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Cyclohexylamine Triazine-Quinoline hydrazone Schiff base a Fluorescence Receptor for Co (II) Ion in aqueous medium

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Abstract
(Z)-6-(2-((2-Chloroquinolin-3-yl)methylene)hydrazineyl)-N2,N4-dicyclohexyl-1,3,5-triazine-2,4-diammine (SBL), was synthesized and characterized using various spectroscopic techniques. The structure was characterized by ^{1}H NMR, ^{13}C NMR, MS and IR spectroscopy. To study its fluorescence and colorimetric properties towards various metal ions, high sensitivity and selectivity were achieved for Co^{2+} over other common metal ions. The limit of detection (LOD) for Co^{2+} determination was found to be as low as 0.22 µM by fluorescence spectroscopy and 0.73 µM by UV–Vis spectroscopy, demonstrating the high sensitivity of the synthesized SBL receptor toward Co^{2+} ions. The 1:1 binding stoichiometry between receptor (1) and Co (II) ion was established using Job's plot. The sensing mechanism is attributed to the coordination of Co^{2+} ions through the quinoline nitrogen, azomethine nitrogen, hydrazone nitrogen, and triazine nitrogen donor centers of the receptor, resulting in suppression of the PET process and enhancement of fluorescence emission. Complexation suppresses the photo-induced electron transfer (PET) process, resulting in a significant change in fluorescence intensity.

Keywords: Fluorescence chemosensor, colorimetric sensor, hydrazone, quinoline

Introduction
Cobalt is an important transition metal that plays a crucial role in biological systems as a key component of vitamin B_{12} and various metalloenzymes. However, excessive cobalt exposure may lead to cardiomyopathy, thyroid dysfunction, neurological disorders, and environmental toxicity [1, 2]. Therefore, the development of highly sensitive and selective chemosensors for Co^{2+} detection has attracted considerable attention for environmental and biological monitoring [3, 5]. Due to the presence of unpaired electrons, Co^{2+} is reported as a sensor that operates through fluorescence mechanisms. Nevertheless, fluorescence enhancement-based sensors are generally considered more advantageous because of their higher sensitivity and lower background interference. Consequently, "OFF–ON" fluorescent sensors for Co^{2+} ions remain relatively rare [6].

The 1,3,5-triazine ring is an important heterocyclic scaffold in medicinal and materials chemistry because of its planar aromatic structure, versatile substitution pattern, and favorable coordination properties [7]. Incorporation of hydrazine functionality onto the triazine ring provides an efficient binding site capable of forming stable complexes with transition metal ions and undergoing condensation reactions to generate hydrazone derivatives [7, 13]. Furthermore, quinoline Schiff base with triazine hydrazone contributes additional coordination sites and extends the conjugated framework, thereby enhancing the photophysical properties of the resulting receptor [8]. The addition of cyclohexylamine groups improves lipophilicity and structural flexibility, which may further influence metal-ion recognition behavior.

Based on these considerations, we designed a new class of Triazine hydrazone quinoline fluorescent receptors containing cyclohexylamine substituents for the selective recognition of Co^{2+} ions. The sensing mechanism is primarily based on suppression of photoinduced electron transfer (PET) and chelation-enhanced fluorescence (CHEF) upon metal-ion binding [9] In the unbound state, the receptor exhibits relatively weak fluorescence due to PET-mediated non-radiative decay pathways, when coordination occurs with Co^{2+} ions due to presence of nitrogen donor atoms in quinoline, hydrazone, and triazine molecules, PET is an effectively suppressed and molecular rigidity increase that gives enhanced fluorescence emission and an OFF→ON sensing response [9, 11].

This provides lower detection limits and it is more selective towards Co^{2+} ions over there competitive metal ions, and it shows accurate sensing performances in mixed aqueous media suitable for environmental monitoring applications [12, 14].

Hence, we report the synthesis, spectral measurements and photophysical properties of a novel SBL receptor. The receptor was synthesized stepwise starting from cyanuric chloride involving hydrazine hydrate substitution followed by Quinoline incorporation and further substitution of cyclohexylamine. UV–Visible and fluorescence titration studies were done to investigate the sensitive behavior of other ions towards Co^{2+} ions. The binding stoichiometry and selectivity toward Co^{2+} ions in the presence of competing metal ions were detected. Later, the structure–property relationships sensing the performance of the receptor were discussed, showing its potential application for the selective detection of cobalt (II) ions in aqueous media.

