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Fluorescent probe sensor based on (R)-(–)-4-phenyl-2-oxazolidone for effective detection of divalent cations

Shvamal Baruah | Merangmenla Aier | Amrit Puzari 💿

Department of Chemistry, National Institute of Technology Nagaland, Chumukedima, Dimapur, Nagaland, India

Correspondence

Amrit Puzari, Department of Chemistry, National Institute of Technology Nagaland. Chumukedima, Dimapur, Nagaland, India. Email: amrit09us@yahoo.com

Abstract

Significant progress attained in sensor science in recent years has resulted in the development of highly efficient fluorescence probes for sensing metal ions. Fluorescent molecular probes based on (R)-(-)-4-phenyl-2-oxazolidone are reported here. Fluorescence studies indicated that the molecular probe could be used successfully to sense divalent metal cations such as Cu²⁺, Co²⁺, Pb²⁺, and Zn²⁺. The addition of divalent metal cations to the molecular probe produced a specific interaction pattern under UV-visible and fluorescence spectroscopy. These molecules could detect metal cations using fluorescence quenching. Stern-Volmer plots were used to determine quenching rate coefficients, which were calculated to be 2×10^{1} , 1.06×10^{3} and 7.39×10^2 M⁻¹ s⁻¹ for copper, cobalt, and zinc respectively. Calculation of limit of detection for heavy metal cations revealed that the reported molecular probes improved the limit of detection compared with available standard data. Limit of quantitation values were also well within the permissible range. The frontier energy gap of highest occupied molecular orbital to the lowest unoccupied molecular orbital was evaluated using the density functional theory approach and Gaussian 09 W software, which complemented the coordination of azetidinones with divalent metal ions.

KEYWORDS

(R)-(-)-4-phenyl-2- oxazolidone, fluorescent probe, metal cations, optical sensor, quenching

1 | INTRODUCTION

Chemosensor is a term used in host-guest chemistry describing a molecular structure in close association.^[1] Sensors are molecules in a system that on stimulation by any form of energy undergo a characteristic change of their own state resulting in one or more other changes.^[2] Chemosensors are used for ion recognition, and have increasingly found application in various fields.^[3] The roles of ions as cations and anions are more prevalent in industry^[4], and in farming^[5], as well as in the environment.^[6] Ni²⁺, Cu²⁺, Co²⁺ and Zn²⁺ are the most relevant cations in different fields^[7] and are additionally significant for the life cycle of cells.^[8] Macromolecular receptors, which are proteins in nature, generally influence cellular biological process.^[9] Several biochemical transformations are triggered by their cationprotein complexes.^[10] Therefore cationic species recognition has become an important research objective.^[11] In recent years, the selective recognition of cations by synthetic receptors has accelerated.^[12] To design artificial receptors with optimal selectivity toward a particular ion, several strategies need to be followed.^[13] Receptors with their various functionalities can be organized to complement the size and shape of the analyte.^[14] For overall receptor-ion interactions, the topology of the receptor is important.^[15] Receptors can form complexes with many cations ranging from transition metals to alkali^[16] and alkaline earth metals^[17] due to the special characteristics of their flexible donor atoms^[18] plus geometrical and interaction complementarity between the associating partners. This is generally implied by recognition, i.e. the optimal information content of a receptor concerning associating partners.^[19] The 'lock and key' concept represents the double complementarity principle extending over energetic features, as well as over geometric ones.^[20] The steric fit concept of Emil Fischer^[21], electrostatic interactions (ion–ion, ion–dipole, and dipole– dipole)^[22], hydrogen bonding ^[23], π – π stacking interactions^[24], dispersion and induction forces (van der Waals forces)^[25], and hydrophobic or solvatophobic effects are also components of recognition.^[26] Heterocyclic supramolecular entities are an important precursor for application studies in various research fields.^[27] These types of molecules have been successfully employed in molecular recognition²⁸ and for sensing applications.^[29] A diverse range of chemosensor probes has been developed based on various structures ranging from small molecules ^[30], metal complexes^[31], macrocycles through to polymers^[32], carbon nanotubes^[33], quantum dots^[34], and nanoparticles.^[35] These systems have been developed for sensing and detection of substrates of varying sizes and charges, ranging from cations^[36] and anions^[37] to small molecules such as explosives^[38] and biologically important motifs.^[39]

Oxazolidinone-based compounds have the scope for application in various fields including molecular recognition and sensory applications.^[40] Therefore, many research projects are still needed to develop new molecules based on oxazolidinone for application in diverse fields. (R)-(–)-4-phenyl-2 oxazolidone-based molecular building blocks have been rarely described and therefore can be considered as potential molecules for application studies in various research fields. The study presented here outlines the synthesis of a series of azetidinones based on (R)-(–)-4-phenyl-2 oxazolidone and describes the investigation of their application as sensory materials for detection of cations using UV-vis and fluorescence spectroscopy.

