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Phenothiazine-pyridyl chalcone: an easily accessible colorimetric and fluorimetric ‘on–off’ dual sensing probe for Cu^{2+}

Sabir H. Mashraqui *, Tabrez Khan, Subramanian Sundaram, Shailesh Ghadigaonkar

Department of Chemistry, University of Mumbai, Vidyanagari, Santacruz (E), Mumbai 400 098, India

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Abstract

Phenothiazine-pyridyl chalcone, a new internal charge transfer (ICT) probe, has been designed that allows naked eye detection and a reversible, ratiometric ‘on–off’ sensing capability for biologically and environmentally significant Cu^{2+} in $CH_3OH–H_2O$ (4:1 v/v) under buffered conditions. The association constants determined using spectrophotometric and the fluorimetric techniques follow the order $Cu^{2+} > Ni^{2+} > Co^{2+} > Zn^{2+} > Cd^{2+} > Mg^{2+} \approx Ba^{2+} \approx Ca^{2+} \approx K^{+} \approx Na^{+} \approx Li^{+}$.

Cu^{2+} is one of the most significant metal ions in biological systems.[1] Copper is vital for haemoglobin synthesis, correct nerve functioning and bone development.[2] Copper is also implicated in lipid metabolism,[3a] inflammatory disorders[3b] and Alzheimer’s disease.[3c] Cu^{2+} proteins are involved in oxygen binding, electron transfer and the activation of small molecules.[4] Cu^{2+} in concentrations exceeding cellular needs can give rise to pathological conditions and it is also a significant environmental pollutant.[5] Consequently, strong interest exists in the development of selective Cu^{2+} sensors for biological and environmental applications. A variety of Cu^{2+} probes exhibiting either fluorescence ‘on–off’ or ‘off–on’ signalling modes have been developed.[6] However, colorimetric Cu^{2+} sensors offering ratiometric response are fewer comparatively.[7] Colorimetric probes are currently attracting increasing attention, since they can be tailored to allow ‘naked eye’ detection and ratiometric sensing[8] of the analyte.

In our quest to exploit intramolecular charge transfer (ICT) as a promising design strategy to assess selective metal ion sensors,[9] we have synthesized a phenothiazine-pyridyl chalcone derivative **3** as a new ICT chromophore and studied the effects of various metal ions on its photophysical properties. Structurally, receptor **3** is characterized by the presence of a phenothiazine ring as a fluorophore whereas the pyridyl ketone moiety is the metal chelating site. The synthesis of **3**[10] was readily achieved by condensing the known *N*-methyl-3-phenothiazine carboxaldehyde **1**[11] with 2-acetylpyridine **2** in 5% alcoholic NaOH at rt (Scheme 1). The design concept relies on the anticipation that metal ion coordination with **3** would provoke enhanced ICT from the donor phenothiazine ring to the acceptor, namely the metal-bound pyridyl ketone. The ensuing photophysical perturbations of host **3** could be used to harness its potential in metal sensing applications.

Metal ions of biological and environmental interest investigated for their photophysical effects on probe **3** include Li^{+}, Na^{+}, K^{+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+} and Cu^{2+}. Both spectrophotometric and fluorimetric techniques were used and titration experiments were uniformly carried out by adding the solutions of metal perchlorates in $CH_3OH–H_2O$ (4:1 v/v) to a solution of probe **3** in the same solvent system buffered by 10 mM Tris(hydroxymethyl) aminomethane (pH adjusted to 7.0 ± 0.1 by adding 0.1 N HCl). The absorption spectrum of **3** displayed a CT band at 434 nm with a molar extinction coefficient $\varepsilon_m = 2.13 \times 10^4\ M^{-1}\ cm^{-1}$. Titration of **3** with 100 equiv ($c = 2.83 \times 10^{-3}$ M) of the above set of metal

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* Corresponding author.
E-mail address: sh_mashraqui@yahoo.com (S. H. Mashraqui).

