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COMMUNICATION

# Simple pyrazoline and pyrazole "turn on" fluorescent sensors selective for $Cd^{2+}$ and $Zn^{2+}$ in MeCN

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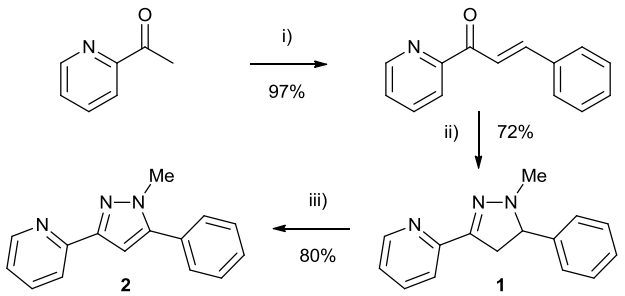
**An efficient two-step synthesis of pyrazoline ligand 1 is described which is an effective "turn on" fluorescent sensor for $Cd^{2+}$ in MeCN. Oxidation to the corresponding pyrazole ligand 2 creates a "turn on" fluorescent sensor now selective for $Zn^{2+}$ and able to distinguish it from $Cd^{2+}$.**

Cadmium is a toxic element widely distributed throughout the environment originating from natural sources and as a pollutant from industrial and agricultural use.[1] Cadmium can accumulate in the body over a period of years and has been linked to various diseases including cancer, even at low-levels of exposure.[1-2] Therefore the facile detection and monitoring of cadmium in various media is of great importance.[3]

Zinc is chemically similar to cadmium due to its position in the periodic table, yet it has a vital role in many biological processes.[4] The detection of zinc in biological systems has become a rapidly emerging field as excess free zinc is toxic.[5] Many $Cd^{2+}$ and $Zn^{2+}$ probes are based on fluorescent sensors which upon chelation to the cation result in a decrease ("turn off") or increase ("turn on") in fluorescence intensity. A large variety of complex and sophisticated $Zn^{2+}$ fluorescent sensors have been reported.[5-6] Various $Cd^{2+}$ sensors have also been reported,[3, 6l, 7] some of which are able to distinguish cadmium from zinc ions.[6l, 7c-f] We report herein a simple two-step synthesis of a "turn on" fluorescent sensor selective for $Cd^{2+}$ which can be readily oxidised in high yield to afford a "turn on" fluorescent sensor selective for $Zn^{2+}$ and able to differentiate it from $Cd^{2+}$.

The ligands are based on the pyrazoline motif as it has been previously reported to possess favourable photophysical properties[8] and chelate a variety of metals,[6a-c, 9] including $Zn^{2+}$.[6a-c, 9a, 9b] It is an attractive and versatile scaffold due to the ease of synthesis from a large range of commercially available acetophenones and benzaldehydes.

Pyrazoline **1** and related pyrazole ligand **2** were synthesised as outlined in Scheme 1. Following previous literature precedent,[10] 2-acetylpyridine underwent Claisen-Schmidt condensation with benzaldehyde in the presence of catalytic NaOH to afford the corresponding chalcone in 97% yield. Treatment of the chalcone with an excess of methylhydrazine gave the pyrazoline ligand **1** as the sole product in 72% yield. Similar transformations suggest the reaction proceeds *via* 1,2-addition followed by cyclisation and not initial 1,4-addition, which could generate a different isomer.[8c] Following a procedure for the oxidation of a 1,2,3,4-tetrahydro-$\beta$-carboline,[11] pyrazoline **1** was readily oxidised with Pd/C to afford




**Scheme 1** Synthesis of pyrazoline **1** and pyrazole **2**. i) PhCHO, 10% NaOH(aq), 24h, ii) $H_2NNHMe$, EtOH, 3h, iii) 10 mol% Pd/C, 200 °C, 4h

pyrazole **2** in 80% yield. This simple synthesis is highly scalable as it uses common commercially available starting materials and all the products are highly crystalline.

