Synthesis and Spectroscopic Studies of Novel Rhodanine Azo Dyes: An Excellent Selective Chemosensor for Naked-eye Detecting of Cu\(^{2+}\) Ion

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Abstract

This study is first report on the synthesis of novel rhodanine based azo dyes and their fluorogenic and chromogenic sensing behaviors with metal ions (Li\(^{+}\), Na\(^{+}\), K\(^{+}\), Rb\(^{+}\), Pb\(^{2+}\), Ni\(^{2+}\), Zn\(^{2+}\), Cu\(^{2+}\)) by using UV-vis and fluorescence spectroscopy techniques. The results of spectroscopic experiments for rhodanine based azo dye \(1a\) exhibited excellent selectivity for Cu\(^{2+}\) over the other metal ions. Furthermore, anti-bacterial studies of rhodanine azo dyes were performed towards some selected bacteria via microbroth dilution method. Obtained results showed that rhodanine ionophore \(1a\) showed strong antibacterial activity against \(S.aureus\).

Keywords: Rhodanine, copper(II) ion, chemosensor, azo-dye, antibacterial activity.

1. Introduction

Molecular recognition is the first step in the design and synthesis of molecular sensors for detection of anions, metal cations or neutral species in organic or aqueous media. The molecular sensor is designed to exhibit a physical response which must be easily detected in the presence of these target species. Rhodanine is an important part of heterocyclic azo dyes, which has been mainly used to determine noble metals.\(^{1,2}\) According to the relationship between structures and chemiluminescence performances of organic azo dyes, many active groups have been introduced to rhodanine matrix to obtain satisfactory chemiluminescence properties.\(^{3}\) Azo compounds, based on rhodanine, play a important role as chelating agents for a large number of metal ions.\(^{4,5}\) The complexation studies of the rhodanine based azo dyes with various metal ions along with uranyl, ruthenium, copper, cobalt and nickel ions are documented in the literature.\(^{6-9}\) Chemical properties of rhodanine and its derivatives are of interest due to coordination capacity and their use as metal extracting agents.\(^{10}\) Careful examination of the literature reveals that considerable work has been reported on the spectrophotometric studies of the heterocyclic azo compounds, their metal complexes and their analytical applications.\(^{11}\) But little information has appeared in the literature concerning azo compounds derived from rhodanine and their metal complexes. It is also accepted that knowledge of the stability constants of such azorhodanines and their metal complexes may eventually help to throw some light on the deactivation of essential trace metals in biological systems.\(^{12}\) Furthermore, heterocyclic compounds based on thiazolidine are biologically active compounds having five membered rings, with different heteroatoms. A wide range of pharmacological activities has been attributed to these molecules. Among these include antimicrobial,\(^{13}\) anticancer,\(^{14,15}\) antiviral,\(^{16}\) anticonvulsant,\(^{17-19}\) antitubercular,\(^{20}\) antifungal,\(^{21,22}\) anti-inflammatory,\(^{23}\) analgesic\(^{24}\) and antiproliferative\(^{25,26}\) activities.

Up to now, although several works about the effect of the rhodanine based azo dyes on the both metal complexation and pharmacological activities has been repor-
There is no other reported chemosensor study between metal cations and rhodanine based azo dyes. Therefore, the aim of the present study was to explore the effect of rhodanine based azo dyes on the selective chemosensor studies for some selected metal cations such as Li\(^+\), Na\(^+\), K\(^+\), Rb\(^+\), Pb\(^{2+}\), Ni\(^{2+}\), Zn\(^{2+}\) and Cu\(^{2+}\).

2. Experimental

2.1. Materials

All of the reagents used in this study were obtained from Merck or Fluka and used without further purification. All the metal salts were used as perchlorate compounds (MClO\(_4\), M : Li\(^+\), Na\(^+\), K\(^+\), Rb\(^+\), Pb\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\)). Thin layer chromatography (TLC) was performed using silica gel on glass TLC plates (silica gel H, type 60, Merck). Column chromatographic separations were performed on Merck Silica gel-60 (230–400 mesh).

2.2. Instrumentation

\(^1\)H NMR spectra were obtained using a Varian 400 MHz spectrometer operating at 400 MHz. IR spectra were obtained on a Perkin Elmer spectrum 100 FTIR spectrometer (ATR). UV-vis absorption and fluorescence spectra were recorded on a Shimadzu UV-1700 spectrophotometer and on a Perkin-Elmer LS-55 fluorescence spectrometer, respectively. Elemental analyses were performed using a Leco CHNS-932 analyzer. Melting points were determined on a Gallenkamp apparatus.

