

Research Article

Green Fluorescence Property of Chromone-based Hydrazone Towards Zn²⁺ Ion

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ABSTRACT

Excessive concentration of Zn in the environment may lead to bad toxicological responses that can affect human health and create numerous challenges in the environment. Therefore, it is essential to develop a sensitive method such as selective chemosensors based on fluorescence properties, which does not require laborious work in order to detect Zn metal ion. A formylchromone-thiosemicarbazide with two chloro derivatives namely, DFCTSC was synthesised from reflux reaction of 6,8-dichloroformylchromone (DCFC) and thiosemicarbazide (TSC) in ethanol. The characterisation of the ligand structure was determined by using spectroscopic techniques such as Ultraviolet-Visible spectroscopy (UV-Vis), Fourier Transform Infrared spectroscopy (FT-IR) and Nuclear Magnetic Resonance spectroscopy (NMR). Meanwhile, the fluorescence property of DFCTSC in the presence of Zn²⁺ ion was recorded using a fluorescence spectrophotometer. The result showed DFCTSC has a good fluorescence behaviour towards Zn²⁺ ion by giving turn-on green fluorescence at peak 500 nm and emitting light when it is observed under UV light. The behaviour displayed the ability of the ligand to be used as a potential fluorescent chemosensor for detecting Zn²⁺ ion.

Keywords: Chromone-based hydrazone, fluorescence property; chemosensor; Zn²⁺ ion

1. INTRODUCTION

Zn is the second most abundant transition metal ion in the human body and other mammals, and it plays a significant role in various physiological and pathological processes such as brain function, gene transcription, and in various biological systems including brain, intestine pancreas, and retina (Sun et al., 2014; Asayama et al., 2017). In general, a normal quantity of Zn is essential and beneficial for human health in our everyday life. Excessive concentration of Zn in the environment may lead to bad toxicological responses that can affect human health and create numerous challenges in the environment (Mitra et al., 2022). Toxicity

is exhibited with an overloading amount of Zn, causing some poisoning symptoms and various degenerative conditions, such as diabetes and Alzheimer's disease, Parkinson's disease, Menkes disease (Pezzi et al., 2017; Hernández-Camacho et al., 2020). Over the past ten years, Zn sensing has drawn more attention from the chemical and biological sciences. Among the numerous heavy metals in the environment, zinc can build up in agricultural soils and have adverse effects on crops and food waste (Rashid et al., 2023). Hence, it is critical to develop selective and practical sensors for determining the amount of zinc in the environment. The recommended dietary allowance (RDA) for zinc was 8 mg/day for females over the age of 19 and 11 mg/day for males, with the maximum concentration of zinc in water established by the Water Quality Criteria (WQC) at 120 ug/L (Zhang et al., 2018).

Numerous analytical methods have been developed for the detection of Zn, including atomic absorption spectrometry, inductively coupled plasma mass spectroscopy (Tomer et al., 2021), colorimetric analysis, fluorescent detection, electrochemistry, and spectrophotometry mainly including their expensive instruments, high maintenance cost and difficult sampling methods constraints their usage (Nam et al., 2023). For sensing metal ions in the aqueous phase, fluorescence analysis is one of the suggested analytical methods that has the advantages of high sensitivity, fast response time, specificity, and manageability (Lai et al., 2023). Fluorescence-responsive compounds have become more necessary for numerous technological applications, such as lighting and switching devices, as well as bio-imaging and analytical probes. Furthermore, an analytical probe for detection is an inexpensive, fast, and easy method that is essential for metal ion chemosensors and attracts interest from researchers (Shi et al., 2023).