2 | EXPERIMENTAL

2.1 | Materials and methods

All chemicals used for the synthesis were procured from Sigma-Aldrich or TCI, Japan and were used as received. The reaction progress was monitored using thin layer chromatography (GF254). Infrared (IR) spectra were recorded on a Cary 630 Fourier transform infrared (FTIR) spectrometer and the values were expressed as ν_{max} cm⁻¹. ¹H nuclear magnetic resonance (NMR) and ¹³C (¹H) NMR spectra were recorded on a Bruker AvIII HD- 300 MHz FT NMR instrument and Varian Mercury Plus 300 MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectral data were recorded on a Waters UPLC-TQD (ESI-MS) instrument and Triple Quadrupole (LC-MS/MS) mass spectrometer. Elemental analysis was performed with a Thermo Finnigan Flash EA 1112 CHN/CHNS/O analyzer and PerkinElmer PR 2400 series II elemental analyzer. A Cary Eclipse fluorescence spectrometer was used to study fluorescence spectroscopic properties. Similarly, a Cary 100 UV-vis spectrophotometer was used for the UV-vis absorption spectra within the wavelength range 200-800 nm. For the UV-vis and fluorescence measurements, stock solutions of azetidinones (0.01 M) and metal ions (0.01 M) were prepared in H₂O/methanol (50/50, v/v). A circular dichroism (CD) study was performed using a J-815 spectropolarimeter and a JASCO spectrophotometer at room temperature (25°C).

2.2 | General procedure for the synthesis of imines

The synthesis of imines was performed following a published procedure.^[41] Aromatic aldehyde (2.0 mmol) was dissolved in 1.0 ml methanol taken in a test tube. An equimolar amount of amine was dissolved separately in another test tube containing 1.0 ml of 0.02 M H₂SO₄ solution. The solutions were then mixed thoroughly in a 25.0 ml round bottomed flask. The reaction mixture was then transferred into a microwave oven and maintained at 40°C. The reaction was allowed to continue for 15 min under microwave irradiation. Methanol was removed from the reaction mixture under reduced pressure and the product of the reaction was extracted with dichloromethane (2 × 10.0 ml). The solvent was removed under reduced pressure to obtain the product. Products were further purified by passing through a small column of silica gel using a 10% ethyl acetate solution in hexane as the eluent.

2.2.1 | Analytical data for 2,4-dichlorobenzyliminepropane

Yield: 79.5%. Appearance: colourless liquid. IR (ν_{max} cm⁻¹): 1578 (CH=N), 3089 (C-H), 1461, 1386, 1035, 918, 833, 717, 557. ¹H NMR (CDCl₃, 400 MHz) δ = 8.22 (s, 1H, 1CH imine), 7.23, 7.35, 7.44 (m, 3H, 3CH arom.), 3.54 (t, 2H, 1CH₂), 1.72 (m, 2H, 1CH₂), 0.96 (t, 3H, 1CH₃). ¹³C NMR (CDCl₃, 100 MHz) δ = 158.00, 130.56, 134.38, 133.01, 129.54, 127.11, 63.42, 23.95, 11.83. Mass 216 (M), 218 (M + 2). Elemental anal. Calcd. for C₁₀H₁₁NCl₂, C-55.56, H-5.09, N-6.48%. Found C-54.94, H-5.23, N-6.56%.

2.2.2 | Analytical data for 2,4-dichlorobenzyliminebutane

Yield: 78.9%. Appearance: colourless liquid. IR (ν_{max} cm⁻¹): 1585 (CH=N), 3099 (C-H), 1393, 1083, 815, 738, 521. ¹H NMR (CDCl₃, 400 MHz) δ = 8.62 (s, 1H, 1CH), 7.76, 7.33, 7.27 (m, 3H, 3CH), 3.68 (t, 2H, 1CH₂) 3.62 (t, 2H, 1CH₂), 1.65 (m, 2H, 1CH₂), 0.95 (t, 3H, 1CH₃). ¹³C (¹H) NMR (CDCl₃, 100 MHz) δ = 156.63, 136.82, 132.15, 130.62, 59.11, 33.05, 20.62, 14.03. Mass 232 (M + 2). Elemental anal. Calcd. for C₁₁H₁₃NCl₂, C-57.39, H-5.65, N-6.09%. Found C-56.87, H-5.32, N-6.39%.