2.3. Synthesis

Rhodanine based azo dyes (1a and 2a-c) were synthesized according to the following modified literature procedure (Scheme 1).\(^{27,28}\) All of the reactions were monitored with thin layer chromatography.

2.4. Synthesis of Rhodanine Based Azo Dyes 1a and 2a-c

2.4.1 General Procedure

25 ml of distilled water containing hydrochloric acid (12 M, 32.19 mmol) were added to corresponding amine compounds such as 2-naphthylamine or o-, p-, m-amino phenol (5 mmol) respectively. To the cooled (0 °C) and stirred mixture, a solution of sodium nitrite (5.1 mmol, in 10 mL of water) was added dropwise for 10 min. Then, to the formed diazonium chloride mixture, a solution of 2-thioxo-4-thiazolidinone (5 mmol) in 25 mL of glacial acetic acid was added dropwise for 20 min with cooling so that the mixture was maintained 0–5 °C. The mixture containing both the rhodanine and the diazonium salt was then added with stirring to a cold solution of 15 grams of sodium acetate in 50 mL of water. After addition, the resulting slurry was stirred for 2 hours at 5 °C. The mixture was allowed to stand for overnight. Then the obtained dark red precipitates were filtered, washed several times with water. The crude product was purified by recrystallization from hot ethanol.

5-(naphthalen-2-yl)diazenyl-2-thioxothiazolidin-4-one (1a)

Yield = 65%, dark red; Mp 107 °C; IR (\(\nu_{\max}\), cm\(^{-1}\)):

1721 (C=O), 1665 (C=C), 1450 (N=N), 1325 and 1211 (C=S).

\(^1\)H NMR (CDCl\(_3\)):

\(\delta\) 8.53 (bs, 1H, NH), 6.85–6.96 (m, 7H, ArH), 3.51 (bs, 1H, CH).

Anal. Calc.: C\(_{13}\)H\(_9\)N\(_3\)O\(_2\)S\(_2\). C, 54.34; H, 3.16; N, 14.62%. Found: C, 54.29; H, 3.12; N, 14.68%.

5-(2-hydroxyphenyl) diazenyl-2-thioxothiazolidin-4-one (2a)

Yield = 68%, dark red; Mp 80–82 °C; IR (\(\nu_{\max}\), cm\(^{-1}\)):

1720 (C=O), 1671 (C=C), 1440 (N=N), 1317 and 1195 (C=S).

\(^1\)H NMR (CDCl\(_3\)):

\(\delta\) 10.03 (s, 1H, OH), 8.71 (s, 1H, NH), 6.85–6.96 (m, 7H, ArH), 3.51 (bs, 1H, CH).

Anal. Calc.: C\(_9\)H\(_7\)N\(_3\)O\(_2\)S\(_2\). C, 42.68; H, 2.79; N, 16.59%. Found: C, 42.59; H, 2.77; N, 16.63%.

5-(3-hydroxyphenyl) diazenyl-2-thioxothiazolidin-4-one (2b)

Yield = 59%, red; Mp 86–89 °C; IR (\(\nu_{\max}\), cm\(^{-1}\)):

1720 (C=O), 1658 (C=C), 1445 (N=N), 1315 and 1211 (C=S).

\(^1\)H NMR (CDCl\(_3\)):

\(\delta\) 10.03 (s, 1H, OH), 8.71 (s, 1H, NH), 6.77–6.90 (m, 4H, ArH), 3.33 (s, 1H, CH).

Anal. Calc.: C\(_9\)H\(_7\)N\(_3\)O\(_2\)S\(_2\). C, 42.68; H, 2.79; N, 16.59%. Found: C, 42.59; H, 2.77; N, 16.63%.
(C=S). 1H NMR (CDCl3): δ 8.81 (s, 1H, OH), 8.58 (s, 1H, NH), 7.02 (s, 1H, ArH) 6.87–7.00 (m, 3H, ArH), 3.56 (s, 1H, CH). Anal. Calc.: C9H7N3O2S2. C, 42.68; H, 2.79; N, 16.59%. Found: C, 42.61; H, 2.84; N, 16.66%.