In recent years, so many academic fields have been studied on chromone derivatives and hydrazine development of chemosensors of metal ions. The development of chemosensors for efficient detection of specific metal ions of their simple synthesis, outstanding ability to complex an emerging area in chemistry based on their potential analytical applications in many different fields including chemistry, and biology (Hazra & Roy, 2021). Flavonoids such as flavones, isoflavones, and flavonols have a core fragment called the chromone ring system (Singh & Malhotra, 2017). Chromone derivatives exhibit significant biological properties such as the ability to form metal chelates of chromone metal complexes, analytical reagents for transition elements, anti-HIV, and anti-inflammatory properties (Li et al., 2016; Salar et al., 2017), chromone derivatives have received a great deal of attention in recent years. Moreover, chromone derivatives are widely used in pharmacological and sensing applications in fluorescent and colorimetric chemosensors, with the ability to sense a variety of metal ions in chemistry and biological samples (Tomer et al., 2021). Therefore, the search for new chemosensor metal ions sensing is still an ongoing challenge. The coordination of Zn with chromone-based ligands can be simplified due to the presence of strong N and O donor atoms. It has become desirable to be studied on its interaction of M-N and M-O binding that enhance its photochemical properties. Until now, these reported sensors have been designed using a range of mechanisms such as Photoinduced Electron Transfer (PET), Metal-Ligand Charge Transfer (MLCT), Chelation-Enhanced Fluorescence (CHEF), Intramolecular Charge Transfer (ICT), C=N isomerisation and Excited-State Intramolecular Proton Transfer (ESIPT). By using this process, a chemosensor can be made with an 'off-on' mode that can change from non-fluorescence to fluorescence when metal ions are present (Tamil Selvan et al., 2018).

In this study, we report a chromone-based ligand as a turn-on fluorescent chemosensor for determining the presence of Zn^{2+} ion. The ability of the ligand to sense Zn^{2+} ion selectivity as well as the ability to detect coordination metal ions have been assessed.

2. MATERIALS AND METHODS

2.1. Apparatus

All starting materials and solvents for synthesis were purchased from Across and Aldrich and were used without further purification. The ligands of 6,8-dichloroformylchromone (DCF) and thiosemicarbazide (TSC) were synthesised as ligands according to the previous literature (Arifin et al., 2018). FT-IR spectra were recorded using a Perkin Elmer spectrophotometer in the 400–4000 cm⁻¹ range. Electronic spectra of the ligand in DMSO were obtained using the Cary 60 (Agilent) spectrophotometer. The JNM-ECX-500 (Joel) spectrometer was used to collect the ¹H-NMR spectra, and the solvent was dimethylsulphoxide-d₆. The fluorescence spectra of the ligand in DMSO were recorded using a Cary Eclipse (Agilent) spectrophotometer.

2.2. *Synthesis of ligand, DCFCTSC*

The ligand DCFCTSC (Figure 1) was synthesised by dissolving 1 mmol of 6,8-dichloroformylchromone (DCFC) in 15 mL ethanol and added gradually into 15 mL of an acidified ethanolic solution of 1 mmol thiosemicarbazide (TSC). The mixture was stirred for 24 hours. Then, the yellow precipitate was filtered off, washed several times with cold ethanol, and after that dried in a desiccator for 24 hours at room temperature. The ligand was characterised by CHNS, FTIR, UV-Vis, and ¹H NMR.

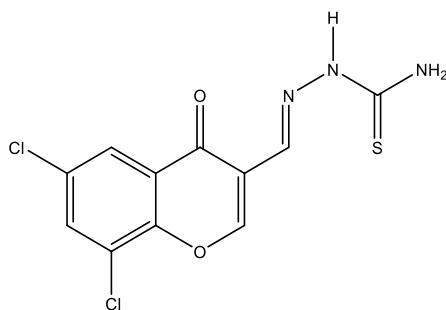


Figure 1. Structure of DCFCTSC

2.3. *Analysis of fluorescence properties*

The stock solution (1 mM) of metal acetate salts and DCFCTSC ligand were prepared in DMSO. The selectivity study was carried out by adding 15 µL of the probe solution (DCFCTSC) into the cuvette, followed by the appropriate aliquot of metal ion stock solution. The solution was diluted with the DMSO solution to 3 mL and needed a five-minute rest period. The slit widths for emission and excitation were set to 5 nm, meanwhile a wavelength of 371 nm was selected as the excitation during fluorescence analysis. At room temperature, the experiment was conducted.

3. RESULTS AND DISCUSSION

3.1. *Characterisation of ligand*

The structure of the ligand was explained by CHNS, FTIR, UV-Vis spectra, and ¹H NMR data. The experimental CHNS data were compared to their calculated assignments. Found (Calcd.) %; C:41.93 (41.79), H:1.93 (2.23), N: 12.75 (13.29), S:9.36 (10.14). The calculated values were in good agreement with the experimental values. Meanwhile, the FTIR spectrals of the ligand showed characteristic absorption bands at 3406 cm⁻¹ and 3214 cm⁻¹ which were assigned to ν(N-H) and ν(N-H₂), respectively. The absorption bands at 1645 cm⁻¹, 1605 cm⁻¹