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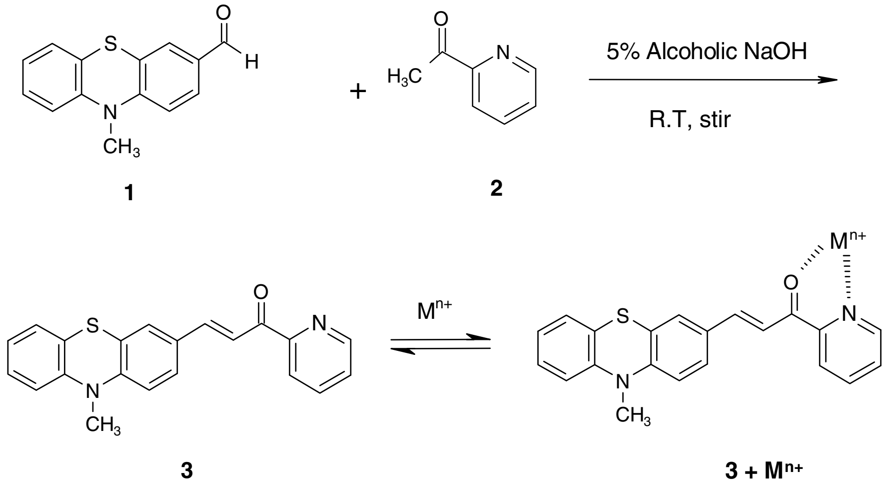

Scheme 1. Synthesis and proposed interaction of the CT probe **3** with metal ions, M^{n+}.

ions revealed dramatic changes only with Cu^{2+} both in the shape and in the maximum of the original CT band of **3**. Li^+, Na^+, K^+, Ba^{2+}, Ca^{2+}, Mg^{2+} and Cd^{2+} resulted in no detectable changes in the 434 nm absorption. However, Co^{2+}, Zn^{2+} and Ni^{2+} (Fig. 1) induced a marginal red shift of 2–4 nm, accompanied by a slight decrease in the band intensity by 5–20%. In addition, these cations also displayed a weak shoulder ($\varepsilon_m \leqslant 0.21 \times 10^4$ M^{-1} cm^{-1}) in the region 540–550 nm.

In contrast, incremental additions of Cu^{2+} (0–2.83×10^{-3} M) to a solution of **3** (2.83×10^{-5} M) resulted in a progressive decline in absorbance at 434 nm with a new red shifted ICT band, which increased in intensity at 560 nm. As depicted in Figure 2, at a limiting concentration of 2.83×10^{-3} M of Cu^{2+}, the original band of **3** was totally replaced by a 560 nm absorption band of comparable intensity ($\varepsilon_m = 2.11 \times 10^4$ M^{-1} cm^{-1}). A graphical representation of the steady changes in the UV–visible profile of **3** upon adding Cu^{2+} is depicted in the inset of Figure 2. Three isosbestic points are observed at $\lambda = 336$, 400 and 486 nm, indicating the formation of a well-defined complex between **3** and Cu^{2+}. From the Job plot,[12] we determined the stoichiometry of complexation to be 1:1 (see Supplementary data). It is clear that relative to the various metal ions examined, only Cu^{2+} elicited dramatic response in the UV–visible spectrum of **3**.

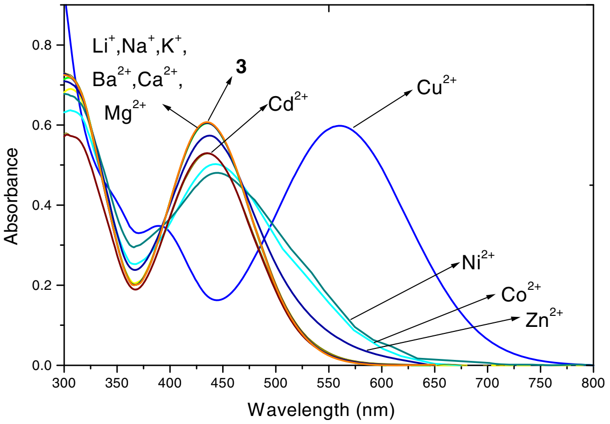

Fig. 1. Absorption spectra of **3** (2.83×10^{-5} M) in the presence of selected metal ions (2.83×10^{-3} M) in CH_3OH–H_2O (4:1 v/v) at pH 7.0 ± 0.1.