Experimental
Fluorescence measurements were carried out using a Varian Cary Eclipse fluorescence spectrophotometer equipped with a xenon discharge lamp and a 1 cm quartz cuvette. UV–Visible absorption spectra were recorded on an Schimadzu 2400 UV–Visible spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded at 293 K on a bruker avance Neo 500MHz using DMSO-d_6 as solvent and tetramethylsilane

(TMS) as an internal standard. FT-IR spectra were obtained using FT-IR spectrophotometer IR affinity in the range 4000–400 cm^{-1}. High-resolution mass spectra (HRMS) were recorded using electrospray ionization mass spectrometry (ESI-MS).

Synthesis Method

Synthesis of 2-Hydrazino-4,6-dichloro-1,3,5-triazine

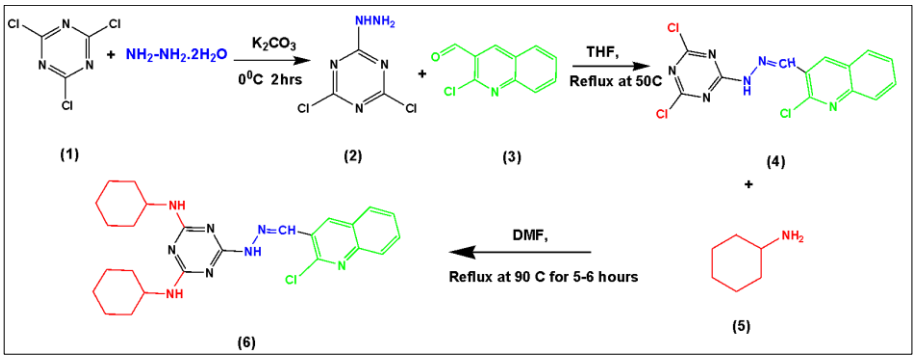
A stirred solution of cyanuric chloride (1.0 equiv, 1) in dry acetone was cooled at 0–5 °C in an ice bath. Hydrazine hydrate (1.0 equiv, 2) was dissolved in a small volume of acetone and added dropwise in above solution over 15–20 min while maintaining the temperature below 5 °C. The reaction mixture was stirred for 2 h at same temperature. The precipitated solid was obtained and filtered, washed with cold water, dried under vacuum to afford 3-2-hydrazino-4,6-dichloro-1,3,5-triazine as a white to off-white solid.

Synthesis of Hydrazone Intermediate

2-Hydrazino-4,6-dichloro-1,3,5-triazine (1.0 equiv, 3) was dissolved in absolute ethanol and stirred at room temperature. To this solution, 2-chloro-3-formylquinoline (1.0 equiv, 4) was added, followed by small amount of potassium carbonate. The reaction mixture was refluxed for 4–6 h. Upon completion, the reaction mixture was cooled to room temperature and poured into ice-cold water. The resulting solid was filtered, washed with water, 2-[(2-chloroquinolin-3-yl)methylenehydrazino]-4,6-dichloro-1,3,5-triazine (5).

Synthesis of Final Dianilino Derivative

The hydrazone intermediate (1.0 equiv, 6) was dissolved in DMF, cyclohexylamine (7) and potassium carbonate were added and the reaction mixture was stirred initially at room temperature and subsequently refluxed for 6–8 h. The reaction progress was monitored by TLC. After completion, the reaction mixture was cooled and filtered to remove inorganic salts and the crude product was poured into ice water. The (Z)-6-(2-((2-Chloroquinolin-3 yl)methylene)hydrazineyl)-N2,N4-dicyclohexyl-1,3,5-triazine-2,4-diammine (Schiff Base Ligand, 8) was collected by filtration, washed thoroughly with water, dried and purified by column chromatography.