and 1309 cm^{-1} were attributed to $\nu(\text{C=O})$, $\nu(\text{C=N})$ and $\nu(\text{C=S})$, respectively (Abdelrahman et al., 2021). DCFTSC showed high absorbance intensity peaks at 293 nm and 329 nm. The highest energy bands are assigned to $\pi-\pi^*$ transitions within the aromatic rings, while the moderate energy bands can be assigned to $n-\pi^*$ transitions within C=O, C=N and C=S groups (Table 1). Meanwhile, the resonances of ^1H NMR spectra of the ligands were recorded in dimethyl sulfoxide-d₆ (DMSO) solution. The N-H signal was observed as a single peak in the region $\delta = 11.491\text{ ppm}$. The other single peak appearing in the region 8.588 ppm was attributed to H-C=N. The chemical shift in the region $\delta = 7.363 - 7.969\text{ ppm}$ was observed and was assigned to the aromatic ring protons (Table 2).

3.2. *Fluorescence property*

The fluorescence property was investigated in order to obtain information on the fluorescent behaviour of DCFCTSC toward Zn^{2+} ion. For this purpose, 1.0 eq. ligand was reacted with 1.0 eq. Zn^{2+} ion in DMSO and diluted to 3 mL. The reaction between DCFCTSC and Zn^{2+} ion gives out an intense green fluorescence under UV light (Figure 2). Fluorescence spectra were obtained by exciting DCFCTSC at 371 nm. New emission peak observed at 500 nm causes a significant fluorescence emission.

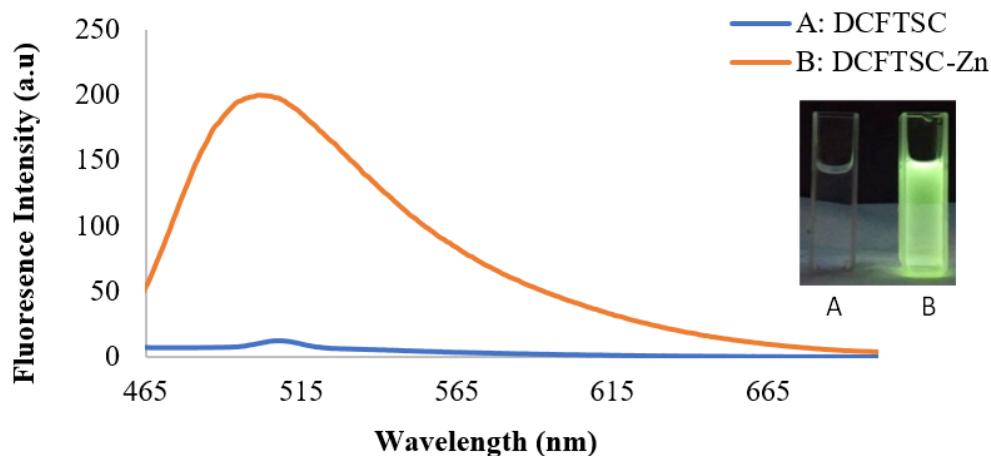


Figure 2. Fluorescence emission spectra of DCFCTSC with Zn^{2+} ion (1.0 eq.) at 500 nm ($\lambda_{\text{ex}}=371\text{ nm}$). Inset: colour of DCFCTSC (colourless) and DCFCTSC-Zn (green fluorescence) under UV

3.3. *Selectivity study*

Further study of the fluorescence properties of the ligand was conducted via selectivity studies. Selectivity is a vital factor that must be considered for the construction of fluorescence probes. In this experiment, 1.0 eq. DCFCTSC was reacted with 1.0 eq. other metal ions such as Fe^{2+} , Fe^{3+} , Cu^{2+} , Co^{2+} , Hg^{2+} , Mn^{2+} , Mg^{2+} , Na^+ , Ni^{2+} , Al^{3+} and Pb^{2+} ions in DMSO and diluted to 3 mL. Figure 3 shows the selectivity study of ligand DCFCTSC in the presence of several metals. Ligand DCFCTSC indicates an interesting behaviour towards Pb^{2+} , Mn^{2+} and Zn^{2+} ions by giving the highest intensity with emission peak at 500 nm compared to other metal ions. Although DCFTSC shows notable fluorescence behaviour towards the three (3) elements, this research focuses specifically on the fluorescence behaviour of DCFCTSC in the presence of Zn^{2+} ion. This focus aims to explore its potential as a selective fluorescent chemosensor for Zn^{2+} ion detection, laying the groundwork for its development as an alternative method for Zn^{2+} ion determination.

