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A Highly Selective Fluorescence Turn-on Probe for Zn^{2+} Based on New Diaryloxadiazole Chelate

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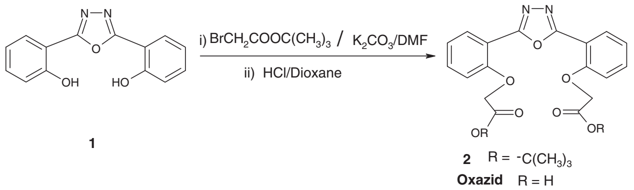
A new chelating diaryloxadiazole probe, oxazid displayed emission red shift and 16-fold emission enhancement upon binding with Zn^{2+} in buffered condition, while competing Cd^{2+}, Ca^{2+}, Mg^{2+}, and several potentially quenching metal ions posed no significant optical interferences. Experimental evidences indicated 1:1 complexation, and the computational work revealed formation of a stable octahedral geometry.

Metal-ion-triggered optical modulation of photoemittive receptors constitutes one of the key approaches for the recognition of chemically and biologically significant metal ions.[1] Zinc, an essential micronutrient, regulates many cellular and enzymatic processes.[2] However, Zn^{2+} metabolic disorder is associated with many debilitating neurological conditions.[3] Consequently, a heightened interest persists in designing Zn^{2+}-selective optical probes.[4] Among the known Zn^{2+} luminophores, some prominent examples include TSQ, RhodaZin-3, ZinPyr-1, Rhodafluor-2, and ZnAF-1.[5] These probes are constructed by hosting nitrogenous zinc chelators, such as dipicolylamine, iminodiacid or cyclen onto the photoemittive fluorescein, rhodamine, or quinoline platforms.[5] Despite continuing advances, the cross affinities, especially from biologically abundant Ca^{2+} and Mg^{2+} and toxic Cd^{2+}, frequent emission quenching observed from Co^{2+}, Ni^{2+}, and Cu^{2+}, and the need for nonaqueous measurements forms limitations in many cases. Thus, it was of interest to a explore new class of fluorescent chelators which might exhibit selective discrimination of Zn^{2+} over competing metal ions, thereby enabling useful sensing properties.

Diaryloxadiazole motif is a stable fluorophore, which others[6] and we[7] have previously incorporated as a reporter group to design certain luminescent metal-ion sensors. With intent to extending the range of the reporter ligands for Zn^{2+}, we now describe a new diaryloxadiazole chemoreceptor, oxazid, carrying oxyacetic acid chelates for the selective "off–on" signaling for Zn^{2+} under semiaqueous buffer conditions.

Synthesis of oxazid involved O-alkylation of 2,5-di(2-hydroxyphenyl)-1,3,4-oxadiazole (**1**) with *t*-butyl bromoacetate in K_2CO_3/DMF to form diester **2**, which upon acid-catalyzed hydrolysis afforded the target, oxazid[8] (Scheme 1). With regard to the sensing mechanism, we presumed that metal-ion binding might induce the chelation-enhanced fluorescence due to the conversion of the conformationally flexible probe into the conformationally restricted metal ion complex.[9]

The fluorescence sensitivities of oxazid toward selected metal ions of biological and/or environmental relevance were evaluated in $MeCN–H_2O$ (7:3 v/v, buffered by 10 mM Tris-HCl, pH 7.2)[10] Excitation of oxazid at 314 nm resulted in a weak emission band at 352 nm with quantum yield Φ_f of 0.017 measured with respect to anthracene. The low Φ_f may be attributable in part to the nonradiative aryl-ring-rotation processes in free probe. The emission profile of oxazid (1×10^{-6} M) was not significantly perturbed when 500 equiv of Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+} as their perchlorates were introduced.[9] In contrast, addition of just 100 equiv of Zn^{2+} induced marked changes both in the intensity and energy of the probe's emission.



Scheme 1. Synthetic sequence toward diaryloxadiazole receptor, oxazid.

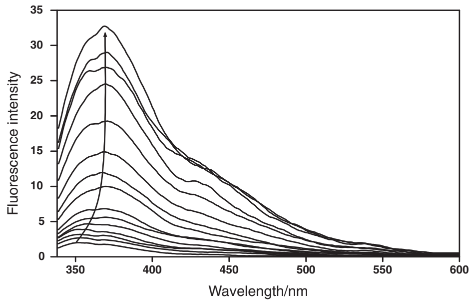


Figure 1. Fluorimetric titration of oxazid (1×10^{-6} M) with incremental Zn^{2+} ($0–10^{-4}$ M) in $MeCN–H_2O$ (7:3 v/v, buffered by 10 mM Tris-HCl).