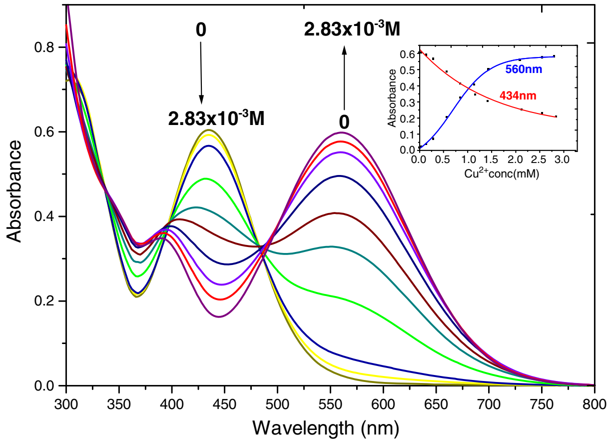

Fig. 2. Spectrophotometric titration of **3** (2.83×10^{-5} M) with Cu^{2+} (0–2.83×10^{-3} M). Inset: Absorbance plot of **3** against different concentrations of Cu^{2+} at λ_{max} at 434 and 560 nm.

From the spectrophotometric titrations, we calculated the detection limit[13] of Cu^{2+} to be 1.08×10^{-6} M (see Supplementary data), which compares favourably with the values reported for many Cu^{2+} selective sensors.[7] The red shift of 126 nm resulted in a colour change from deep yellow to blue, allowing for the ready visual detection of Cu^{2+} (see Supplementary data). With the exception of the anthraquinone based sensor reported by Kumar,[14] the red shift of 126 nm experienced by **3** is possibly one of the highest reported so far for Cu^{2+}.

Moreover, these spectrophotometric responses were fully reversible, since the addition of an aqueous solution of EDTA (2.83×10^{-4} M) led to an instant loss of the 560 nm band and the reappearance of the original 434 nm absorption of the free host (see Supplementary data). This experiment demonstrates that receptor **3** could serve as a reversible colorimetric molecular sensor for Cu^{2+} conforming to the requirements of naked eye detection and potential reutilization.

The red shift of 126 nm of **3** in the presence of Cu^{2+} indicates an enhanced charge transfer from the donor, phenothiazine ring to the metal-bound acceptor in the ground state. While the structure of $\mathbf{3}+Cu^{2+}$ complex is still to be chemically characterized, the proposed bidentate chelation illustrated in Scheme 1 is supported by the IR-band shift of the –CO group from 1667 cm^{-1} in **3** to 1595 cm^{-1} in the $\mathbf{3}+Cu^{2+}$ complex.

It can be seen that the changes observed in the absorption spectral profile of **3** at the two respective wavelengths of 434 and 560 nm are significantly more pronounced for Cu^{2+} compared to the other metal ions examined (Fig. 3). Thus, in addition to naked eye detection, the spectrophotometric signalling can also be employed for the ratiometric sensing of Cu^{2+}. Furthermore, to validate the utility of **3** as a selective ratiometric Cu^{2+} sensor, competitive experiments were performed by adding Cu^{2+} to a solution of **3** containing equivalent amounts of Li^{+}, Na^{+}, K^{+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+} and Ni^{2+}. No significant changes were observed in the A_{560}/A_{434} ratios in comparison with that observed with Cu^{2+} alone (see Supplementary data).

Excitation of **3** at its λ_{max} 434 nm produced a broad emission band at 640 nm with a Stokes shift of 206 nm. The quantum yield (Φ_f) of **3** was determined to be 0.0133 with reference to the integrated fluorescence spectra of neutral red at pH 7.0 in methanol.[15] As shown in Figure 4, the addition of the metal ions induced varying degrees of emission quenching depending on the identity of the metal ion. The addition of 100 equiv (2.83×10^{-3} M) of Li^{+}, Na^{+}, K^{+}, Ba^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}, Mg^{2+} Co^{2+} and Ni^{2+} resulted in ca. only 6–36% quenching of **3** (2.83×10^{-5} M). Indeed, no further quenching of **3** was detected on the addition of up to 3000 equiv of these cations. However, Cu^{2+} at just 100 equiv (2.83×10^{-3} M) with respect to probe **3** led to a strong fluorescence quenching of 80%.