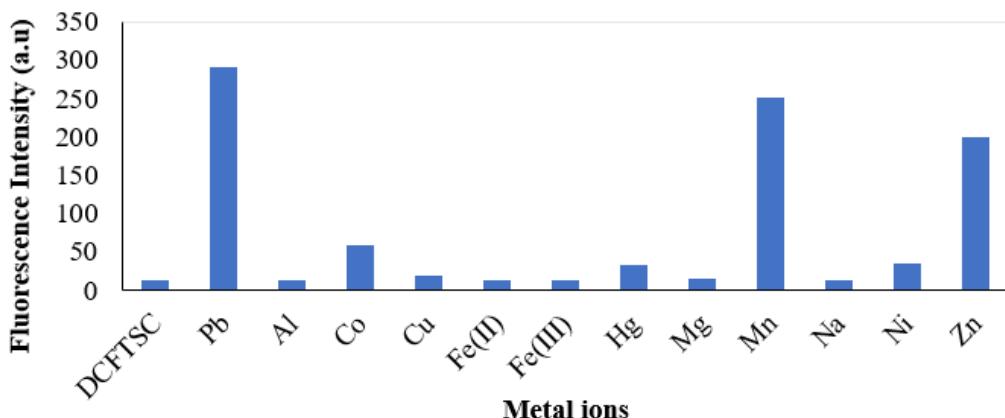


Figure 3. Fluorescence selectivity of DCFCTSC (1.0 mM) in DMSO with different metal ion at 500 nm ($\lambda_{ex}=371$ nm)

3.4. *Fluorescence titration*

The fluorescence titration of DCFCTSC towards Zn^{2+} ion was carefully examined to investigate the sensitivity of the turn-on fluorescence probe for Zn^{2+} ion detection by adding 0-1.0 eq. Zn^{2+} ion solution to the ligand solution of 25 μ L and diluted to 3 mL using DMSO. As Zn^{2+} ion was titrated, the intensity of fluorescence emission for the ligand was increased gradually (Figure 4). Figure 4 illustrates the value of R^2 of 0.9818 obtained in the fluorescence titration result of DCFCTSC and shows a good correlation in its trend line. Therefore, DCFCTSC has the potential to be developed as an alternative chemosensor due to the good selectivity towards Zn^{2+} ion. The coordination of DCFCTSC to Zn^{2+} ion through donor atoms was believed to enhance the fluorescence output via Chelation-Enhanced Fluorescence (CHEF) effect by interrupting the Photoinduced Electron-Transfer (PET) process, thus giving the “turn on” fluorescence signal in the presence of Zn^{2+} ion (Nootem et al., 2021). Therefore, binding studies were carried out for further investigation.