The pyrazoline ligand **1** has been previously reported complexed with ruthenium, although no other metals were described.[9f] Similarly, the synthesis of pyrazole **2** was recently reported by an alternative route although no chelation properties were reported.[12] We now describe the investigation of pyrazoline **1** and pyrazole **2** with various cations‡ analysed by UV/Vis, NMR and fluorescence spectroscopy.

UV/Vis spectroscopy was performed in MeCN, as similar ligands were investigated in this solvent,[6a] and in the presence of Group 1 and 2 metals produced negligible results with both ligands. Upon the addition of various transition metals, however, both ligands **1** and **2** showed spectral changes consistent with chelation. The effect of $Zn^{2+}$ and $Cd^{2+}$ with pyrazoline **1** is given in Figure 1 and is representative of the various metals examined with ligands **1** and **2** (the complete list is available in the Electronic Supplementary Information). The absorbance at 320 nm ($\varepsilon$ = 14800 $M^{-1}cm^{-1}$) is due to the pyrazoline ligand **1** only and disappears upon the addition of $Zn^{2+}$ or $Cd^{2+}$. The formation of a new band at 360 nm with $Zn^{2+}$ ($\varepsilon$ = 8650 $M^{-1}cm^{-1}$, Figure 1A) and 350 nm with $Cd^{2+}$ ($\varepsilon$ = 7650 $M^{-1}cm^{-1}$, Figure 1B) is evident, which increased proportionally up to 1.0 equivalent of the metal ion and levelled off thereafter, suggesting a 1:1 ratio of the metal to ligand. Job plot analysis also suggests the formation of a 1:1 complex with a 0.5 molar ratio of pyrazoline **1** and $Zn^{2+}$ or $Cd^{2+}$ (Figure 1).

This is consistent with a crystal structure of a similar pyrazoline ligand in a 2:2 complex with $Zn^{2+}$, with chloride atoms acting as bridging ligands.[9a] Attempts to obtain a crystalline

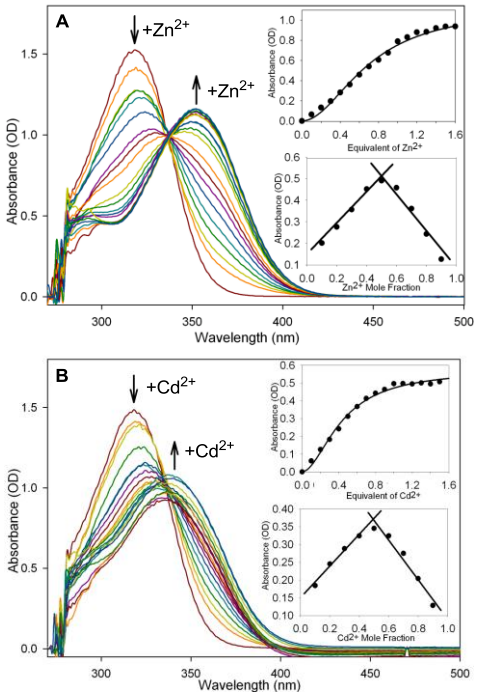



**Figure 1** Absorbance spectra of pyrazoline **1** (MeCN, 500 $\mu$M) with the addition of 0-1.5 equiv. (0.1 increments) of $Zn^{2+}$ (**A**) and $Cd^{2+}$ (**B**). Insets at $\lambda_{em}$ = 370 nm. Lower inset Job plot

complex of pyrazoline **1** with $Zn^{2+}$ using the previously reported conditions (EtOH/$H_2O$, reflux, 24 h),[9a] instead resulted in a $Zn^{2+}$ complex with the *pyrazole* ligand **2** (Figure 2). This is presumably the result of aerobic and/or zinc mediated oxidation during the harsh recystalisation process. Interestingly, the asymmetric unit was seen to contain four independent Zn(pyrazole)$Cl_2$ motifs. Three of these are 1:1 monomers but the fourth, located proximate to a crystallographic inversion centre, forms a 2:2 dimer, with bridging chloride atoms (ESI, Figure 2 only shows one of the monomeric 1:1 structures for clarity).