2.2.3 | Analytical data for 3,4-dichlorobenzyliminepropane

Yield: 81.0%.Appearance: colourless liquid. IR (ν_{max} cm⁻¹): 1578 (CH=N), 3089 (C-H). ¹H NMR (CDCl₃, 400 MHz) δ = 8.23 (s, 1H, 1CH imine), 7.27, 7.36, 7.43 (m, 3H, 3CH arom.), 3.53 (t, 2H, 1CH₂), 1.72 (m, 2H, 1CH₂), 0.96 (t, 3H, 1CH₃). ¹³C (¹H) NMR (CDCl₃, 100 MHz) δ = 158.00 (CH=N), 130.56, 127.11, 129.52 (aromatic), 134.38, 133.01 (C-Cl), 63.45 (-CH₂-N), 23.94 (-CH₂), 11.82 (-CH₃). Mass 216 (M), 218 (M + 2). Elemental anal. Calcd. for C₁₀H₁₁NCl₂, C-55.56, H-5.09, N-6.48%. Found C-54.94, H-5.23, N-6.56%.

2.3 | Synthesis of (2-oxo-4-phenyl-oxazolidin-3yl) acetic acid

(R)-4- phenyl-2-oxazolidone (2.0 mmol) was dissolved in 3.0 ml of methanol taken in a 50.0 ml round bottomed flask, followed by the addition of an equimolar amount of chloroacetic acid (2.0 mmol). Sodium ethoxide (3.0 mmol) was added to the reaction mixture and the reaction mixture was kept at 40°C for 24 h. The progress of the reaction was monitored by thin layer chromatography. Methanol was removed from the reaction mixture and the product of the reaction was extracted with diethyl ether (3 × 10 ml) and was dried using anhydrous sodium sulfite. Finally, the solvent was removed under reduced pressure to obtain the product.

2.3.1 | Analytical data for (2-oxo-4-phenyl-oxazolidin-3yl)acetic acid

Yield: 69.5%. Appearance: white crystal. Melting point (m.p.): 85°C. IR (ν_{max} cm⁻¹): 3450 (-OH), 2989, 2879, 1678 (C=O). ¹H NMR (CDCl₃, 400 MHz): 11.00 (carboxyl -OH), 7.42, 7.41, 7.40, 7.39, 7.38, 7.36 (aromatic), 4.72, 4.13 (methylene). ¹³C (¹H) NMR (CDCl₃, 100 MHz) δ = 171.32 (carboxylic), 49.76 (-CH₂), 162.00 (amide), 74.03 (-CH₂ aliphatic), 141.82, 130.44, 130.17, 129.58, 127.12 (aromatic -CH), Mass 221 (M), Elemental anal. Calcd. for C₁₀H₁₁NCl₂, C-59.72, H-4.90, N-6.30%. Found C-58.90, H-5.21, N-7.20%.

2.4 | Synthesis of (R)-(–)-4-phenyl-2-oxazolidonebased azetidinones

(2-Oxo-4-phenyl-oxazolidin-3yl)acetic acid (2.0 mmol) was dissolved in 3.0 ml of methanol taken in a 250.0 ml round bottomed flask followed by the addition of an equimolar amount of thionyl chloride (2.0 mmol). The reaction mixture was kept at 40°C for 1 h. This was followed by the addition of an equimolar amount of imine and triethylamine as a base. The reaction mixture was further stirred for 4 h at 40°C. The progress of the reaction was monitored by thin layer chromatography. Methanol was removed from the reaction mixture under reduced pressure and the product was extracted with diethyl ether (3 × 10 ml). The product was dried using anhydrous sodium sulfite. Finally, the solvent was removed to obtain the product.^[42]

2.4.1 | Analytical data for 4-(2,4-dichloro-phenyl)-3-(2-oxo-4-phenyl-tetrahydro-furan-3-yl)-1-propylazetidin-2-one (C1)

Yield: 72.0%. Appearance: Creamy yellow crystal. Melting point (m.p.): 82°C. IR (ν_{max} cm⁻¹): 3375, 3059, 3011, 2920, 2863, 1725, 1650 (C=O), 1590, 1480, 1438, 1217, 1073, 767 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz: 7.55, 7.50, 7.49, 7.48, 7.45, 7.36 (m, 8H, CH aromatic) 7.35, 7.27, 5.02 5.01 4.93 (d, 2H, 1CH₂ aliphatic) 4.81, 4.73, 3.27 (t,