5-(4-hydroxyphenyl) diazenyl-2-thioxothiazolidin-4-one (2c)

Yield = 66%, red; Mp 101–104 °C; IR (v_max, cm⁻¹): 1717 (C=O), 1665 (C=C), 1445 (N=O), 1321 and 1213 (C=S). 1H NMR (CDCl3): δ 8.21 (s, 1H, OH), 8.10 (s, 1H, NH), 7.00–7.13 (m, 4H, ArH), 3.50 (s, 1H, CH). Anal. Calc.: C9H7N3O2S2. C, 42.68; H, 2.79; N, 16.59%. Found: C, 42.66; H, 2.82; N, 16.65%.

2. 5. Absorption and Fluorescence Measurements

Stock solutions of the metal perchlorates and rhodanine basedazo dyes 1a or 2a-c were prepared 7.0 × 10⁻³ M and 7.0 × 10⁻⁴ M in DMF, respectively. For UV-vis and fluorescence measurements, sample solutions were obtained by mixing appropriate amount of stock solution of receptors 1a or 2a-c with stock solution containing metal perchlorate, and finally diluted with DMF to make the solution having desired concentrations of chemosensor and metal ions in the solution. For the Job plot analyses, a series of solutions in DMF with varying concentrations of receptors 1a and Cu²⁺ were prepared, and the measurements were performed under the condition of [1a] + [Cu²⁺] = 7.0 × 10⁻⁵ M in final solution (5 mL). All the measurements were taken at room temperature about 25 °C. UV-vis absorption spectra and fluorescence spectra of the mixed solutions were recorded against DMF at the end of the completed reaction between the metal ions and 1a. For the fluorescence measurements, excitation wavelength was 293 nm, with a slit width of 5 nm.

2. 6. Antibacterial Studies

The minimal inhibition concentration (MIC) values of the chemicals were studied for the microorganisms. The inocula of microorganisms were prepared from 12-h McFarland standard turbidity. Mueller Hinton Broth (100 μL) were placed into each 96 wells of the microplates. Chemical solutions at a concentration of 20 mg mL⁻¹ were added into the first rows of microplates and two fold dilutions of the chemicals were made by dispensing the solutions to the remaining wells. Culture suspensions (100 μL) were inoculated into each wells. Gentamicin solution was used as positive control. The sealed microplates were incubated at 37 °C for 18 h. Microbial growth was determined by adding 20 μL of 2,3,5-triphenyl-tetrazolium chloride (0.5%) after incubation to each well and incubating for 30 minutes at 37 °C.

3. Results and Discussion

Rhodanine based azo dyes 1a and 2a-c were synthesized according to modified literature procedure as shown in Scheme 1. The formation of 5-arylidenethrobanines and 5-arylazo rhodanines can be carried out both in an alkaline medium and in acetic acid in the presence of sodium acetate, while the corresponding 5-substituted derivatives of thiazolidine-2,4-dione and 2-thiohydantoin can be obtained only in an alkaline medium. All of the newly synthesized compounds 1a and 2a-c were characterized by IR, ¹H NMR and elemental analysis. Spectroscopic data were in full agreement with those expected. In the IR spectra of the compounds 1a and 2a-c, peaks were seen around 1710–1720 cm⁻¹ attributable corresponding C=O bond and 1320 and 1210 cm⁻¹ attributable C=S bond stretching. Additionally, the occurrence of new bands around 1440–1450 cm⁻¹ region attributable azo N=N bond stretching in the IR spectra of the compounds 1a and 2a-c confirmed the completion of the reactions. In the ¹H NMR spectra of newly synthesized rhodanine based azo dyes 1a and 2a-c, new peaks attributable aromatic protons were observed around 6.90–7.10 ppm. Furthermore, it was observed that new signals attributed to the phenolic hydroxyl protons were seen around 10.03 ppm for 2a, 8.81 ppm for 2b and 8.21 ppm for 2c respectively. Although, rhodanine ring existed in three tautomeric forms: ketone, enol and thioenol, due to a significant polarity of the rhodanine N–H bond and to the possibility of the proton migration to the oxygen or sulfur atoms of the C=O and C=S groups, we found that the most stable form of the rhodanine ring in crystalline state was the ketone form for compounds 1a and 2a-c. This is not surprisingly results. Because, it is well known that rhodanine ring exists mainly as the ketone form, which is due to the low probability of keto-enol and keto-thioenol transformations. The limiting stage of the tautomeric transformation is the heterolytic dissociation of the rhodanine N–H bond. Also, this situation is supported by quantum-chemical calculations in literature.