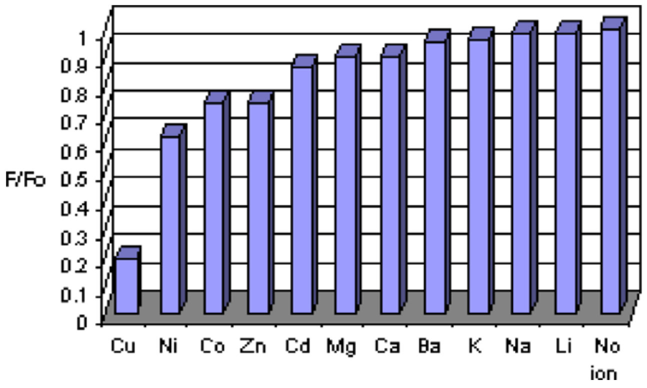

Fig. 4. Responses of various metal ions (2.83×10^{-3} M) on the fluorescence intensity of **3** (2.83×10^{-5} M).

As depicted in Figure 5, the fluorimetric titration of **3** (2.83×10^{-5} M) with Cu^{2+} showed a progressive decline in the fluorescence intensity at $\lambda_{em}=640$ nm until, at a limiting concentration of 3.0×10^{-3} M of Cu^{2+}, a plateau was reached with ca. 80% quenching. The emission profile of copper bound **3** remained practically unchanged in the company of 3 mM each of Ni^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, Mg^{2+}, Ba^{2+}, Ca^{2+}, K^{+}, Na^{+} and Li^{+} indicating the high selectivity of Cu^{2+} over these cations (see Supplementary data). A maximum perturbation of the absorption spectrum together with a prominent quenching by Cu^{2+} supports its strong affinity for probe **3** relative to the other metal ions tested.

Further, the complexation was also found to be fluorimetrically reversible since the addition of EDTA fully revived the original fluorescence of **3** (Fig. 6). Thus, **3** also has a potential ability as a selective 'on–off' sensor for the imaging of Cu^{2+} even in the presence of higher concentrations of other competing metal ions.

The stability constants ($\log K_s$) for metal ion complexations, determined using nonlinear curve fitting method,[16] together with Φ_f values are listed in Table 1. The binding interactions follow the order $Cu^{2+}>Ni^{2+}>Co^{2+}>$

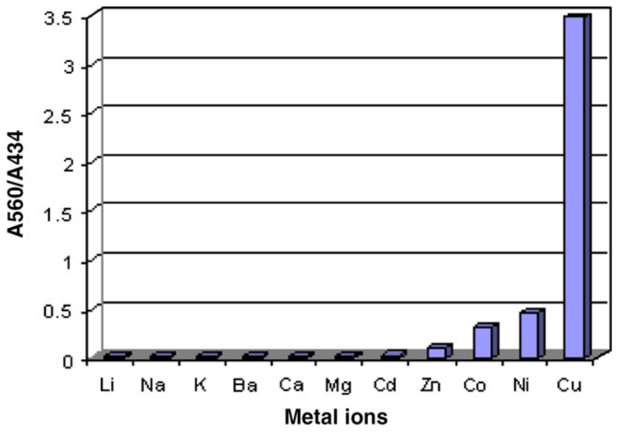

Fig. 3. Ratiometric response of **3** (2.83×10^{-5} M) towards various metal ions (2.83×10^{-3} M).

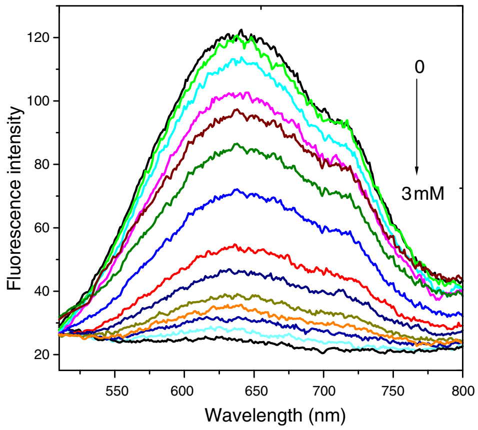

Fig. 5. Fluorometric titration of **3** (2.83×10^{-5} M) with Cu^{2+} (0–3.0×10^{-3} M), excitation at the isosbestic point, $\lambda_{ex}=400$ nm.