Scheme 1: Synthesis of Schiff Base Ligand

Spectral Characterization

FT-IR (KBr, νmax, cm^{-1}): 3428 (N–H stretching), 3236 (hydrazone N–H), 3068 (aromatic C–H), 2928, 2854 (aliphatic C–H), 1618 (azomethine C=N), 1565 (triazine C=N), 1512 (quinoline ring vibration), 1456 (C=C aromatic stretching), 1318 (C–N stretching), 1236 (N–N stretching), 812 (s-triazine ring breathing), 756 (C–Cl stretching).

^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 10.39 (s, 1H, NH–N=CH), 10.10 (br s, 2xH, NH attached to triazine), 9.00 (s, 1H, CH=N), 8.66 (d, J = 8.2 Hz, 1H, quinoline-H), 8.05–7.06 (m, 6H, quinoline aromatic protons), 3.07–3.05 (m, 2H, cyclohexyl N–CH), 1.80–1.20 (m, 20H, cyclohexyl CH_2).

^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 170.2, 168.5 (triazine C=N), 154.3 (quinoline C=N), 147.8 (azomethine carbon, CH=N), 136.5, 132.4, 129.3, 127.7, 126.1, 125.4, 124.2, 122.8, 121.5 (quinoline aromatic carbons), 52.1 (cyclohexyl N–CH), 33.8, 32.5, 25.8, 24.9 (cyclohexyl CH_2 carbons).

HRMS (ESI-MS): m/z: 478.24 $[M]^+$, 479.24 $[M+1]^+$, 480.23 $[M+2]^+$.

Sample Preparation for Photo Physical Measurement

All the stock solutions were prepared in doubled distilled water and A.R grade DMF solution. The nitrate salts of Ag^+, Al^{3+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mg^{2+}, Na^+, $Ni^{2+}$and Zn^{2+} were used to prepare the stock solutions with concentration 1×10^{-3} M in water. The synthesized receptor was dissolved in a mixed solution of DMF:H_2O (1:1, v/v) to give a stock solution with concentration 1×10^{-4} M. The stock solutions were used after appropriate dilution.

Results & Discussions

Acid titrations show that the receptor was not sensitive to pH variations within the range of 4.0–10.0. No major changes in fluorescence intensity or UV–Visible absorbance were observed in this pH range, which indicates that addition of proton alone does not cause any effect on the electronic structure of the receptor. This pH stability is highly required for sensing applications in environmental and biological systems, where near-neutral conditions are mostly seen.

Optical Response toward Co^{2+} Ions

The fluorescent sensitive behavior of the receptor toward Co^{2+} ions was tested in DMF:H_2O (1:1, v/v) containing 0.005 M Tris–HCl buffer at pH 7.0. Upon slow and steady addition of Co^{2+} ions, the receptor demonstrated OFF–ON fluorescence response. The fluorescence intensity increased importantly from 0.22 CPS for the free receptor to 2.85 CPS at 462 nm in the presence of Co^{2+} ions that demonstrates 12.95-fold enhancement (Fig. 1). Simultaneously, the UV–Visible absorption spectrum displays an important bathochromic shift with increase in absorbance intensity that shows strong interaction between receptor and Co^{2+} ions (Fig.

2). The induced colour change was clearly visible to the naked eye, allowing both colorimetric and fluorometric detection of cobalt ions. The fluorescence titration profile suggested progressive formation of a receptor Co^{2+} complex with increasing metal-ion concentration [15].

Selectivity and Interference Studies

The selectivity of the receptor toward Co^{2+} ions was tested in the presence of various competing metal ions including Ag^{+}, Al^{3+}, Ca^{2+}, Cd^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Mg^{2+}, Na^{+}, Ni^{2+} and Zn^{2+}. Among all the tested metal ions, Co^{2+} produced the highest intensity (2.85 CPS) at 462 nm, whereas the remaining metal ions generated weak intensity ranging from 0.14 to 0.28 CPS (Fig. 3). Competitive experiments further revealed that the response of Co^{2+} was retained even in the presence of excess concentrations of potentially interfering ions. Following addition of Co^{2+} to solutions containing competing metal ions, color intensities remained within the range of 2.58–2.74 CPS, indicating only minimal interference. These results demonstrate the high selectivity and anti-interference capability of the receptor toward Co^{2+} ions. The arrangement of donor atoms within the receptor framework provides a favorable coordination environment for cobalt ions, thereby facilitating preferential recognition under neutral conditions [16, 17].