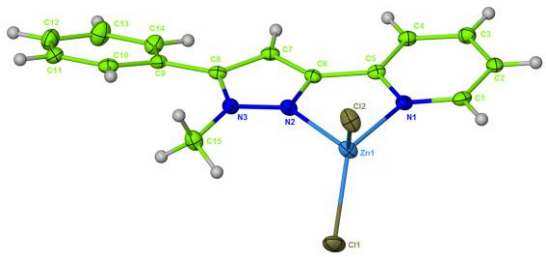


**Figure 2** Ortep-3 representation of one of the three 1:1 monomers containing pyrazole ligand **2** and $Zn^{2+}$ in the asymmetric unit of the crystal structure, with the ellipsoids represented at 30% probability[13]

The interaction of the pyrazoline **1** and pyrazole **2** ligands with $Zn^{2+}$ and $Cd^{2+}$ was also analysed by $^1$H NMR spectroscopy. The addition of $Cd^{2+}$ to pyrazole **2** is representative of the results obtained and is shown in Figure 3 (complete study shown in ESI). Significant chemical shifts of the pyridine and pyrazole protons were observed upon the addition of the metal, broadening and moving downfield as previously reported for other sensors upon chelation to $Zn^{2+}$ [6g-k, 7c] and $Cd^{2+}$ [6j, 6l, 7a-c] (Figure 3).

Pyrazoline **1** and pyrazole **2** were examined by fluorescence

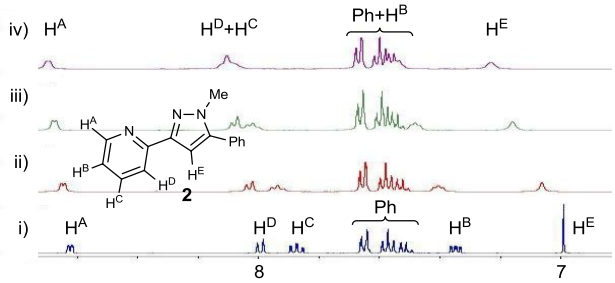



**Figure 3** Partial $^1$H NMR spectra of i) pyrazole **2** (DMSO-$d_6$, 63 mM) with ii) 0.9, iii) 2.0 and iv) 3.0 equiv. $Cd^{2+}$

spectroscopy in MeCN at 20 $\mu$M, as previously reported with similar ligands.[6a] No change in fluorescence was observed with various Group 1, 2 and transition metals, while exposure to $Zn^{2+}$ or $Cd^{2+}$ produced a large increase in fluorescence for both ligands **1** and **2** (Figure 4 and ESI). This result is particularly pleasing as it demonstrates that although there is no selectivity in the absorbance spectroscopy of either ligand with various metals, they are however only fluorescent in the presence of either $Zn^{2+}$ or $Cd^{2+}$. Moreover, with pyrazole **2** they fluoresce at different wavelengths providing a "turn on" fluorescent sensor that can distinguish between $Zn^{2+}$ and $Cd^{2+}$ in MeCN. The effect of different solvents on fluorescence was investigated with ligand **1** and **2** with $Zn^{2+}$ and gave variable results, with complete fluorescence quenching observed in the presence of water (ESI).

