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Two-Photon Fluorescent Probes of Biological Zn(II) Derived from 7-Hydroxyquinoline

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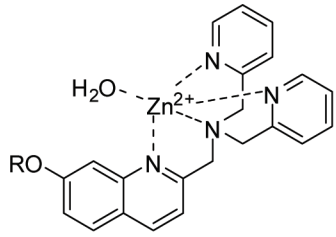
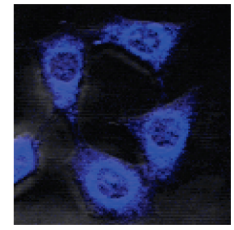
ABSTRACT





A new fluorescent probe for monitoring Zn^{2+} was synthesized based on the structure of 7-hydroxyquinoline. Compared with 8-substituted quinolines, the new probe exhibited higher selectivity for Zn^{2+} over Cd^{2+}. Its fluorescence enhancement (14-fold) and nanomolar range sensitivity (K_d = 0.117 nM) were favorable toward biological applications. Experiments also showed that a cell-permeable derivative of the new probe was potentially useful for two-photon imaging in living cells.

Zn^{2+} is involved in many biochemical processes, such as neurotransmission,[1] cellular metabolism,[2] enzyme regulation,[3] and gene expression.[4] Biological imaging of Zn^{2+} ions can provide direct information on their spatiotemporal distributions in living systems.[5] In recent years, two-photon microscopy (TPM) has gained much interest in biology because this method leads to less phototoxity, better three-dimensional spatial localization, and greater penetration into scattering or absorbing tissues.[6] TPM is considered to be a good noninvasive means of fluorescence microscopy in tissue explants and living animals.[7] The application of the two-photon method to zinc physiology requires the parallel development of novel zinc-specfic fluorescent probes that operate within living cells. Although various Zn^{2+} probes have been designed based on fluorescein,[8] coumarin,[9] quinoline,[10] and other fluorophores, only a few of them have been used for two-photon imaging.[11] The development of novel

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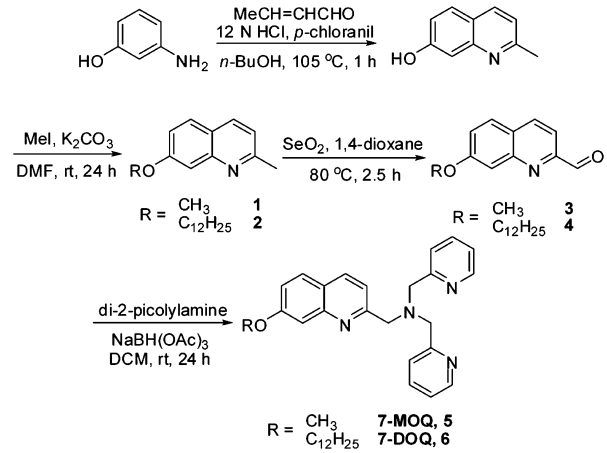
two-photon excitied Zn^{2+} fluorescent probes remains an interesting and important challenge.

It is known that an efficient Zn^{2+} probe for biological applications should have sufficient water solubility, high selectivity, high photostability, and long excitation wavelength to avoid cell damage.[12] Previously TSQ and Zinquin have been found to be good Zn^{2+} probes based on the quinoline structure.[13] To meet the demand of high selectivity toward Zn^{2+}, a strong chelator, i.e., di-2-picolylamine moiety,[15] was incorporated into quinoline.[16] However, the ultraviolet excitation wavelength (~350 nm) of these quinoline-based probes might damage living cells.[14] This problem may be solved with the TPM technique, which employs two lower-energy, near-infrared photons for excitation.

We notice that most Zn^{2+} probes based on the quinoline structure poccess an oxygen or nitrogen atom at the 8-position of quinoline, which participates in the coordiantion with Zn^{2+}.[13,16] It poses an interesting question as to whether the sensing affinity and selectivity for Zn^{2+} will be altered if the substitution position changes. This change may also affect the efficiency of molecular internal charge transfer (ICT) of the chemosensor.[17] Thus, we design and synthesize a novel quinoline-derived Zn^{2+} probe, **7-MOQ**, which carries a methoxy group at the 7-position of quinoline. We anticipate that the change of the substitution position of the electron-donating group may alter the ICT process and ion selectivity.

The synthesis of **7-MOQ** is shown in Scheme 1 (details are available in the Supporting Information). The X-ray crystal structure of the zinc complex with **7-MOQ** (Figure 1) demonstrates that the oxygen atom at the 7-position does not participate in coordination with Zn^{2+}, which differs from the previously reported 8-substituted quinolines.[13,16] The nitrogen atoms of the di-2-picolylamine moiety and quinoline ring participate in zinc coordinations. One H_2O molecule is also found to chelate to Zn^{2+} to complete the five-coordination geometry.