1CH₃), 1.73 (s, 1CH₂), 0.98 (t, 1CH₃). ¹³C (¹H) NMR (CDCl₃, 400 MHz): 165.15 (C=O, azetidinone), 162.54 (C=O, oxazolidinone), 141.01, 135.93, 129.22, 128.13, 127.55, 126.83, 74.17 (-CH₂), 57.25, 52.64, 49.37, 42.44, 21.62, 11.34. Mass 312.11 (C₆H₁₀Cl₂), 268.06 (C₁₂H₁₃ ClN₂O₃). Elemental anal. Calcd. for C₂₁H₂₀N₂O₃Cl₂ C-60.27, H- 4.78, N-6.70%. Found C-58.91, H-5.13, N-6.90%.

2.4.2 | Analytical data for 3[1-butyl-2-(2,4-dichlorophenyl)-4-oxo-azetidin-3-yl]-4-phenyl-azetidin-2-one (C2)

Yield: 73.5%. Appearance: yellow crystal. Melting point (m.p.): 85.5°C. IR (ν_{max} cm⁻¹): 3372, 3058, 3012, 2919, 2872, 1728, 1687 (C=O), 1596, 1488, 1445, 1213, 1071, 753. ¹H NMR (CDCl₃, 400 MHz; 7.57, 7.52, 7.47, 7.43, 7.39, 7.37, 7.34, 7.32 (m, 8H, CH aromatic), 5.54, 5.23, 4.92 (d, 2H, 1CH₂ aliphatic), 3.23, 1.64 (s, 1CH₂), 1.37 (s, 2H, 1CH₂), 0.96 (t, 3H, 1CH₃).¹³C (¹H) NMR (CDCl₃, 400 MHz): 168.34 (C=O, azetidinone), 162.57 (C=O, oxazolidinone), 136.14, 135.76, 135.16, 131.45, 131.15, 130.62, 130.63, 130.32, 130.13, 129.79, 129.54, 128.14, 127.13, 74.06, 57.55, 49.77, 49.58, 49.36, 47.93, 30.64, 20.87, 14.00. Mass 288 (C₁₆H₂₀N₂O₃), 232.08 (C₁₂H₁₂N₂O₃). Elemental anal. Calcd. for C₂₂H₂₂Cl₂N₂O₃, C-61.09, H-5.09, N-6.48%. Found, C-59.60, H-5.72, N-6.54%.

2.4.3 | Analytical data for 4-(3,4-dichloro-phenyl)-3-(2-oxo-4-phenyl-tetrahydro-furan-3-yl)-1-propylazetidin-2-one (C3)

Yield: 74.0%. Appearance: creamy yellow crystal. Melting point (m.p.): 82.3°C. IR (ν_{max} cm⁻¹): 3377, 3061, 3015, 2929, 2873, 1728, 1682 (C=O), 1596, 1491, 1448, 1217, 1073, 758 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz: 7.58, 7.55, 7.43, 7.42, 7.32, 7.33, 7.32, 7.31, 7.30 (m, 8H, CH aromatic), 5.38, 5.23, 5.12, 4.90 (d, 2H, 1CH₂ aliphatic), 4.12, 3.82, 3.23 (t, 2H, 1CH₂), 2.86, 1.76 (s, 2H, 1CH₂), 1.33, 0.93 (t, 3H, 1CH₃). ¹³C (¹H) NMR (CDCl₃, 400 MHz): 165.17 (C=O, azetidinone), 162.58 (C=O, oxazolidinone), 141.01, 140.00, 135.79, 129.13, 128.04, 127.57, 126.72, 74.10, 57.12, 52.56, 49.22, 42.37, 21.57, 11.29. Mass 387 (C₂₁H₂₁ClN₂O₃), 345 (C₁₅H₁₅N₂O₃Cl₂). Elemental anal. Calcd. for C₂₁H₂₀N₂O₃Cl₂. C-60.27, H-4.78, N-6.70%. Found C-59.70, H-6.30, N-6.66%.

