A novel selective chemosensor for Mg\(^{2+}\) detection based on quinoline-hydrazone-crown ether

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A novel Mg\(^{2+}\)-selective chemosensor based on quinoline-hydrazone-crown ether (L1) has been synthesized and characterized by \(^1\)H NMR, elemental analysis and ESI-mass spectrometry. The absorption and emission spectra of L1 have been investigated in the presence of alkali and alkaline-earth cations (Li\(^+\), Na\(^+\), K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\) and Ba\(^{2+}\)). Mg\(^{2+}\) results in an instant color change of L1 from colorless to yellow in ethanol. Upon binding of Mg\(^{2+}\), a significant fluorescence enhancement is triggered in acetonitrile. Thus, L1 is expected to be used as an interesting Mg\(^{2+}\) sensitive chemosensor. The 1:1 stoichiometry binding mode of L1 with Mg\(^{2+}\) is supported by the Benesi-Hildebrand analysis. The binding constants (logK) in ethanol and acetonitrile are found to be 4.54 and 3.57 by the goodness of the linear fitting of the Benesi-Hildebrand plot from the results of Ultraviolet–visible (UV-Vis) and fluorescence titrations, respectively.

Keywords: Quinoline-hydrazone-crown ether, synthesis, alkali and alkaline-earth cations, Mg\(^{2+}\), chemosensor

The development of molecular probes capable of targeting metal ions selectively has been the main stream in supramolecular chemistry because of implications in cell biology, medicine, analytical chemistry and environmental sciences\(^1\). Due to high sensitivity, ultra-fast response, real-time measurements, convenient operation as well as low costs, the spectral analytical methods based on the absorption and fluorescence spectra are gaining increasing attention for the selective detection of metal ions\(^2,3\). A number of reported chemosensors, particularly for metal ions, have been prepared for the detection of chemical species, by absorption and fluorescence changes induced by the supramolecular host-guest complex formation\(^4-6\). Such supramolecular systems are mainly based on crown ethers, calixarenes, and so on because of easy modifications with functional groups and appropriate chromophores\(^7\). In these systems, the host supplies binding sites for the guest and the chromophore units act as the transducer of the guest binding into the optical signals, respectively\(^8\). While crown ethers show high affinities for alkali metal ions\(^9,10\), in contrast azacrown ethers exhibit superior interactions towards alkaline earth metal ions\(^11,12\). Alkali and alkaline earth metal ions are the most abundant metal ions in physiological systems and necessary for the function of all living cells where they activate many enzymes, participate in the oxidation of glucose to produce adenosine triphosphate and also participate in the transmission of nerve signals\(^13,14\). These cations, excluding Sr\(^{2+}\) and Ba\(^{2+}\), co-exist in the biological systems and are common interfering metal ions.

We are interested in targeting Mg\(^{2+}\) signaling, because it is closely related to human health. As an important trace element, it plays an active role in many biological processes at the cellular level such as proliferation of cells, enzyme-driven biochemical reactions and stabilization of DNA conformation\(^15\). Moreover, Mg\(^{2+}\) is also believed to be an etiological factor in many pathological processes, such as congestive heart failure, cerebral infarction, lung cancer, and muscle dysfunction\(^16,17\). A decrease in Mg\(^{2+}\) concentration has been implicated in the development of cardiac, hypokalaemia, hypocalcaemia and neurological manifestations. Migraines, diabetes, osteoporosis, and coronary heart disease have been associated with chronic low magnesium\(^20,21\). In contrast, high levels of Mg\(^{2+}\) lead to a number of neuronal diseases ranging from hypertension to Alzheimer's disease related to age\(^22\). In this regard, the development of colorimetric and fluorescent chemosensors for Mg\(^{2+}\) has attracted increasing interest in the area of chemical and biological investigation.

8-Hydroxyquinoline derivatives were good photoactive groups, which were widely applied in the
research of analytical detection and emitting materials as chelating agents of metal ions due to excellent coordination activity and unique chelating abilities with different metal ions23. To our knowledge, chemosensors of linking a quinoline derivative group and a benzoazacrown ether ring by an acylhydrazone chain have not been reported. Based on, the 8-hydroxyquinoline derivative group was appended to the azacrown ether ring through an acylhydrazone chain, by which a novel quinoline-hydrazone-crown ether N-(8'-hydroxy-5'-quinoline aldehyde acetylhydrazone)-benzoaza-15-crown-5 (L1) has been synthesized (Scheme I). Moreover, the chemosensing characteristics of L1 were investigated in the presence of alkali and alkaline earth metal ions (Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Sr²⁺ and Ba²⁺) using Ultraviolet-Visible (UV-Vis) and fluorescence measurements.