Scheme 1. Synthesis of **7-MOQ**



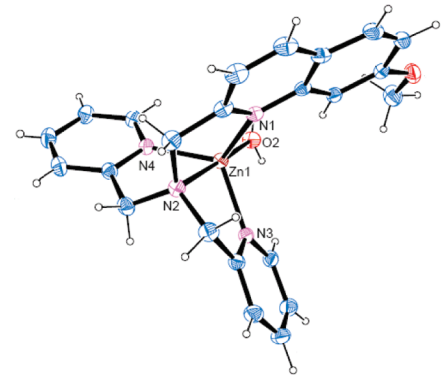


Figure 1. X-ray crystal structure of the zinc complex of **7-MOQ**.

The ^1H NMR spectra of **7-MOQ** (Figure 2) indicate that upon coordination to Zn^{2+} in DMSO-d_6 the protons at the *ortho*-position of pyridines shift downfield from 8.50 to 8.72 ppm. The proton at the 3-position of quinoline also shifts downfield, suggesting the interaction between the fluorophore and Zn^{2+}.

The spectroscopic properties of **7-MOQ** were measured in aqueous buffer (25 mM HEPES, 0.1 M $NaClO_4$, 5% v/v

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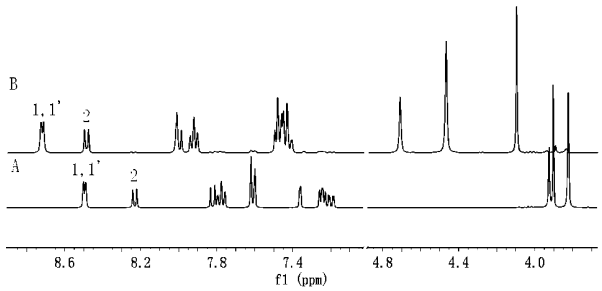

Figure 2. ^{1}H NMR spectra of the **7-MOQ** (A) and **7-MOQ** with 1.0 equiv of Zn^{2+} in DMSO-d_6 (B).

DMSO, pH 7.4, 25 °C). The UV−vis absorption spectrum of **7-MOQ** exhibits a maximum absoption at 236 nm (Figure S1, Supporting Information). As shown in Figure 3, free

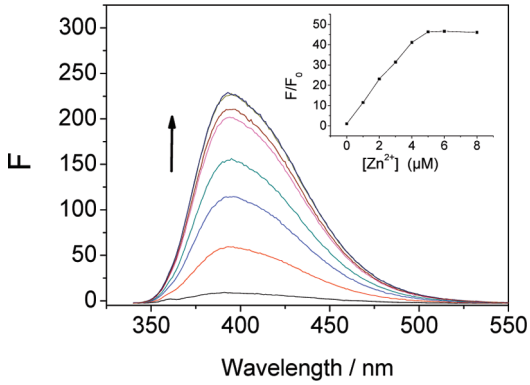

Figure 3. Fluorescence responses (λ_{ex} = 320 nm) of 5 μM **7-MOQ** upon the addition of Zn^{2+} (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.6 equiv) in the HEPES buffer (25 mM HEPES, 0.1 M $NaClO_4$, 5% (v/v) DMSO, pH = 7.4, I = 0.1).

7-MOQ showed weak fluorescence emisssion at 393 nm upon excitation at 320 nm ($\varepsilon = 3.09 \times 10^4$ M^{-1} cm^{-1}, Φ_0 = 0.039). Upon addition of Zn^{2+} (0−1.6 equiv), the emission intensity increases significantly. This enhancement can be explained by the blocking of the photoinduced electron-transfer (PET)[18] pathway of **7-MOQ** due to the binding with Zn^{2+}.

The maximum fluorescence emission intensity was obtained when the Zn^{2+} concentration increased to 1.0 equiv (Φ_{Zn} = 0.556). A Job's plot (Figure S3, Supporting Information) also indicates the 1:1 binding model between **7-MOQ** and Zn^{2+}. It is important to point out that the fluorescence enhancement (14-fold) of **7-MOQ** is higher than the previously reported 8- methoxylated Zn^{2+} sensors (4−6-fold). The apparent dissociation constant (K_d) of **7-MOQ** for Zn^{2+} is calculated to be 0.117 nM (Figure S5, Table S1, Supporting Information). This value indicates that the probe can be used in the subnanomolar range, which affords sufficient sensitivity for application in living cells.[19]

To check the pH effect on the fluoresence response, the fluorescence spectra of **7-MOQ** with saturating Zn^{2+} under different pH conditions were examined (Figure S4, Supporting Information). It is found that the fluorescence intensity remains constant for pH > 5. Therefore, the probe can be used to monitor intracellular Zn^{2+} without being affected by the physiological pH change.