3 | RESULTS AND DISCUSSION

3.1 | Synthesis of (R)- (–)-4-phenyl-2- oxazolidonebased azetidinones

A series of (R)-(-)-4-phenyl-2- oxazolidone-based azetidinones was prepared using the synthesis procedure outlined in Scheme 1. The reaction involved *in situ* conversion of (2-oxo-4-phenyl-oxazolidin-3yl)-acetic acid to its corresponding acid chloride by reacting with

4

CI





SCHEME 1 Synthesis of (R)- (–)-4-phenyl-2-oxazolidone-based azetidinones

thionyl chloride and then treating the product with an aromatic imine in the presence of triethylamine as base, leading to the formation of the compounds (C1–C3) in 76%, 77.5%, and 76.5% yields, respectively (Figure 1).

The molecules were characterized using ¹H NMR, ¹³C (¹H) NMR, FTIR, and LC–MS spectroscopy. Observation of two distinct bands of the two (C=O) groups in the FTIR spectra of each compound strongly supported the formation of the product. ¹H and ¹³C (¹H) NMR spectra of the compounds also supported the formation of azetidinone rings in the molecules, as represented in Figure 1. The chemical shift positions of the azetidinone protons in the ¹H NMR spectra were observed in the range 4.9–5.2 ppm. Chemical shift position for the azetidinone carbons in ¹³C (¹H) NMR spectra was observed in the range 155–160 ppm.

3.2 | Circular dichroism study of the molecules

Circular dichroism spectroscopy is an extensively used technique in the study of chiral molecules of all types and sizes. The technique has gained far more importance in the study of large biological molecules. The secondary structure or conformation of supramolecules can be analyzed using this technique. The technique reveals the structural, kinetic, and thermodynamic information of macromolecules. CD signals are observed only when there is absorption of radiation, and therefore the spectral bands are easily assigned to distinct structural features of the molecule. An advantage of the CD technique in the study of proteins is that complementary structural information can be obtained from several spectral regions. Circular dichroism spectra recorded for the compounds (R)-(–)-4-phenyl-2 oxazolidone and 4-(2,4-dichloro-phenyl)-3-(2-oxo-4-phenyl-tetrahydrofuran-3-yl)-1-propyl-azetidin-2-one are represented in Figure 2.

The absorption region at 240 nm and below in Figure 2 indicated the *cis* steric orientation of the aromatic chromophore. This directly reflects the difference in folding of the overall structure. From Figure 2(a), which represents the CD spectra of the parent (R)-(–)-4-phenyl-2 oxazolidone, it was observed that the *cis* isomer has strong peaks in the region 230–240 nm. Whereas, for the compound synthesized from (R)-(–)-4-phenyl-2 oxazolidone, i.e. for the azetidinone as represented in Figure 2(b), strong absorption peaks were observed below 230 nm. Therefore the study shows that incorporation of azetidinone into the structure influences the positive sign of the band relative to the parent compound regardless of the relative orientation of the ring substituents.

3.3 | The change of electronic absorption by divalent metal cations

It has been observed that the azetidinones reported in this study, on interaction with metal cations exhibit significant changes in their

FIGURE 1 Chemical structures of (R)-(–)-4-phenyl-2-oxazolidone-based molecules (a) C1 (b) C2 and (c) C3



FIGURE 2 Circular dichroism spectra of (a) (R)-(-)-4-phenyl-2 oxazolidone (b) 4-(2,4-dichlorophenyl)-3-(2-oxo-4-phenyltetrahydrofuran-3-yl)-1-propylazetidin-2-one (for the experiment methanol was used as solvent) electronic absorption spectra. Such changes in electronic absorption spectra can be attributed to the specific interaction of the metal cation with the azetidinone moiety as represented in Scheme 2.



SCHEME 2 Schematic diagrams showing the interaction of azetidinones with metal cations

Specific interaction patterns of these azetidinones observed with the metal cations can be exploited for sensing such metal cations and the same was established experimentally. Additionally, the molecules exhibited important photoluminescence characteristics, reflecting the scope for using these molecules as optical sensors and fluorescence probes.

3.4 | Cation sensing

For biological metabolism Fe, Zn, Cu, Co, Cr, Mn, and Ni, are essential in trace amounts; however higher doses of these ions in living organisms may cause toxic effects. Conversely, metals like Pb, Hg, Cd, and



FIGURE 3 UV-vis absorption pattern from interactions of (a) C1 with Cu^{2+} (b) C2 with Co^{2+} (c) C3 with Zn^{2+} (d) C1 with Pb^{2+} and (e) C3 with Cu^{2+}

As are positively toxic and are not suitable for biological functions. Therefore, scientific research focused on cation sensing in the biological system has become an important research objective. Here we investigated the cation sensing ability of (R)-(–)-4-phenyl-2 oxazolidone-based azetidinones for the metal cations Cu^{2+} , Co^{2+} , Zn^{2+} , and Pb^{2+} . The study was performed by introducing a dilute aqueous solution of the metal salt (0.01 M) to a solution of C1, C2, or C3 in methanol (0.01 M) under UV-vis spectroscopy. The UV-vis spectra obtained for these four metal cations are represented in Figure 3.