Fluorimetric titration of oxazid (Figure 1) revealed linear enhancement in emission intensity with incremental addition of Zn^{2+}. At saturating 10^{-4} M of Zn^{2+}, a high 16-fold emission enhancement ($\Phi_f = 0.264$), accompanied with 17 nm red shift was observed. Figure 2a shows that relative to other metal ions examined, only Zn^{2+} is capable of inducing high fluorescence "off–on" signaling response. To further demonstrate the selective interaction with Zn^{2+}, initially the fluorescence of the probe (1×10^{-6} M) in the presence of a matrix consisting of 5×10^{-4} M each of Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+} was recorded. As shown in Figure 2b, the matrix barely impacted the fluorescence intensity (<1.5-fold enhancement) of the probe. Subsequently, addition of just 10^{-4} M of Zn^{2+} induced emission enhancement by ca. 14-fold. This finding clearly validates the selective binding of Zn^{2+} even though other metals may be present in excess

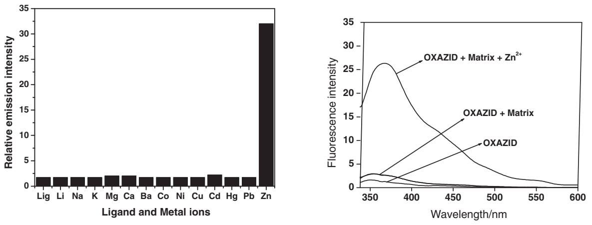


Figure 2. a) Relative emission intensity of oxazid (1×10^{-6} M) with metal ions (100 equiv), b) competitive matrix experiment: emission spectra of oxazid alone (1 µg), oxazid + 5×10^{-4} M each of Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, and Hg^{2+} and oxazid + matrix + Zn^{2+} (10^{-4} M).

concentrations. The Job plot indicated 1:1 binding stoichiometry, and the stability constant, $\log K$ for Zn^{2+}, was determined to be 3.72.[8] For other metal ions tested, the emission changes were relatively insignificant to allow a reliable measure of their $\log K_S$. The detection limit of Zn^{2+} determined from the fluorescence data was found to be 5.75×10^{-7} M.[8]

The shift of the IR band from 1694 cm^{-1} in the free receptor to 1600 cm^{-1} in the oxazid–zinc complex confirms the coordination of carboxylic acids with Zn^{2+}.[8] The ^1H NMR spectrum of the probe displayed a singlet for $–OCH_2CO_2–$ at δ 4.76, but in the Zn^{2+} complex, this signal, while retaining its singlet character, was upfield shifted to δ 4.41. In addition, relative to the free probe, the aryl ring protons in the zinc complex were also upfield shifted in the range of δ 0.13 to 0.22.[8] These NMR data together with Job's plot imply that both the $–OCH_2CO_2H$ groups are probably bound to a single, centrally located Zn^{2+} in a 1:1 complexation mode.

To theoretically study the energetic and geometric features of the oxazid–Zn^{2+} complex, calculations were performed using DFT (RI-BP86/def2-TZVP) within the program TURBOMOLE[11a] version 5.10 taking into account solvent effects (water) using the COSMO approximation.[11b] Depending on the orientations of the oxadiazole ring, several possible geometries of 1:1 complexation were analyzed. However, we found only one computationally suitable conformation which had the oxygen atom of the oxadiazole ring pointing to the Zn^{2+} (**1**·ZnA, Figure 3). Inclusion of an explicit water molecule in the inner coordination sphere of the Zn^{2+} (**1**·ZnB) afforded extra 5.4 kcal mol^{-1} stabilization.[12] The ability of the probe to form stable octahedral complex could account for the selective zinc coordination.

Understandably, Cu^{2+}, Co^{2+}, and Ni^{2+}, which predominately form either the square-planar or tetrahedral coordination, fail to interact. Moreover, in the Zn^{2+} octahedral complex, the rotations around aryl–oxadiazole bonds would be severely restricted. This phenomena might contribute to emission amplification by enhancing the radiative channels over thermal quenching processes prevailing in the conformationally flexible probe.[8]

In conclusion, we have reported a new, structurally simple diaryloxadiazole chelate which affords high fluorescence turn-on response for targeting Zn^{2+}. Noteworthily, many potentially interfering or quenching metal ions displayed no significant optical perturbations even in relatively higher concentrations. The present probe, carrying wholly "oxygen"-based ligand system should be a welcome new addition to the repertoire of the potentially useful zinc fluorescence sensors.

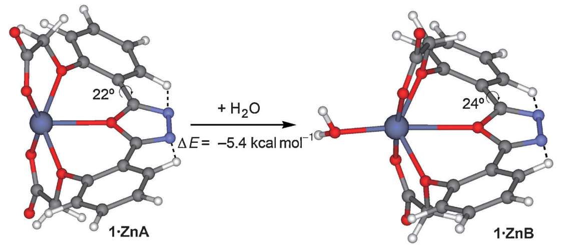


Figure 3. Optimized structures of oxazid–Zn^{2+} complex and their difference in energies.

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