



3-(2-Pyridyl)-2-pyrazoline derivatives: novel fluorescent probes for Zn²⁺ ion

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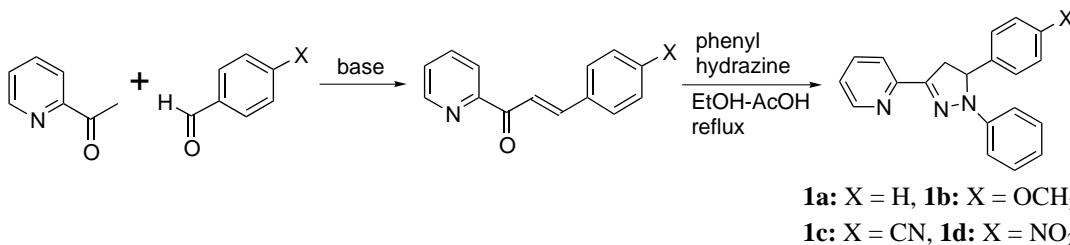
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Abstract—Spectroscopic studies revealed that 3-(2-pyridyl)-2-pyrazoline derivatives have rather strong affinity toward divalent transition metal ions, but have almost no interaction with alkali and alkaline-earth metal ions. In the case of the 5-(4-cyanophenyl) derivative, enhancement of the fluorescence intensity was observed upon addition of the Zn²⁺ ion, while most of other transition metal ions caused complete quenching. © 2001 Elsevier Science Ltd. All rights reserved.

1,3,5-Triaryl-2-pyrazolines are well-known fluorescent compounds with high quantum yields and are widely used as whitening or brightening reagents.¹ In addition, these compounds have been utilized as fluorescence probes in some elaborated chemosensors.² In most, such sensors, an acceptor (=ligand) and a fluorophore (=pyrazoline) are covalently linked to form PET systems. On the other hand, the fluorescent 3-(2-pyridyl) analogues³ of triarylpyrazolines themselves can serve as *N,N'*-type bidentate ligands for metal ions. In these intrinsic fluorescent ligands, the metal ion-binding may affect intramolecular charge transfer and consequently induce spectral changes both in absorbance and emission. The foregoing may be also applicable to the sensing of metal ions.⁴ However, to the best of our knowledge,

only a few examples⁵ have been reported on the interactions between pyridylpyrazoline derivatives and metal ions. In this study, we newly synthesized several 1,5-diphenyl-3-(2-pyridyl)-2-pyrazoline derivatives **1a–d** and investigated their complexation properties with metal ions. In these compounds, *para*-substituents on the 5-phenyl group were introduced in order to modulate the spectroscopic properties of the resulting metal complexes as well as those of ligands.

Pyridylpyrazoline derivatives **1a–d** were prepared from the corresponding chalcones according to the reported method⁶ with a slight modification (Scheme 1). Pure products were obtained in moderate yields after recrystallization from ethanol.[‡]



Scheme 1.

Keywords: chelation; fluorescence; pyrazolines; zinc.

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[‡] All new compounds gave satisfactory ¹H NMR, MS, ESI-MS, and elementary analysis data.

Binding affinities of pyridylpyrazoline derivatives **1a–d** toward divalent transition metal ions, Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+} were evaluated by UV–vis spectroscopy measurements. Upon addition of these metal ions, the absorption spectrum of each derivative changes in a similar manner as shown in Fig. 1 (**1c**). The addition of metal ions causes a decrease in optical density of the absorption maximum (360 nm for **1c**), which may correspond to the π – π^* transitions, with a peak broadening except for the Cu^{2+} ion. In this case, a new absorption band appears. These results indicate that derivatives **1a–d** have high binding affinity toward these metal ions. And these distinct changes in the spectra allow us to estimate the stability constants and stoichiometries of the metal complexes of **1a–d** by means of spectrophotometric titration. By contrast, the addition of alkali metal ions, Li^+ , Na^+ , K^+ and alkaline earth metal ions, Mg^{2+} , Ca^{2+} causes almost no change in the absorption spectra of **1a–d**. This observation implies rather weak interactions of **1a–d** with alkali and alkaline earth metal ions.