**Experimental Section**

All chemicals and analytical grade solvents were obtained from commercial suppliers and used without further purification except absolute ethanol, absolute acetonitrile, and 1,4-dioxane. Solutions of alkali and alkaline earth metal ions (Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Sr²⁺ and Ba²⁺) were prepared from the corresponding metal chloride salts. ¹H NMR spectra were recorded on a Bruker Advance III 500 MHz instrument spectrometer with TMS as internal standard. Elemental analyses were done on Vario instrument Micro Elemental analyzer. UV-Vis spectra were recorded on Hitachi U-2900 UV-Vis spectrophotometer. Fluorescence spectra were recorded on Hitachi F-4600 Fluorescence spectrophotometer. For the fluorescence measurement, the excitation and emission slit widths were both 5 nm. Mass spectra were obtained using Thermo LCQ Advantage–MAX LC–MS spectrometer in ESI mode. Melting points were determined using a Beijing XT5 microscopic melting point apparatus.

**Synthesis of intermediates and L1**

Benzoaaza-15-crown-5, 1 was prepared using a slightly modified literature method reported earlier24.

**Synthesis of N-(ethyl acetate) benzoaza-15-crown-5, 2**

Benzoaaza-15-crown-5, 1 (1.6 g, 6 mmol), and anhydrous potassium carbonate (0.86 g, 6.2 mmol) were dissolved in dry acetonitrile (30 mL), and stirred at RT for 15 min. Ethyl bromoacetate (0.66 mL, 6 mmol) were added to this solution. The reaction mixture was stirred and refluxed on an oil-bath for 8 h. After the reaction, the mixture was cooled to RT, and filtered. The filtrate was concentrated to yellow viscous liquid. After standing undisturbed for 12 h at RT, a crude solid product formed. The final product as a white solid (0.8864 g) was obtained by recrystallization from heptane. Yield 44.7%. m.p.48.2~51.1°C. Anal. Calcd for C_{18}H_{27}NO_6: C, 61.17; H, 7.70; N, 3.96. Found: C, 61.15; H, 7.49; N, 3.99%.

**Synthesis of N-(acetyl hydrazine) benzoaza-15-crown-5, 3**

Compounds 2 (0.8863 g, 2.51 mmol) were added in a 50 mL flask with 5 mL of dry ethanol as solvent, then the solution was stirred at RT for 15 min. At the end of this period, 80% of hydrazine hydrate (15 mL, 0.25 mol) were added to this solution. The reaction mixture was refluxed on an oil-bath for 5 h. A crude solid was obtained by distillation under reduced pressure and placed at RT. The desired product as a white crystal (0.512 g) was obtained by recrystallization from heptane. Yield 62.7%. m.p.58.7~60.1°C. Anal. Calcd

![Scheme I — Synthetic routes for L1](image-url)
for C_{16}H_{25}N_{2}O_{3}: C, 56.62; H, 7.424; N, 12.38. Found: C, 56.59; H, 7.415; N, 11.98%. ¹H NMR (500MHz, CDCl₃) (Supplementary data, Figure S2): δ 7.271 (t, J = 5.0Hz, 4H, 2×-CH₂), 7.007 (d, J = 8.0Hz, 1H, QL-4-H), 6.944 (m, 1H, QL-6-H), 6.880~6.934 (m, 4H, 4×Ar-H), 9.446 (s, 1H, -NH-).

**Synthesis of 5-formyl-8-hydroxyquinoline, 4**

5-Formyl-8-hydroxyquinoline 4 was synthesized according to the reported procedure. A solution of 8-hydroxyquinoline (10.0 g) in anhydrous ethanol (40 mL) was added to a three-necked flask equipped with a dropping funnel and then a NaOH (10 g) solution in distilled water (25 mL) was added. The mixture was heated to reflux and then CHCl₃ (18.5 mL) was distilled off, the residue was dissolved in 300 mL of water and neutralized carefully with hydrochloric acid (0.01 mol) until neutral to slightly acid pH (5~6) and then filtered off and dried. The precipitate was extracted with petroleum ether for 3 days at 60~90°C, and purified further by crystallization from absolute ethanol to obtain the desired product as an orange-red crystal.