3. 1. UV-Vis Studies

The sensing ability of chemosensor 1a for various alkali and heavy metal ions (Li⁺, Na⁺, K⁺, Rb⁺, Pb²⁺, Ni²⁺, Cu²⁺, Zn²⁺) in DMF was investigated by UV-vis measurements. In the absence of the metal ions, rhodanine based azo dye 1a showed an absorption band at 414 nm attributed to the n-π* transitions of the rhodanine moiety. Upon addition of Cu²⁺, the band at 414 nm decreased and a new red-shifted absorption band at 492 nm appeared as shown in Fig. 1a. Correspondingly, the solution color changed from orange to dark brown. The significant color change indicates that compound 1a is a sensitive naked-eye indicator for Cu²⁺ (Fig. 1b). On the other hand, no significant change at 414 nm was observed upon the addition of the
other metal ions, indicating that receptor 1a shows high selectivity for Cu$^{2+}$ over these metal ions. This indicated that Cu$^{2+}$ binding with chemosensor 1a blocked the electron withdrawing ability of the rigid naphthalene group and resulted in a shorter absorption wavelength.

Fig. 2a shows UV-vis absorption spectra of receptor 1a with various concentrations of Cu$^{2+}$. The absorption peak at 414 nm gradually decreased with increasing amounts of Cu$^{2+}$ (0–6 eq.). A well-defined isosbestic point at 450 nm indicates the formation of a new compound. By plotting the changes of receptor 1a in the absorbance intensity at 492 nm as a function of Cu$^{2+}$ concentration, sigmoidal curve was obtained and the stoichiometry between receptor 1a and Cu$^{2+}$ ion was determined as 1:1 complex formation as shown in Fig. 2b. To evaluate the stoichiometry between 1a and Cu$^{2+}$, Job’s plot analysis was also executed. The maximum point occurred at the molar fraction of [1a] / ([Cu$^{2+}$] + [1a]) of ~ 0.53, confirming the 1:1
stoichiometry (Fig. 2c). The association constant of the complex of compound 1a and Cu\(^2\+) was determined to be 7.2 × 10\(^4\) M\(^{-1}\) by the Benesi-Hildebrand equation.\(^{36}\)

\[
\frac{1}{\Delta A} = \frac{1}{(Ke[D]_0[A]_0)} + \frac{1}{(c[D]_0)} \tag{1}
\]

where \([D]_0\) is the initial concentration of receptor 1a (donor), \([A]_0\) is the initial concentration of Cu\(^{2+}\) (acceptor), is the molar coefficient of the complex, \(K\) is the association constant of the complex, \(\Delta A\) is the change in the absorbance of the interaction of receptor 1a and Cu\(^{2+}\) after adding Cu\(^{2+}\) ions.

On the other hand, the absorption behaviors of the other rhodanine derivatives (2a-c) were investigated under the same conditions. The UV-vis spectra of receptors 2a-c showed new absorption bands at 300 nm, 298 nm, 299 nm, respectively. Excess perchlorate salts of 6 eq. Cu\(^{2+}\) were tested to evaluate the metal ion-binding properties of receptors 2a-c. Interestingly, we found that receptors 2a-c do not show any UV and color (light yellow) changes, whereas receptors 1a show a significant selectivity for Cu\(^{2+}\) over the other metal ions. The results obtained are summarized in Table 1. Upon the addition of Cu\(^{2+}\), UV-vis absorption spectra of the Cu-complexes exhibited similar photochemical changes compared to those of receptors 2a-c.