Stoichiometry and Binding Strength

The binding stoichiometry between the receptor and Co^{2+} ions was detected using Job's method of continuous variation. The Job's plot exhibited a mole fraction corresponding to a 1:1 receptor Co^{2+} complex that shows the formation of a single dominant species in solution (Fig. 4). The binding affinity of the receptor toward Co^{2+} ions was further tested using Benesi–Hildebrand, Scatchard and Connor's methods. The calculated association constants (Ka) were found to be 1.15×10^{4} M^{-1}, 1.20×10^{4} M^{-1} and 1.18×10^{4} M^{-1}, respectively. These values confirm the reliability of the binding analysis and suggest the formation of a stable receptor Co^{2+} complex in solution.

The analytical sensitivity of the receptor was assessed through fluorescence titration experiments. The limit of detection (LOD) and limit of quantification (LOQ) for Co^{2+} ions were determined to be 0.22 µM and 0.73 µM, respectively, this shows that receptor is suitable for determination of Co^{2+} ions at microlevels [18].

Proposed Sensing Mechanism

The fluorescence enhancement observed upon addition of Co^{2+} ions can be rationalized on the basis of a photoinduced electron transfer (PET) and chelation-enhanced fluorescence (CHEF) mechanism. In the absence of Co^{2+} ions, the receptor displays relatively weak fluorescence owing to PET-mediated non-radiative decay pathways involving the donor nitrogen atoms (Fig. 1). Upon coordination with Co^{2+} ions through the quinoline nitrogen, azomethine nitrogen, hydrazone nitrogen and triazine nitrogen donor atoms, the PET process is effectively suppressed. Simultaneously, complex formation increases the rigidity of the molecular framework and reduces non-radiative relaxation pathways. As a result, fluorescence emission is significantly enhanced, producing the observed OFF–ON response. The 1:1 binding stoichiometry and substantial fluorescence enhancement are fully consistent with a chelation-driven sensing mechanism [19, 20] (Fig.2,3& 4).

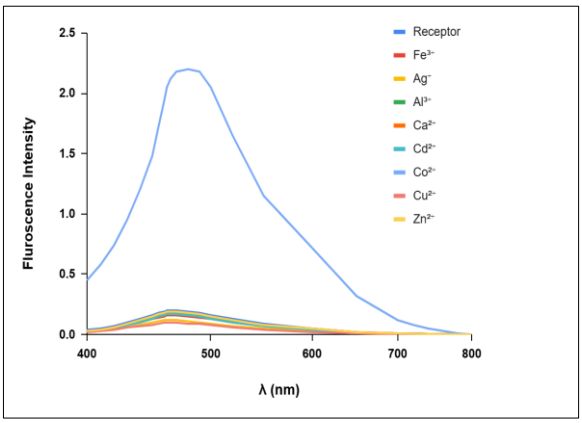


Fig 1: Fluorescence intensity of receptor (1×10^{-4} M, λex = 462 nm) upon addition of various metal ions (1×10^{-4} M) in DMF:H_2O (1:1, v/v).

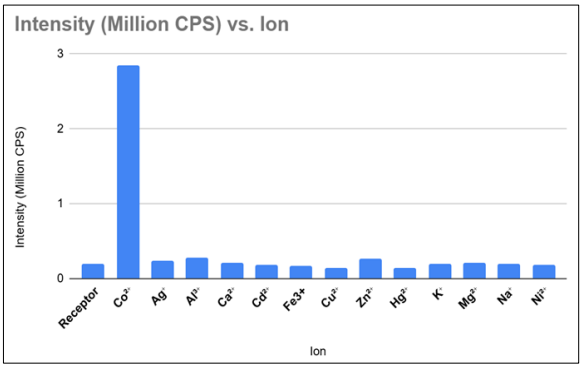


Fig 2: UV-Visible intensity of receptor (1×10^{-4} M, λex = 462 nm) upon addition of various metal ions (1×10^{-4} M) in DMF:H_2O (1:1, v/v) at wavelength 462 nm.