A specific absorption pattern was observed for interaction of the azetidinone molecules with metal cations. For each metal cation, the absorption band observed at 360 nm for the azetidinone unit disappeared on interaction with the cation. Absorption peaks observed at 310 nm for the azetidinone molecule exhibited a blue shift from 310 nm to 300 nm. The intensity of the absorption band gradually decreased with time (Figure 3a). A similar observation was noted for the Co²⁺ cation (Figure 3b). In this case, the intensity of the absorption band also gradually decreased with time, as is the case for the absorption spectra of the Zn²⁺cation and Pb²⁺cation. Figure 3(c) and Figure 3(d), respectively. The observed blue shift in the UV-vis absorption peak at 310 nm and the subsequent decrease in the absorption intensity of the peak at 300 nm suggested the ability of the azetidinones to sense metal cations. Interaction of the metal cation with the organic molecular entity (C1, C2, and C3) led to the formation of a ligand to metal charge transfer complex, in which the metal cation acts as acceptor and ligand molecule as a donor. The absorption peaks observed at 220, 275, 300 and 360 nm in Figure 3 (a) are due to $n-\pi^*$ and $\pi-\pi^*$ transitions. After interaction with Cu²⁺ ion solution, the absorption peak at 300 nm was degraded up to 50% and the peak at 360 nm was completely degraded in 45 min. Similarly. for the Co^{2+} cation, the absorption peak at 300 nm was degraded up to 90% and the one at 360 nm was fully degraded after 60 min. We also observed that for the Zn²⁺ ion, the interaction led to almost complete degradation of both the peaks at 300 nm and 360 nm. For other heavy metals like the lead (Pb) cation, for interaction with azetidinone C1, there was intercrossing and an isosbestic point was observed around 380 nm. The appearance of the isosbestic point indicates that the interaction took place through a two-step process involving an equilibrium. However, nonappearance of isosbestic point in other cases does not mean that the interaction of the two species has not proceeded through equilibrium. Similarly, for copper cations, C3 showed an exclusive ligand to metal charge transfer (LMCT) interaction (Figure 3e). As metal ions have an empty d orbital, therefore the d-d transition can also contribute to the decrease in the absorption peaks. Therefore, it can be stated that the forbidden d-d transition has resulted in a decrease in absorption intensity.

3.5 | Binding mode study

To find out the stoichiometry of binding between the receptor and the metals, the Job's method of continuous variation was utilized as shown in Figure 4. For the study, a constant total concentration



FIGURE 4 Job's plot for the stoichiometry of binding between C3 and Cu^{2+}

of the ligand and the metal $[C3-Cu^{2+} (10 \text{ ml})]$ was considered along with a continuous variable mole fraction of the guest. From the figure, it was observed that the maximum absorption intensity was attained with a mole fraction of 0.5 for C3. This fact confirmed that there was a 1:1 stoichiometry for the C3-Cu²⁺ complex. Similar 1:1 stoichiometry was also observed for the metal cations Ni, Co, and Zn.

3.6 | Fluorescence study

Fluorescence response is generally considered more sensitive than UV-vis absorption and therefore sensing and binding properties of the molecules were examined using fluorescence spectroscopy. Reported azetidinones are expected to have fluorescence properties as they contain the electron-rich conjugated aromatic system. As expected, they showed a significant change in their absorption spectra in the presence of certain divalent transition metal cations. The changes in the emission spectra of the ligands in the presence of those metal ions were also measured. Fluorescence spectra of C1 and C3 along with metal cations like Cu²⁺, Co^{2+,} and Zn²⁺ are represented in Figure 5.