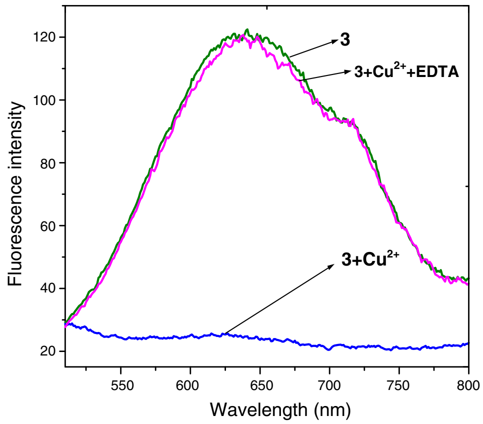

Fig. 6. Fluorescence spectra of **3**, **3** + Cu^{2+} and **3** + Cu^{2+} + EDTA.

Table 1
Relative quantum yields (Φ_f) and stability constants ($\log K_s$) of **3** in the presence of metal ions in MeOH–H_2O (4:1 v/v)

Compound	Φ_f	$\log K_s$ (absorption)	$\log K_s$ (fluorescence)
3	0.0133	—	—
3 ⊂ Li^+	0.0130	>0.1	>0.1
3 ⊂ Na^+	0.0129	>0.1	>0.1
3 ⊂ K^+	0.0128	>0.1	>0.1
3 ⊂ Ba^{2+}	0.0128	>0.1	>0.1
3 ⊂ Ca^{2+}	0.0126	>0.1	>0.1
3 ⊂ Mg^{2+}	0.0126	>0.1	>0.1
3 ⊂ Cd^{2+}	0.0122	0.42	0.36
3 ⊂ Zn^{2+}	0.0106	1.05	0.97
3 ⊂ Co^{2+}	0.0106	1.31	1.22
3 ⊂ Ni^{2+}	0.0092	1.62	1.55
3 ⊂ Cu^{2+}	0.0026	2.86	2.78

$Zn^{2+} > Cd^{2+} > Mg^{2+} \approx Ba^{2+} \approx Ca^{2+} \approx K^+ \approx Na^+ \approx Li^+$. Reasonable correlations between the spectrophotometrically and the fluorimetrically derived $\log K_s$ values imply that the binding interactions both in the ground and in the excited states are of comparable strength. The more than an order higher $\log K_s$ of Cu^{2+} signifies its superior binding interaction and selectivity compared to the coordinatively competing and biologically co-existing Co^{2+}, Zn^{2+}, Mg^{2+} and Ca^{2+} ions including toxic Ni^{2+} and Cd^{2+}.

In conclusion, we have designed a simple, easily accessible ICT probe **3** that undergoes reversible visual changes and fluorescence 'on–off' signalling for the detection of Cu^{2+}.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.04.041.

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10. *N*-Methyl phenothiazine-3-carboxaldehyde **1** (0.241 g, 1 mmol) and 2-acetylpyridine **2** (0.114 g, 1 mmol) were stirred overnight in 15 ml of 5% alcoholic NaOH at room temperature. The precipitated solid was filtered and washed with cold ethanol. Crystallization from $CHCl_3$ + pet ether (3:2 v/v) gave **3** as an orange crystalline solid in 70% yield (0.245 g). Mp 150–152 °C. IR (KBr, cm^{-1}): 1667, 1592, 1570, 1459, 1399, 1328, 1217, 1024, 795, 760. ^1H NMR (400 MHz, $CDCl_3$): δ 3.40 (s, 3H), 6.81 (t, 2H, $J = 9.36$ Hz), 6.96 (t, 1H, $J = 7.61$ Hz), 7.10 (m, 2H), 7.50 (m, 3H), 7.85 (m, 2H), 8.17 (m, 2H), 8.74 (d, 1H, $J = 4.29$ Hz). ^{13}C NMR ($CDCl_3$) 35.43, 113.76, 114.16, 114.32, 118.49, 122.46, 123.57, 126.48, 126.92, 127.13, 127.32, 129.26, 129.37, 136.78, 144.45, 143.55, 147.58, 148.48, 148.63, 154.12, 188.88. *m*/*z*: 345 $(M+H)^+$. Anal. Calcd for $C_{21}H_{16}N_2OS$: C, 73.26; H, 4.65; N, 8.14; S, 9.30. Found: C, 73.45; H, 4.85; N, 8.49; S, 9.51.
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