**Synthesis of L1**

The synthetic route of crown ether L1 was shown in Scheme I. Compounds 3 (158.3 g, 0.47 mmol) were added in a three-necked flask with 10 mL of dry ethanol as solvent, and the solution was stirred at RT for 15 min under the protection of N₂. Then an ethanol solution (10 mL) of 5-formyl-8-hydroxyquinoline was added dropwise by dropping funnel to this solution. The reaction mixture was refluxed for 5 h. After placed for 12 h, a yellow solid was observed. The final product as a yellow crystal (73.5 mg) was filtered, dried and recrystallized from benzene. Yield 31.5%. m.p.174.9~176.5°C. Anal. Caled for C_{26}H_{30}N_{2}O_{6}: C, 69.359; H, 4.074; N, 8.07%. Found: C, 69.36; H, 4.054; N, 8.05%. ¹H NMR (500MHz, CDCl₃) (Supplementary data, Figure S3): δ 7.647~7.672 (m, 1H, QL-3-H), 7.993 (d, J = 8.0Hz, 1H, QL-7-H), 7.271 (d, J = 8.5Hz, 1H, QL-4-H), 6.944~6.994 (m, 4H, 4×Ar-H), 8.860~8.870 (m, 1H, QL-5-H), 9.615 (d, J = 8.5Hz, 1H, QL-6-H), 11.070 (s, 1H, -NH-). ESI-MS: m/z calcd for C_{26}H_{30}N_{2}O_{6}: [M+H]+, 495.22; [M+Na]+, 517.21. Found (Supplementary data, Figure S5): [M+H]+, 495.55; [M+Na]+, 517.27.

**Results and Discussion**

**UV-Vis spectral studies of L1**

The UV-Vis profiles of L1 were investigated in ethanol in the presence of biologically significant metal ions such as Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Sr²⁺, and Ba²⁺ as their chloride salts. The spectral changes were shown in Figure 1 (A). Li⁺, Na⁺ and K⁺ failed to elicit appreciable changes in the UV-Vis spectra, suggesting that L1 did not form complexes or rather weak binding interactions with alkali metal ions. When alkaline earth metal ions were used as the guest, varying degrees of changes in both peak positions and peak intensities were observed in the

**Figure 1** — (a) Absorption spectra of L1 (1.4882×10⁻⁴ M) in ethanol and L1 containing 10 equiv. of Li⁺ (1.504×10⁻⁴ M), Na⁺ (1.4820×10⁻⁴ M), K⁺ (1.4774×10⁻⁴ M), Mg²⁺ (1.481×10⁻⁴ M), Ca²⁺ (1.455×10⁻⁴ M), Sr²⁺ (1.512×10⁻⁴ M) and Ba²⁺ (1.4876×10⁻⁴ M); (b) The color changes of L1 upon addition of 10 equiv. of various metal ions.
absorption spectra of L1. The magnitude in the guest-induced spectral variation became gradually small with increasing the ionic radius of the alkaline earth metal ion. These results indicated that L1 formed complexes with alkaline earth metal ions with larger binding affinity as compared to those with alkali metal ions. That might be associated with the ionic radius in accordance with the size of the crown ring and a larger binding affinity to divalent cations. Moreover, UV-Vis spectra of L1 was more significantly perturbed in the presence of Mg\(^{2+}\), as shown by the fact that pronounced red shifts in original absorbance peaks of L1 were observed in the presence of Mg\(^{2+}\), concomitantly resulting in the solution color change from colorless to yellow (Figure 1 (B)). All of these suggested that L1 displayed superior binding interaction for Mg\(^{2+}\) relative to alkali metal ions and other alkaline earth metal ions. The results also showed that L1 can be considered as a specialized selective colorimetric chemosensor for Mg\(^{2+}\) in ethanol.