Fluorescence titration of 0.01 M solution of C1 and C3 in methanol was carried out with metal cations Cu^{2+} , Co^{2+} , and Zn^{2+} . Upon titration of molecules C1 and C3 with various metal cations, significant changes in the spectral intensity were observed. Fluorescence titration spectra for the three metal cations are shown in Figure 6. Taking the example of Cu^{2+} , a decrease in emission intensity was observed for ligand C3 upon addition of Cu^{2+} ion solution. This dramatic quenching of fluorescence was monitored for the ligand C3 in the presence of Cu^{2+} at varying concentrations. Here, on increasing the metal ion concentration, a sharp quenching of the fluorescence was observed. This sharp quenching of fluorescence was attributed to the formation of a



FIGURE 5 Fluorescence spectra of (a) C1 and (b) C3 (0.01 M) with copper, cobalt, and zinc cation (0.01 M) in MeOH-H₂O (1/1, v/v) solutions



FIGURE 6 Fluorescence titration (10 and 20 μ l titration) spectra of C3 (0.01 M) with (a) copper, (b) cobalt, and (c) zinc cations (0.01 M) in MeOH-H₂O (1/1, v/v) (excitation wavelength was fixed at 360 nm)

complex between the ligand and metal ion. But the pattern of changes in fluorescence spectra of ligand C3 was different for cobalt (Co) and zinc (Zn) cations. The metal ions under study produced a varying degree of luminescence quenching effects in which the quenching effect was more prominent for the Cu²⁺ ion, indicating more effective binding of Cu²⁺ ion with the azetidinone fluorophore than the Co²⁺ and Zn²⁺ ions. However, Co²⁺ and Zn²⁺ also produced significant quenching effects. This fact implied that the azetidinone molecule can successfully be used for detection of metal cations.

Fluorescence titration revealed that the azetidinones are capable of detecting specific changes in fluorescence properties even at very low concentrations of metal ions. Therefore, these ligands can be efficiently used as fluorescent probes for detection of certain specific transition metal ions in the presence of other metal ions, implying the potential applicability of these species as fluorescence-enhanced detectors for heavy metals.

Here the fluorophore receptor conjugate, with a benzene ring as the fluorophore unit, serves as a chemosensor for detection of analyte. Strong and selective interaction between the metal ion analyte and the fluorophore receptor conjugate assumes unique coordination geometries which allows tuning of the photophysical properties. However, depending on the occupancies of the d-orbitals, the interaction will vary from one metal ion to another and this fact explains the selective nature of metal ion detection by the azetidinones. On the mechanistic front it can be ascertained that binding of the receptor unit with the metal ion through lone pairs of electrons attached to the two carbonyl groups, led to lowering of the energy for orbitals containing them. This might induce effectively one electron transfer from the receptor HOMO to the fluorophore HOMO, leading to fluorescence quenching. Therefore the process follows a static quenching mechanism. The resulting complexes formed had their own unique properties that influenced the observed quenching process.



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The quenching rate coefficient was determined using the Stern-Volmer relationship. It provides the kinetics of a photophysical intermolecular deactivation process. The Stern-Volmer equation is given below:

$$F_0/F = 1 + K_Q \cdot [Q]$$

where F_0 = intensity or rate of fluorescence without a quencher, F is the intensity or rate of fluorescence with the quencher, K_Q is the quencher rate coefficient and Q is the concentration of the quencher. The Stern–Volmer plot obtained for the fluorescence titration of C3 with copper (20 µl titration) is shown in Figure 7.

Quenching rate coefficients calculated for the metal cations were $2 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$, 1.06×10^3 and $7.39 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$, respectively, for copper, cobalt and zinc, indicating the presence of a static quenching process.

As a representative example, the interaction of C3 with different metal cations was compared and plotted in the form of a histogram, as shown in Figure 8. It is evident from the histogram that, comparatively, the interaction of the C3 molecule with Cu^{2+} cations produced more prominent changes in intensities for both wavelengths, followed by Zn^{2+} cations.



FIGURE 8 Histogram for comparison of photoluminescence titration spectra of C3 with copper, cobalt, and zinc cation

Metal	Standard deviation (Sa)	Slope (b)	Limit of detection	Limit of quantitation
Copper (Cu)	16.64	0.365	0.085 mg/L	0.289 mg/L
Cobalt (Co)	6.37	0.129	0.086 mg/L	0.463 mg/L
Zinc (Zn)	6.46	0.130	0.096 mg/L	0.314 mg/L

FIGURE 7 Stern–Volmer plot for C3 with (a) copper, (b) cobalt, and (c) zinc (10 and 20 μl titrations)

100

[Q]

(c)

150

200

50

0

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ΔE-4.81 eV



(b)

HOMO -13.20 eV

LUMO -8.39 eV Intensities were plotted in the histogram particularly at the 360 nm wavelength (at which the increase in the excitation intensity was observed) and at 462 nm (at which the decrease in the emission intensity was observed). The histogram illustrates that the responses of copper and zinc cations produce more prominent responses for photoluminescence sensing in the fluorescence spectra. In addition, a significantly higher intensity variation was observed in the emission at 462 nm for the Cu^{2+} cation. The preference of Cu^{2+} suggests that the large ionic radius of Cu^{2+} has a complementary size to the pseudocavity formed by the ligand for encapsulation in the binding sites. Therefore it can be put forward as an example of better binding of Cu^{2+} with the receptor according to its size rather than its acidity.