The addition of 5 equivalents of $Zn^{2+}$ to pyrazoline **1** resulted in an 8 fold increase in fluorescence at 460 nm, whereas 5 equivalents of $Cd^{2+}$ gave a 14 fold increase in fluorescence also at 460 nm with a Stokes shift of 100 nm (Figure 4A and 5). As previously observed in the UV/Vis study, Job plot analysis is consistent with a 1:1 ratio of metal to ligand for both cations

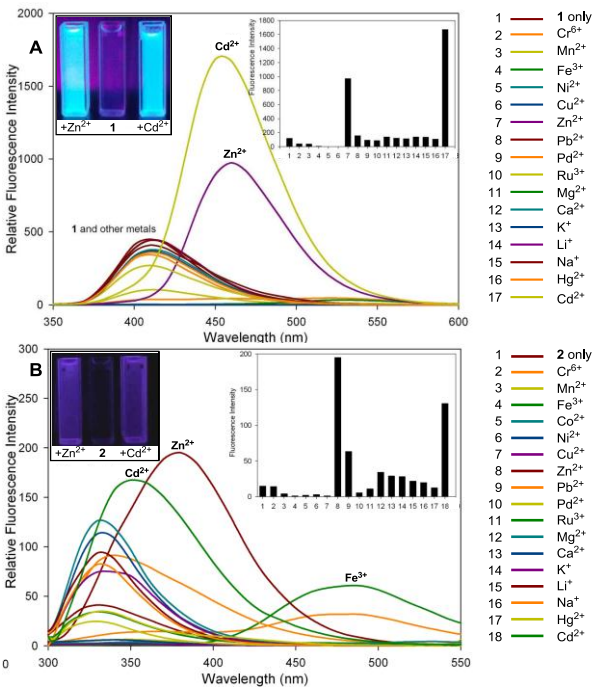



**Figure 4** Fluorescence spectra of pyrazoline **1** (**A**, $\lambda_{ex}$=320 nm) and pyrazole **2** (**B**, $\lambda_{ex}$=285 nm, MeCN, 20 $\mu$M), upon addition of 5 equiv. of metal. Metal screen at $\lambda_{em}$=460 nm (**A**) and 380 nm (**B**). Inset shows fluorescence with $Zn^{2+}$ and $Cd^{2+}$ at 313 nm (**A**) and 254 nm (**B**)

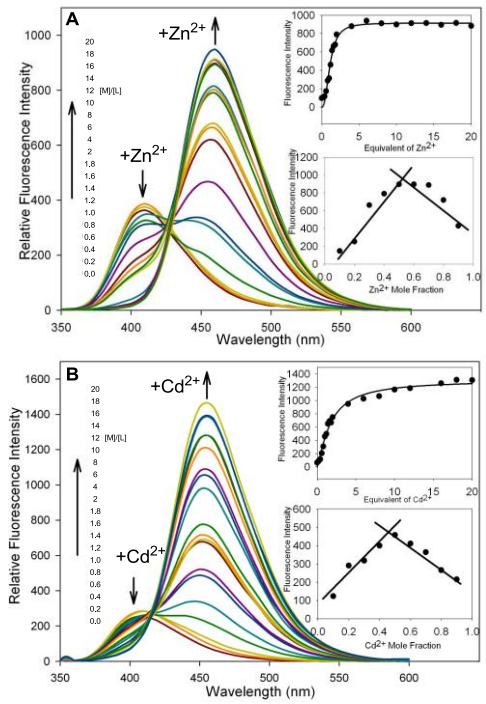



**Figure 5** Fluorescence spectra of pyrazoline **1** (MeCN, 20 $\mu$M, $\lambda_{ex}$=320 nm) upon addition of 0-20 equiv. $Zn^{2+}$ (**A**) and $Cd^{2+}$ (**B**). Insets at $\lambda_{em}$=460 nm upon addition of cation. Lower inset Job plot