To further investigate the interaction between sensor L1 and Mg\(^{2+}\), UV-Vis titration was carried out by incremental addition of Mg\(^{2+}\) dissolved in ethanol (up to maximum complexation) into a fixed concentration of L1 (Figure 2). With the increase of Mg\(^{2+}\) concentration, the original absorbance peaks at 248 nm, 278 nm and 349 nm decreased gradually, while three new red shifted absorbance peaks emerged at 263 nm, 309 nm and 405 nm with increasing intensity. When L1 was treated with approximately 1 equiv. of Mg\(^{2+}\), the absorbance at 405 nm would keep stable and remain almost unchanged, suggesting that L1 achieved maximum complexation with 1 equiv. of Mg\(^{2+}\) (Figure 2, inset). In addition, five clear isosbestic points were observed at 254 nm, 272 nm, 293 nm, 333 nm and 375 nm, showing that L1 formed a complex with Mg\(^{2+}\). The results indicated that a three-dimensional structure was induced by the chelation of L1 with Mg\(^{2+}\) along with Π-Π* transition of the C=N–N=C groups coupled with charge transfers from ligands to metal ions (L1→Mg\(^{2+}\)). This contributed to the generation of a larger conjugated system, and finally led to the red shifts of the absorption peaks of L1.

L1 coordinated with Mg\(^{2+}\) in a 1:1 stoichiometry. The binding stability constant \(K_L\) was determined to be 4.54 (Supplementary data, Figure S6, Table S1 and Table S2). These were confirmed from UV-Vis spectrophotometric titration data by the Benesi-Hildebrand method\(^{27,28}\). When assuming a 1:1 association between L1 and Mg\(^{2+}\), the modified Benesi-Hildebrand equation have been given as follows (see the Supplementary data):

\[
\frac{1}{A_A} = \frac{1}{A_{A_0}} + \frac{K\left(A_{A_0} - A_A\right)\left[Mg^{2+}\right]}{A_{max} - A_A} \text{ ... (1)}
\]

\(A_{A_0}\) was the absorbance of L1 without Mg\(^{2+}\), \(A\) was the absorbance of L1 obtained with Mg\(^{2+}\), \(A_{max}\) was the absorbance of L1 in the presence of excess amount of Mg\(^{2+}\), \(K\) was the binding stability constant between L1 and Mg\(^{2+}\), and \([Mg^{2+}]\) was the total concentration of Mg\(^{2+}\) added (mol·L\(^{-1}\)). As shown in Figure S6, the plot of \(1/(A-A_{A_0})\) against [Mg\(^{2+}\)] showed a good linear relationship, indicating that L1 indeed associated with Mg\(^{2+}\) in a 1:1 stoichiometry. The larger stability constant was consistent with the stronger binding interaction of L1 with Mg\(^{2+}\).

In order to evaluate the preferential selective binding of Mg\(^{2+}\), we performed the competitive spectral analysis in ethanol in the presence of other interfering ions (Li\(^{+}\), Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\), Sr\(^{2+}\) and Ba\(^{2+}\)). L1 (15 µM) was treated with 1 equiv. of Mg\(^{2+}\) in the company of other metal ions (10 equiv), respectively. As shown in Figure 3, their absorption intensities at 405 nm were recorded, respectively. The presence of other interfering metal ions could not induce any apparent change in the UV-Vis spectrum of L1, indicating that coexistence with competitive metal ions could not make substantial interferences in Mg\(^{2+}\) sensing. This competitive experiment served to
demonstrate superior binding interaction of \( L_1 \) with \( \text{Mg}^{2+} \) relative to alkali metal ions and other alkaline earth metal ions.

**Fluorescence spectral studies of \( L_1 \)**

The fluorescence spectra of \( L_1 \) were studied in acetonitrile in the presence of \( \text{Li}^+ \), \( \text{Na}^+ \), \( \text{K}^+ \), \( \text{Mg}^{2+} \), \( \text{Ca}^{2+} \), \( \text{Sr}^{2+} \) and \( \text{Ba}^{2+} \). The spectral changes were shown in Figure 4. On the addition of \( \text{Li}^+ \), \( \text{Na}^+ \) and \( \text{K}^+ \), the fluorescence intensity of \( L_1 \) remained practically unchanged. Similarly, addition of \( \text{Sr}^{2+} \) and \( \text{Ba}^{2+} \) did not alter either the fluorescence intensity or fluorescence maxima, indicating the absence of any significant interaction with these metal ions. By contrast, only \( \text{Mg}^{2+} \) caused a remarkable fluorescence increasing at 520 nm except for a slight emission intensity increase at 520 nm for \( \text{Ca}^{2+} \). These results suggested that \( L_1 \) preferred the divalent ions, rather than univalent ones, and the ion with smaller ionic radii, especially \( \text{Mg}^{2+} \). Thus, in accord with the results obtained from the absorption spectra, the fluorescence spectral changes also indicated that \( L_1 \) displayed superior binding interaction for \( \text{Mg}^{2+} \) relative to alkali metal ions and other alkaline earth metal ions, and \( L_1 \) can be a fluorescent sensor for \( \text{Mg}^{2+} \) in acetonitrile.