The titration of **7-MOQ** with different metal cations was conducted to examine the selectivity of the probe (Figure 4). Because Ca^{2+}, Mg^{2+}, and K^+ are abundant in the biological systems, they were tested at a concentration as high as 1 mM. To our delight, these cations do not produce an appreciable change in the fluorescence emission. Therefore, the probe can be used under biological conditions even with an increase of Ca^{2+} concentration. The change of fluorescence emission intensity was also not observed upon the addition of Mn^{2+} and Pb^{2+}. Other first row transition metal ions including Fe^{2+}, Co^{2+}, and Cu^{2+} might form complexes with **7-MOQ** and quench the fluorescence, but their influence in vivo can be neglected due to their low concentration. Only Zn^{2+} and Cd^{2+} induce the enhancement of fluorescence emission intensity. The interference of Cd^{2+} is a well-known problem of zinc fluorescence probes.[8,10,16] Compared to the previous probes, **7-MOQ** exhibits a much less pronounced interference by Cd^{2+}.

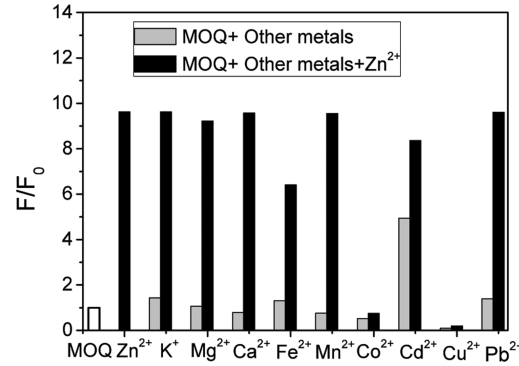

Figure 4. Fluorescence responses of **7-MOQ** upon additions of various metal ions. Experimental conditions: 5 μM **7-MOQ**, 1.0 mM for Mg^{2+}, Ca^{2+}, and K^+, and 5 μM for Mn^{2+}, Fe^{2+}, Co^{2+}, Pb^{2+}, Cu^{2+}, and Cd^{2+}, λ_{ex} = 320 nm.

According to the above experiments, the methoxy group at the 7-position does not participate in the coordination with the zinc ion. To enhance the cell permeability the methoxy

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group was converted into dodecyloxy (**7-DOQ**). A preliminary study of **7-DOQ** in living cells (A431) was carried out by using confocal microscopy (Figure 5). Two-photon

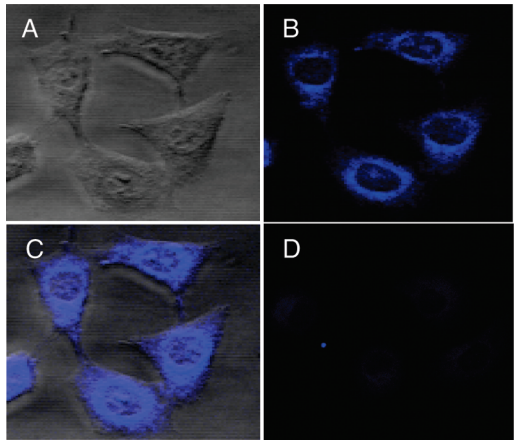

Figure 5. (A) Bright-field image of A431 cells labled with 30 μM **7-DOQ** after 30 min of incubation, λ_{ex} = 800 nm. (B) TP image after a 30 min treatment with zinc(II)/pyrithione (50 μM, 1:1 ratio). (C) The overlay of (A) and (B). (D) TP image of cells that are further incubated with 50 μM TPEN for 10 min.

microscopy (TPM) imaging of the **7-DOQ**-labled cell displays a very weak intracellular staining after incubation at 37 °C for 30 min. The system then exhibits a strong blue fluorescence with the addition of 1:1 zinc(II)/pyrithione (50 μM). Subsequent treatment with the metal ion chelator TPEN (50 μM) reverses the fluorescence intensity to the baseline level. These results indicate that **7-DOQ** is cell permeable and suitable for TPM imaging of intracellular Zn^{2+} flux.

Cytotoxicity is a potential side effect of the probe that must be controlled when dealing with living cells. To this end, we have conducted MTT assays in HeLa cells. The cell viability remains 90% after treatment with **7-DOQ** (30 μM) for 24 h. This cytotoxicity test indicates that low-micromolar concentrations of **7-DOQ** are essentially nontoxic for at least 24 h incubation and can be safely used for two-photon bioimaging.

In conclusion, a novel 7-substituted, quinoline-based fluorescent probe is designed and synthesized. It displays a high selectivity and sensitvity for Zn^{2+} in a neutral buffer aqueous solution. Because the oxygen atom at the 7-position does not participate in the coordination with zinc ion, the new probe shows different properties as compared to the previously reported 8-substituted, quinoline-based Zn^{2+} sensors. The new probe exhibits a 14-fold fluorescence enhancement in response to Zn^{2+}. It has a dissociation constant of 0.117 nM with Zn^{2+} and a higher selectivity toward Zn^{2+} over Cd^{2+}. Furthermore, we demonstrate that a cell-permeable derivative of the new probe can be used for imaging Zn^{2+} in living cells with two-photon microscopy.

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Supporting Information Available: Experimental details and compound characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

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