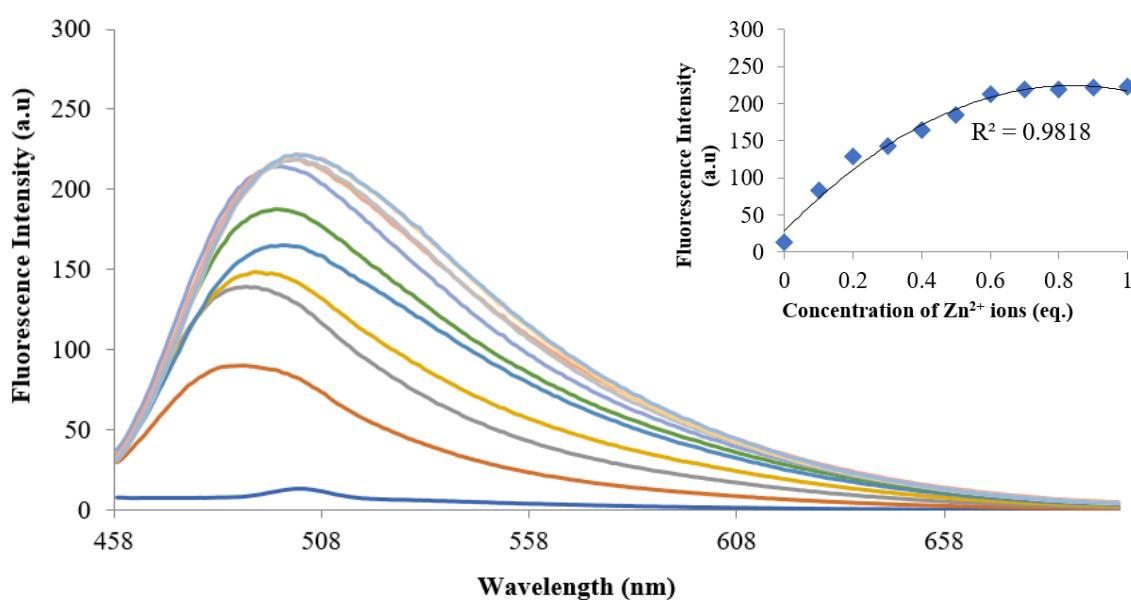


Figure 4. Fluorescence emission spectra of DCFCTSC (1.0×10^{-3} M) at 500 nm in DMSO with the

increasing amount of Zn^{2+} ion (0-1.0 eq.) and ($\lambda_{ex} = 371$ nm). Inset: Calibration curve of titration fluorescence spectra of DCFCTSC with Zn^{2+} ion (0-1.0 eq.)

3.5. Binding study

3.5.1. FTIR

The explanation of the ligand structure in the presence of Zn^{2+} ion was determined by the FTIR spectra in Table 1. The addition of Zn^{2+} ion caused the shifting of the peak attributed to $\nu(C=O)$ from 1645 cm^{-1} to 1642 cm^{-1} and $\nu(C=N)$ from 1605 cm^{-1} to lower energy of 1582 cm^{-1} which indicates the coordination of carbonyl oxygen of chromone and azomethine nitrogen with zinc (Selvamurugan et al., 2016). The shift in $\nu(C=S)$ 1309 cm^{-1} to 1169 cm^{-1} might indicate the coordination of zinc with thione-to-sulphur. Formation of new bands attributed to $\nu(M-O)$ and $\nu(M-N)$ at 651 cm^{-1} and 424 cm^{-1} , respectively, suggests the formation of metal zinc with carbonyl oxygen from chromone group and azomethine nitrogen (Kargar et al., 2021).

Table 1. IR Table for ligands and their complexes

Compound	IR Spectra (cm^{-1})					
	$\nu(N-H)$	$\nu(C=O)$	$\nu(C=N)$	$\nu(C=S)$	$\nu(M-O)$	$\nu(M-N)$
DCFCTSC	3406	1645	1605	1309	-	-
DCFCTSC-Zn	-	1642	1582	1169	651	424

3.5.2. UV-Vis

The UV-Visible absorption studies are well recognised for understanding the interaction of Zn^{2+} ion with the Schiff base ligands reacted with 1.0 eq. The electronic spectrum observed from the free ligand DCFCTSC exhibited two peaks at 289 nm and 324 nm at room temperature (Figure 5). The highest energy bands are assigned to $\pi-\pi^*$ transitions of the conjugated aromatic ring and $n-\pi^*$ transition of $C=O$, $C=N$ and $C=S$ groups in DCFCTSC ligand (Lee et al., 2021). However, the absorption band at 289 nm and 324 nm decreased in the presence of Zn^{2+} ion, while a new band attributed to charge transfer appeared at 371 nm. This is indicating that the coordination of the DCFCTSC ligand with Zn^{2+} ion lead to the formation of metal complexes, resulting in the fluorescence emission of the ligand (Wang et al., 2015).