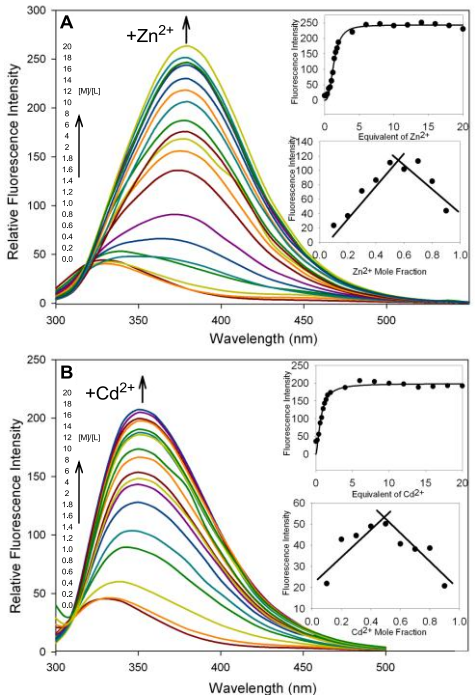



**Figure 6** Fluorescence spectra of pyrazole **2** (MeCN, 20 $\mu$M, $\lambda_{ex}$=285 nm) upon addition of 0-20 equiv. $Zn^{2+}$ (**A**) and $Cd^{2+}$ (**B**). Insets at $\lambda_{em}$=380 nm (**A**) and 350 nm (**B**) upon addition of cation. Lower inset Job plot

(Figure 5 insets). Similar pyrazolines have been previously reported as ligands[9a, 9b] or sensors[6a-c] for $Zn^{2+}$. The data presented here however shows that the pyrazoline ligand **1** is more sensitive towards $Cd^{2+}$ than $Zn^{2+}$ and could be a useful "turn on" fluorescent sensor for cadmium.

Conversely, the related pyrazole ligand **2** displays increased fluorescence in the presence of $Zn^{2+}$ than $Cd^{2+}$ (Figure 4B and 6). A 13 fold increase in fluorescence at 380 nm is observed with $Zn^{2+}$ with a Stokes shift of 90 nm, whereas $Cd^{2+}$ only exhibits a 5 fold increase in fluorescence at 350 nm with a Stokes shift of 60 nm. Titration with $Zn^{2+}$ and $Cd^{2+}$ is again consistent with the 1:1 stoichiometry previously observed by the UV/Vis analysis and X-ray crystallography of pyrazole **2** with $Zn^{2+}$ (Figure 6 insets). Although it is challenging to selectively distinguish $Zn^{2+}$ from $Cd^{2+}$ ions due to similar physical properties, the 30 nm difference between the $Zn^{2+}$ (380 nm) and $Cd^{2+}$ (350 nm) emission maxima enables the pyrazole ligand **2** to distinguish these cations. In addition, the pyrazole ligand **2** exhibits increased sensitivity for $Zn^{2+}$ providing an effective "turn on" fluorescent sensor for $Zn^{2+}$.

Following previous reports,[6c, 14] the detection limits of $Zn^{2+}$ and $Cd^{2+}$ by the ligands **1** and **2** were calculated and show that the pyrazoline **1** has a detection limit of $Zn^{2+}$ 0.20 $\mu$M and $Cd^{2+}$ 0.12 $\mu$M (ESI). Pyrazole **2** has a detection limit of $Zn^{2+}$ 0.24 $\mu$M and $Cd^{2+}$ 0.34 $\mu$M, again highlighting that pyrazoline **1** is a more effective fluorescent sensor for $Cd^{2+}$ and pyrazole **2** a more effective sensor for $Zn^{2+}$.

Competition assays were performed with ligands **1** and **2** to investigate the effect of detecting $Zn^{2+}$ or $Cd^{2+}$ in the presence of other cations (Figure 7 and ESI). The addition of $Mn^{2+}$, $Pb^{2+}$, $Ru^{3+}$, $Mg^{2+}$, $Ca^{2+}$ and $Hg^{2+}$ to a mixture of pyrazoline **1** and $Cd^{2+}$ results in only minor decreases in fluorescence (Figure 7). The ability to detect $Cd^{2+}$ even in the presence of heavy metals such as $Pb^{2+}$ and $Hg^{2+}$ is of particular interest.