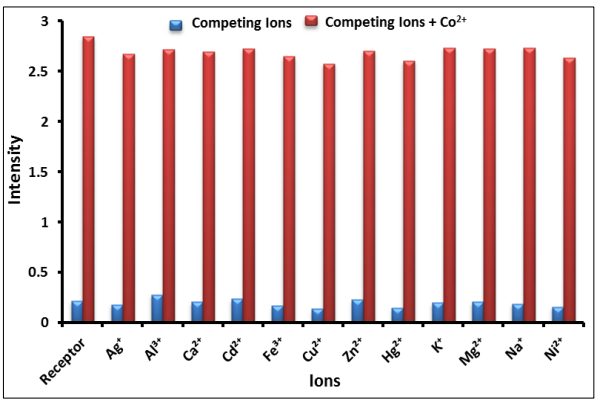


Fig 3: Interference studies of various metal ions (1 equivalent) in presences of Co (II) ion at 462 nm.

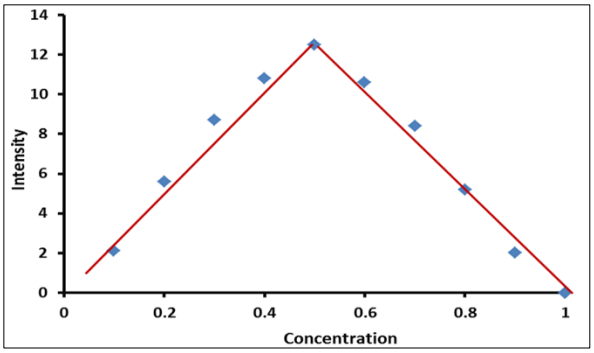


Fig 4: Job's plot for showing 1:1 stoichiometry of receptor (1) and Co (II) ion (host and guest).

Analysis Property

Under optimized conditions (probe concentration 1.00×10^{-4} mol L^{-1}; solvent: DMF/H_2O 1:1 v/v; 0.005 M Tris–HCl buffer, pH 7.0; excitation wavelength 380 nm; emission wavelength 462 nm; room temperature), fluorescence titrations of the receptor with Co^{2+} ions exhibited two linear response regions: $0.10–2.00 \times 10^{-5}$ mol L^{-1} ($R = 0.9981$, $n = 8$) and $2.00–10.00 \times 10^{-5}$ mol L^{-1} ($R = 0.9945$, $n = 8$).

Corresponding UV–Visible absorbance measurements also displayed two linear ranges: $0.50–4.50 \times 10^{-5}$ mol L^{-1} ($R = 0.9953$, $n = 5$) and $4.50–15.00 \times 10^{-5}$ mol L^{-1} ($R = 0.9964$, $n = 12$). Calibration plots were obtained by plotting fluorescence enhancement ($\Delta F = F - F_0$).

(Fig. 5) or absorbance difference ($A - A_0$) against Co^{2+} concentration (Fig. 6) and fitting the data using least-squares linear regression. Residual values were randomly distributed within ±3% across the entire calibration range, confirming excellent linearity.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The analytical sensitivity of the receptor towards Co^{2+} ions was tested under optimized experimental conditions. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the standard expressions LOD = 3σ/s and LOQ = 10σ/s, where σ represents the standard deviation of ten blank measurements and s is the slope of the corresponding calibration curve (Fig. 7). The LOD and LOQ values for Co^{2+}were determined to be 0.22 µM and 0.73 µM, respectively that shows high sensitivity of the receptor toward cobalt (II) ions in aqueous medium.

The low detection limit indicates the potential ability of the receptor for the selective determination of trace levels of Co^{2+} ions in environmental and aqueous samples. The excellent sensitivity can be seen through fluorescence enhancement shown because of the formation of complex with Co^{2+} ions with the receptor through a chelation-enhanced fluorescence (CHEF) mechanism.