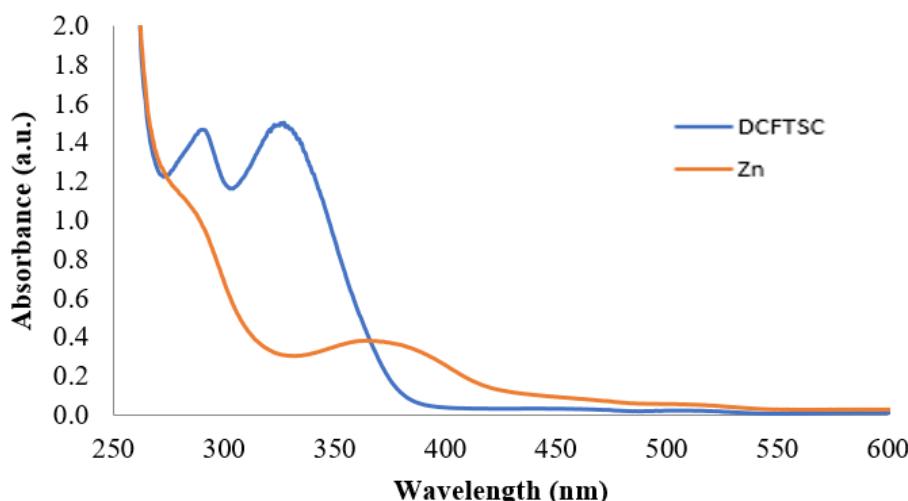


Figure 5. UV-Visible absorption spectra of DCFCTSC in the presence of Zn^{2+} ion

3.5.3. ^1H NMR spectra

^1H NMR spectrum of DCFCTSC in the presence of Zn^{2+} ion was recorded in DMSO-d_6 . In the ^1H NMR spectra the DCFCTSC signal at $\delta=11.491$ ppm, which corresponds to the (N-H) is disappeared in the spectra of DCFCTSC-Zn (Table 2). This clearly indicates that the Schiff base ligand is coordinated as an anionic ligand to Zn^{2+} ion through thiolic sulfur of the thiosemicarbazone side chain (Osman et al., 2021). In the presence of Zn^{2+} ion, there is a slight shift of the imine (H-C=N-) proton from 8.558 to the higher field 8.669 ppm in the obtained ^1H NMR spectra. This shift confirms the coordination of imine nitrogen to Zn^{2+} ion (Gaber et al., 2020).

Table 2. ^1H NMR spectral data of DCFCTSC ligand and the presence of Zn^{2+} ion

Compound	N-H(δ)	HC=N(δ)	H _{aromatic} (δ)
DCFCTSC	11.491	8.588	7.363-7.969
DCFCTSC-Zn	-	8.699	7.171-7.866

The binding studies indicate that the DCFCTSC acts as an ONS donor of tridentate ligand, which coordinates to the Zn^{2+} ion through M-N (imine nitrogen atom), M-O (carbonyl oxygen atom) and M-S (thiolic sulphur atom) as depicted in Figure 6. This coordination of DCFCTSC with the Zn^{2+} ion is thought to enhance the fluorescence output via the Chelation-Enhanced Fluorescence (CHEF) effect. This occurs by disrupting the Photoinduced Electron Transfer (PET) process, thereby producing a "turn-on" fluorescence signal which gives the colour change from colourless to green fluorescence in the presence of Zn^{2+} ions (Li et al., 2016; Nootem et al., 2021).

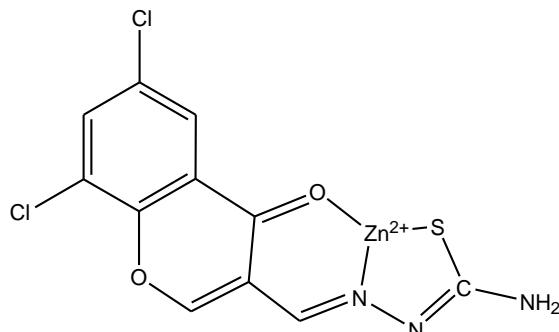


Figure 6. Proposed structure of DCFCTSC-Zn

4. CONCLUSION

In conclusion, we designed chromone-based hydrazone Schiff base which named as DCFCTSC were successfully synthesised by condensing 6,8-dichloro formylchromone (DCFC) and thiosemicarbazide (TSC). DCFCTSC displayed significant enhancement in fluorescence emission at 500 nm due to the inhibition of Photoinduced Electron-Transfer (PET) phenomenon. A turn-on fluorescent probe from colourless to green which contains a Schiff base as a metal binding site for Zn^{2+} ion. The coordination of DCFCTSC to the Zn^{2+} ion through M-N (imine nitrogen atom), M-O (carbonyl oxygen atom) and M-S (thiolic sulphur atom) is believed resulting the enhancement in its fluorescence signal (turn-on fluorescence) due to Chelation-Enhanced Fluorescence (CHEF). Thus, we believe that DCFCTSC has potential to be developed as an alternative fluorescent chemosensor for the determination of Zn^{2+} ion, which is of great concern, due to the release of this metal to the environment and human exposure is increasing day by day.

Declaration of Interest

The authors declare that there is no conflict of interest.

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