3.8 | Estimation of limit of detection (LOD) and limit of quantitation (LOQ) from photoluminescence titration

For a linear calibration curve, LOD and LOQ are expressed as:

 $LOD = 3 S_a/b$

and

 $LOQ = 10 S_a/b.$

where S_a is the standard deviation of the response and b is the slope of the calibration curve. The standard deviation of the response can be estimated by the standard deviation of either y-residuals or y-intercepts, of regression lines. LOD and LOQ data for heavy metals copper (Cu), cobalt (Co) and zinc (Zn) are given in Table 1.

For this calculation, fluorescence titrations of 10 μ l and 20 μ l were employed; 0.01 M metal solutions of Cu, Co, and Zn were continuously added to the ligand solution at a fixed concentration i.e. 0.01 M. Emission intensities were plotted against the molar concentration of the ligand. Linear fitting was performed with the plot to determine the slope.

LOD was found to be 0.085, 0.086 and 0.096 mg/L for copper, cobalt, and zinc, respectively. According to a standard published report, the detection limits of copper, cobalt, and zinc are 1 mg/L, 0.05 mg/L and 3.0 mg/L, respectively.^[43] Therefore comparison with the standard data suggests that azetidinones improved the LOD for these metal cations. Similarly, the LOQ data were also found to be well within the permissible range.

3.9 | DFT study

All calculations were performed using the DFT method with the 6-311G (d, p) basis set using the Gaussian 09w software package.^[44] HOMO-LUMO energies were studied at time-dependent TD-SCF based on the optimized structure. Molecular orbitals generally provide an excellent overview of the reactivity of the molecules. They also give an idea about the active sites by demonstrating the distribution of the frontier orbitals. Therefore for directing a wide range of chemical interactions, HOMO-LUMO frontier energy gap analysis was

performed. The HOMO-LUMO frontier orbital compositions for C1, C2, C3, and C3 complex (with Cu) are depicted in Figure 9(a, b).

All the structures showed conjugation and delocalization of charge over the entire framework. Zero charge density delocalization was observed on the azetidinone ring for all the structures (C1, C2, and C3). After formation of the complex, the charge density moved towards the azetidinone ring. This fact confirmed the coordination of the azetidinone ring with Cu^{2+} .

HOMO-LUMO energy gaps of C1, C2, and C3 were also calculated and were found to be 5.34, 5.34 and 5.46 eV, respectively. Conversely, the energy gap for the C3 complex with Cu²⁺ was found at 4.81 eV. In many studies, it has been reported that the HOMO-LUMO energy gap plays an important role as a stability descriptor. A larger energy gap is associated with a more stable compound. But in our case, the energy gap was less compared with the energy gap of the isolated ligand. This fact revealed that metal chelation reduced the stability of the independent medium. Another fact established from the observation was that charge transfer led to a sharp increase in the electronegativity of the metal that has undergone reduction, leading to a decrease in the energy gap.

4 | CONCLUSION

We developed inexpensive and single optical chemical sensors and/or metal ion receptors based on (R)-(–)-4-phenyl-2-oxazolidone for detection of toxic metal ions. These compounds produced characteristic absorption patterns in the UV-vis region upon interaction with Pb² ⁺, Zn²⁺, Co²⁺, or Cu²⁺ cations, which facilitated easy detection of these cations. Job's plot analysis revealed a 1:1 stoichiometry of the C3-M²⁺ complex that was further supported using DFT studies. Stern-Volmer studies showed that the binding process between C3 and metal ions exhibited a static quenching mechanism. Another important observation was that these compounds could detect metal ions when simultaneously present in a water sample at the ppm level. Therefore their low detection limit at the ppm level makes the compound a promising sensor for metal ions in wastewater and in nutritional samples.

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ORCID

Amrit Puzari D https://orcid.org/0000-0001-9485-6237

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SUPPORTING INFORMATION

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