBS has higher selectivity for only fluoride and leads to a distinct color change that can be observed by the naked eye. Experiments with a series of anions suggested that RBS can efficiently and preferentially bind with the fluoride anion, as established by the UV–Vis and $^1$H NMR results.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2015.06.078.

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