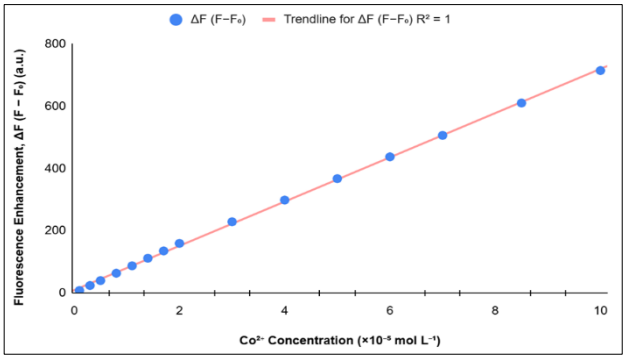


Fig 5: Fluorescence Titration Curve of the Receptor with Co^{2+} Ions

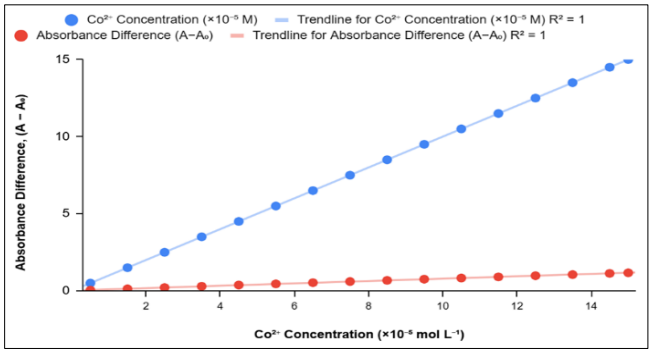


Fig 6: Linear Absorbance Enhancement Profile for Co^{2+} Detection

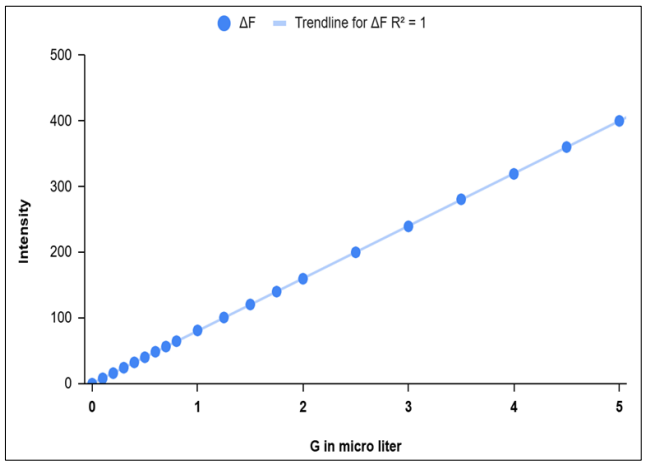


Fig 7: Analytical Calibration Curve for Co^{2+} Sensing

Accuracy and Recovery

The accuracy of the proposed method was assessed by recovery experiments performed at three concentration levels (low, medium and high). Known concentrations of Co^{2+} ions were spiked into blank DMF/H_2O buffer solution and analyzed under optimized conditions. Recoveries ranged from 97.5–102.8% for fluorescence measurements and 96.8–103.4% for UV–Visible absorbance measurements, with RSD values below 2.0% in all cases. These results demonstrate satisfactory accuracy and negligible matrix effects for quantitative determination of Co^{2+} ions.

Conclusion

A novel triazine–hydrazone–quinoline Schiff base receptor incorporating cyclohexylamine substituents was prepared and characterized by FT-IR, ^1H NMR, ^{13}C NMR, HRMS, and elemental analysis. The receptor demonstrated remarkable sensing performance toward Co^{2+} ions in DMF:H_2O (1:1, v/v) medium under neutral conditions. Fluorescence investigations proved about OFF–ON response upon interaction with Co^{2+} ions, resulting in an approximately 12.95-fold enhancement in fluorescence intensity at 462 nm. Among all the investigated metal ions, Co^{2+} produced the strongest fluorescence response, demonstrating excellent selectivity and minimal interference from competing ions. Overall, the combination of high sensitivity, excellent selectivity, rapid response, good reproducibility, and operational stability under near-neutral conditions demonstrates that the synthesized receptor is a promising fluorescent chemosensor for the selective detection of Co^{2+} ions in mixed aqueous media.

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