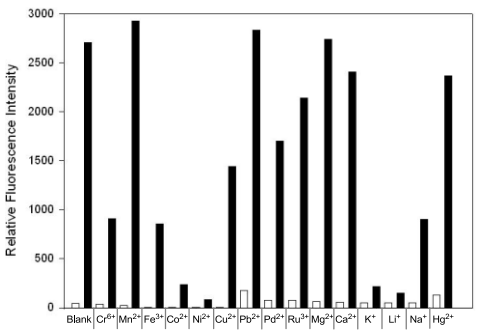



**Figure 7** Competition experiments. The white bar represents pyrazoline **1** (MeCN, 20 $\mu$M, $\lambda_{ex}$=350 nm, $\lambda_{em}$=460 nm) and 5 equiv. of the cation; the black bar is the same plus 5 equiv. $Cd^{2+}$ after equilibrating for 3 min.

Significant fluorescence quenching was observed upon the addition of the paramagnetic metals such as $Fe^{3+}$, $Co^{2+}$ and $Ni^{2+}$ as previously reported with other sensors.[6c-f, 7c] A similar trend was observed for competition experiments with pyrazoline **1** with $Zn^{2+}$, although fluorescence quenching was more pronounced (ESI). Combined with the increased sensitivity for $Cd^{2+}$ over $Zn^{2+}$ (Figure 3), these results suggest that the pyrazoline ligand **1** is more suitable as a $Cd^{2+}$ fluorescent sensor.

Competition assays were also performed with pyrazole **2** in the presence of either $Zn^{2+}$ or $Cd^{2+}$ (ESI). In both cases large variations were observed, although heavy metals such as $Hg^{2+}$ and $Pb^{2+}$ had little or no effect on fluorescence in competition with $Zn^{2+}$ in the presence of pyrazole **2**.

In summary, pyrazoline **1** and pyrazole **2** were prepared in an efficient synthesis and are effective “turn on” fluorescent sensors in MeCN for $Cd^{2+}$ and $Zn^{2+}$ respectively. In addition, the pyrazole

ligand **2** can distinguish between these ions in MeCN and therefore complements other sensors which can differentiate these ions in aqueous media.[6j-l, 7c-f] The ligands are also able to detect $Zn^{2+}$ or $Cd^{2+}$ in the presence of other heavy metals, which is of great importance for industrial applications. Although these simple ligands do not operate in aqueous media and suffer from competition with some biological ions, the modular design and molecular framework of the ligands allows for further functionalisation to fine-tune desirable physicochemical properties, which will be investigated and reported in due course.

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## Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental procedures, characterisation data, $^1$H NMR and $^{13}$C NMR spectra are provided. Data from UV/Vis and fluorescence spectroscopy, NMR titration experiments, competition experiments, X-ray crystallography, calculations of detection limits and extintion co-efficients are also provided. See DOI: 10.1039/b000000x/

‡ The metal complexes used in this study were all metal chlorides (*i.e.* $MCl_x$), except LiBr, $CrO_3$, $Pd(OAc)_2$, $Hg(OAc)_2$ and $Cd(OAc)_2.2H_2O$. The complete list is provided in the ESI.

§ Crystal data for pyrazole **2** complexed with $ZnCl_2$, CCDC 885728. Formula: $C_{60}H_{52}Cl_8N_{12}Zn_4$. $M$ = 1486.22, monoclinic. Unit cell parameters: $a$ = 14.2600(2) Å, $b$ = 26.1400(4) Å, $c$ = 16.8220(2) Å. $\alpha$ = 90°, $\beta$ = 101.476(1)°, $\gamma$ = 90°, $V$ = 6145.15(15) Å$^3$, $T$ = 150(2) K, space group $P2_1/c$, $Z$ = 4, 99 814 reflections collected, 14032 independent reflections [R(int) = 0.0737]. Final R indices [I>2$\sigma$(*I*)] $R_1$ = 0.0452 and $wR_2$ = 0.1015. R indices (all data) $R_1$ = 0.0782 and $wR_2$ = 0.1156.

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