Stability constants for metal complexes of **1** were determined by nonlinear least-squares analysis of the spectrophotometric titration data assuming the formation of 1:1 (ML) and/or 1:2 (ML_2) complexes.⁷ As a result, for Co^{2+} , Ni^{2+} and Zn^{2+} ions, the existence of only one species, ML_2 describes the change in optical density. In the case of the Cu^{2+} ion, the coexistence of ML and ML_2 gives good agreement with the titration results. The results of ESI-MS measurements also support the

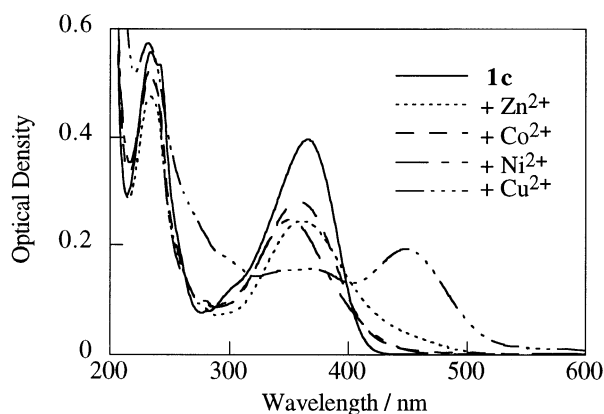


Figure 1. Absorption spectra of **1c** (20 μM) in the presence of various divalent transition metal ions ($[\text{M}]/[\text{L}]=50$) in acetonitrile.

formation of ML_2 complexes for Co^{2+} , Ni^{2+} , Zn^{2+} ions as well as for the Cu^{2+} ion.⁸ The obtained stability constants for the complexes of **1a** and **1c** with metal ions are depicted in Table 1. Generally, the Cu^{2+} ion makes complexes with N,N' -type ligands more tightly than other metal ions (Irving–Williams series).⁸ In the present case, however, overall stability constants for Cu^{2+} complexes (ML_2) are smaller than those for the corresponding Zn^{2+} complexes, respectively. This may be due to the inhibition of the formation of a square planar Cu^{2+} complex, which is energetically more favorable, by the 1-phenyl group of the pyrazoline ring of the ligands. The same phenomenon has been observed for Zinquin, a Zn^{2+} specific fluorophore having a sterically-hindered methyl group.⁹

The fluorescence spectra of **1a–c** change significantly upon addition of divalent transition metal ions, while **1d** is not emissive itself by the presence of a strong electron-withdrawing group (NO_2), which induces intramolecular electron transfer.¹⁰ In the cases of Co^{2+} , Cu^{2+} , Ni^{2+} ions, typical metal-induced fluorescence quenching⁴ is observed for **1a–c**. In addition, for **1a** and **1b**, the fluorescence is also quenched by the Zn^{2+} ion though it is not complete. On the other hand, for **1c**, upon addition of the Zn^{2+} ion, the emission maximum at 479 nm decreases in intensity with the concomitant appearance of a new emission band at around 568 nm. The new band can be assigned to the emission of the

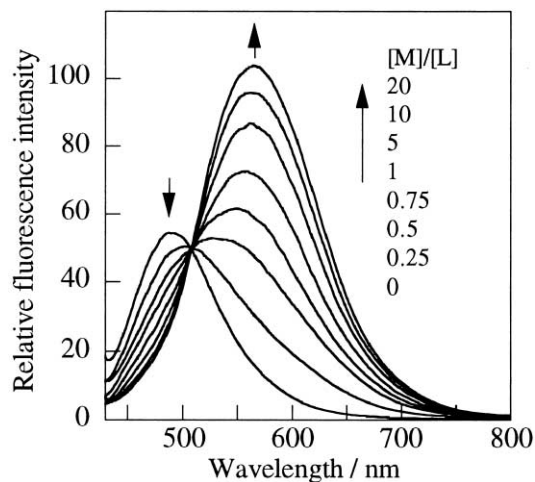


Figure 2. Fluorescence spectra of **1c** (20 μM) in the presence of increasing Zn^{2+} concentrations in acetonitrile. Excitation wavelength was 360 nm.