### 3.2. Fluorescence Studies

The fluorescence spectra of receptor 1a in the absence and presence of various metal ions (Li\(^+\), Na\(^+\), K\(^+\), Rb\(^+\), Pb\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\)) in DMF were recorded at excitation wavelength of 293 nm, and the emission spectrum of free receptor 1a displays a significant band in fluorescence intensity at 360 nm. As shown in Fig. 3a, upon addition of Cu\(^{2+}\), emission intensity of receptor 1a was quenched. In many chemosensing systems, paramagnetic Cu\(^{2+}\) ions can strongly quench the emission of a fluorophore via a photoinduced metal-to-fluorophore electron or electronic energy transfer.\(^{37,38}\) In addition, among the more paramagnetic metal ions, Cu\(^{2+}\) has a particularly high thermodynamic affinity for ligands with \(\text{Zn}^{2+}\) or \(\text{ZnO}^{2-}\) as chelating element, and fast metal-to-ligand binding kinetics.\(^{38,39}\) Zn\(^{2+}\) has a little effect on the fluorescence intensity of receptor 1a. This behavior can be explained by some factors like an increased rigidity in complex structur...
The values of \((I_0/I)\) at 360 nm were plotted against the concentration of \(\text{Cu}^{2+}\). \(I_0\) and \(I\) are the fluorescence intensity of receptor 1a and the fluorescence intensity of \((1a + \text{Cu}^{2+})\), respectively. The curve obtained exhibits a linear range between 0.5 \(\mu\text{M}\) and 5 \(\mu\text{M}\) \(\text{Cu}^{2+}\) with a correlation coefficient of 0.9862 (Fig. 4b). Furthermore, to explore the utility of receptor 1a as a ion-selective fluorescent chemosensor, fluorescence spectral changes of receptor 1a in competitive metal ion complexation were investigated in the presence of \(\text{Cu}^{2+}\) (6 eq.) and miscellaneous cations (6 eq.) including \(\text{Li}^+\), \(\text{Na}^+\), \(\text{K}^+\), \(\text{Rb}^+\), \(\text{Pb}^{2+}\), \(\text{Ni}^{2+}\), and \(\text{Zn}^{2+}\) in DMF. These miscellaneous competitive cations did not lead to any significant spectral change, in the presence of miscellaneous competitive cations, \(\text{Cu}^{2+}\) ions still resulted in the similar changes (Fig. 4c). All these results imply that receptor 1a can be useful as a fluorogenic-sensing material for selective detection of \(\text{Cu}^{2+}\) in the presence of other competitive metal ions.

### 3.3. Antibacterial Activity of Receptors 1a and 2a-c

Antibacterial activities of receptors 1a and 2a-c were investigated by microbroth dilution method according to previously published literature procedure \(^{29}\) towards some selected bacteria such as *Escherichia coli* ATCC 25922, *Staphylococcus aureus* (MSSA) ATCC 25923, *Pseudomonas aeruginosa* ATCC 15442 and *Klebsiella pneumoniae* ATCC 10031. The obtained results are presented in Table 2. All of the receptors were found to be moderate antibacterial against all test bacteria at 1.25 mg \(\text{mL}^{-1}\) dose level. These chemicals were determined as equally effective against pathogen microorganisms tested. MIC values of receptors 2a and 2b were determined as 1.25 mg \(\text{mL}^{-1}\) against *E. coli*, *P. aeruginosa* and *K. pneumoniae*. Comparing the other bacteria and *S. Aureus*, receptor 2c more affected the *S. Aureus* and MIC value was determined as 0.625 mg \(\text{mL}^{-1}\) for *S. aureus*. On the other hand, receptor 1a exhibited very strong antibacterial activity against *S. aureus* at a concentration of 0.0195 mg \(\text{mL}^{-1}\). It manifested a moderate antibacterial activity against *E. coli* at 1.25 mg \(\text{mL}^{-1}\) dose level and against *P. aeruginosa* and *K. pneumoniae* at a concentration of 2.50 mg \(\text{mL}^{-1}\). While receptor 1a showed similar antibacterial activity against *E. coli*, it was less effective against *P. aeruginosa* and *K. pneumoniae* when compared with receptors 2a-c. According to the obtained results, it was determined that compound 1a revealed strong antibacterial activity against *S. aureus* strain used in this study. *S. aureus* is the most sensitive strain against receptor 1a.

### 4. Conclusion

In summary, new rhodanine based azo dye derivatives 1a and 2a-c were prepared and their metal ion-selective chemosensing and antibacterial behaviors were investigated. Receptor 1a exhibited a selective change in UV-vis absorption and fluorescence spectra toward \(\text{Cu}^{2+}\). The metal-binding properties of 1a-Cu\(^{2+}\) complex were determined
ned by spectrophotometric methods. The stoichiometry between receptor 1a and Cu²⁺ was 1:1 complex formation, and the association constant was determined to be 7.2 × 10⁴ M⁻¹ by the Benesi-Hildebrand equation. Addition of Cu²⁺ to receptor 1a solution resulted in an obvious color change from orange to dark brown, which made it possible to detect Cu²⁺ by the naked-eye. Moreover, receptor 1a exhibited a good selectivity toward Cu²⁺ in the presence of competitive cations (Li⁺, Na⁺, K⁺, Rb⁺, Pb²⁺, Ni²⁺ and Zn²⁺). Furthermore, rhodanine based azo dyes 1a showed strong antibacterial activity against S. aureus. As a conclusion, this study can be a good example for designing a variety of optical sensing devices based on metal ion detection.

5. Acknowledgements

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6. References


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