Table 1. Stability constants (K_s) for the metal complexes of **1a,c** in acetonitrile^a

	Zn^{2+}	Ni^{2+}	Co^{2+}	Cu^{2+}	
1a	3.4×10^{11}	5.1×10^9	4.3×10^{10}	2.9×10^{4b}	7.9×10^{4c}
1c	2.9×10^{11}	4.4×10^{10}	1.3×10^9	3.8×10^{4b}	4.1×10^{5c}

^a $\text{M}+2\text{L} \xrightleftharpoons{K_s} \text{ML}_2$.

^b $\text{M}+\text{L} \xrightleftharpoons{K_s} \text{ML}$.

^c $\text{ML}+\text{L} \xrightleftharpoons{K_s} \text{ML}_2$.

[§] For example, ZnL_2 ($\text{L}=\mathbf{1c}$) complex: ESI-MS (m/z): $[\text{M}-\text{ClO}_4]^+$ calcd. for $\text{C}_{42}\text{H}_{32}\text{N}_8\text{ZnClO}_4$, 813.6; found 813.1.

Table 2. Relative fluorescence emission intensities of **1c** at 568 nm in the presence of various metal ions in acetonitrile^a

Metal ion	None	Zn ²⁺	Ni ²⁺	Co ²⁺	Cu ²⁺	Cd ²⁺	Li ⁺	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺
Intensity	15	100	2	2	2	39	16	15	15	16	15

^a The concentration of **1c** was 20 μM . The concentrations of metal ions were 400 μM . The excitation wavelength was 360 nm.

Zn²⁺ complex because its intensity increases with the increase of Zn²⁺ concentration (Fig. 2). Fluorescence quantum yields of free ligands **1a** and **1c** and their Zn²⁺ complexes were calculated according to the known method¹¹ using the value of 1,3,5-triphenyl-2-pyrazoline¹⁰ as a standard. In the case of **1a**, the corresponding Zn²⁺ complex shows considerably low value ($\Phi_f=0.027$) compared with that of the ligand ($\Phi_f=0.830$). Therefore, it is rather difficult to observe the emission originated from the Zn²⁺ complex. On the other hand, for **1c**, the electron-withdrawing group (CN) on the 5-phenyl group decreases the quantum yield of **1c** as in the case of **1d**. On complexation with Zn²⁺, this electron transfer effect may be relieved by the increased charge transfer from the 1-phenyl to the 3-pyridyl group. Consequently, quantum yields of the ligand ($\Phi_f=0.037$) and the complex ($\Phi_f=0.057$) become comparable. This makes it possible to detect the emission from the Zn²⁺ complex without interference from that of the free ligand **1c**.

Although the results of UV–vis measurements indicate that the interactions of **1a–d** with alkali and alkaline earth metal ions are weak, the effects of these ions on the fluorescence of **1** were investigated for **1c**. The fluorescence spectrum of **1c** (20 μM) does not change by the presence of 4 mM of Li⁺, Ca²⁺, Mg²⁺ although higher concentrations (>50 mM) induce an increase in the fluorescent intensity (5–10%) with the red shift (10–20 nm) of the emission maximum. In addition, high concentrations (0.1 M) of Na⁺ and K⁺ ions have no effect on the fluorescence of **1c**. These results suggest that the fluorescences of **1c** and its Zn²⁺ complex are unaffected by the presence of large excess amounts of biologically important metal ions, Li⁺, Na⁺, K⁺, Mg²⁺ and Ca²⁺. Furthermore, the addition of Cd²⁺, which often behaves like Zn²⁺,⁴ increases the fluorescence intensity of **1c** somewhat, showing good selectivity for Zn²⁺ over Cd²⁺. The effects of added metal ions on the fluorescent intensity of **1c** are summarized in Table 2.

In summary, pyridylpyrazoline derivatives, especially **1c**, show specific fluorescent behavior toward the Zn²⁺ ion among divalent transition metal ions, while no interactions exist between **1a–d** and alkali and alkaline earth metal ions. These findings indicate that pyridylpyrazolines **1a–d** are potential compounds for developing efficient fluorescent Zn²⁺ chemosensors,

which are of current interest.^{4,12} In order to improve the Zn²⁺-selectivity and provide good water-solubility to **1**, further studies are underway.

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