For illustration, the fluorescence titrations of \( L_1 \) against incremental addition of \( \text{Mg}^{2+} \) were performed in acetonitrile. As shown in Figure 5, \( L_1 \) showed weak fluorescence signal when it was excited at 370 nm. With addition of increasing concentration of \( \text{Mg}^{2+} \) (0-10 equiv), a new fluorescence peak gradually appeared at 520 nm with increasing intensity. This phenomenon might be ascribed to the hampered PET mechanism due to the formation of \( L_1-\text{Mg}^{2+} \) complex. In the absence of \( \text{Mg}^{2+} \), the fluorescence band of \( L_1 \) at 520 nm was with low intensity because of PET induced by lone pair electrons from the nitrogen atom of \(-\text{C}=\text{N}\). After addition of \( \text{Mg}^{2+} \), the interaction of \( \text{Mg}^{2+} \) and \( L_1 \) resulted in inhibiting the electron transfer from lone pair electrons on \( \text{N} \) of the \(-\text{C}=\text{N}\) group to quinoline ring, as a result, the fluorescence intensity increased significantly at 520 nm.

The binding stoichiometry and binding stability constant (\( \text{lg} K \)) for \( L_1-\text{Mg}^{2+} \) complex were estimated by linear curve fitting analysis from fluorescence spectrophotometric titration data following the
Benesi-Hildebrand equation has been given as follows (see the Supplementary data):

\[ \frac{1}{F} - \frac{1}{F_{\text{min}}} = K(F_{\text{max}} - F_{\text{min}})[M^{2+}] + \frac{1}{F_{\text{max}} - F_{\text{min}}} \]  \hspace{1cm} (2)

Where \( F_{\text{min}} \) was fluorescence intensity of \( L1 \) in the absence of \( M^{2+} \), \( F \) was fluorescence intensity of \( L1 \) obtained at an intermediate \( M^{2+} \) concentration, \( F_{\text{max}} \) was fluorescence intensity of \( L1 \) in the presence of excess amount of \( M^{2+} \). \( K \) was the binding stability constant, and \( [M^{2+}] \) was the total concentration of \( M^{2+} \) in acetonitrile. The goodness of the linear fit of the B-H plot of \( 1/(F-F_{\text{min}}) \) vs. \( 1/[M^{2+}] \) for 1:1 complex formation confirmed the binding stoichiometry between \( L1 \) and \( M^{2+} \). The 1:1 stoichiometric complexation was consistent with the result obtained from the UV-Vis spectrophotometric titration data; the binding stability constant \( K \) was calculated to be 3.57 in acetonitrile solvent (Supplementary data, Figure S7, Table S3 and Table S4). The higher \( K \) for \( M^{2+} \) signified its superior binding interaction and selectivity compared to the coordinatively competing and biologically co-existing other metal ions.

Achieving high selectivity toward \( M^{2+} \) over the other competitive species coexisting was a very important feature to evaluate the performance of the chemosensor. To examine the interference of other relevant metal ions (Li\(^{+}\), Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\), Sr\(^{2+}\) and Ba\(^{2+}\)) on \( L1-M^{2+} \) complex, the competition experiments were carried out in the presence of \( M^{2+} \) and the relevant ions (Figure 6). When 5 equiv. of \( M^{2+} \) was added into the solution of \( L1 \) (7.9265x10\(^{-6}\) M, acetonitrile) in the presence of 6 equiv. of other metal ions, similar fluorescent spectral change was displayed to that with \( M^{2+} \), indicating the exclusive sensitivity of \( L1 \) toward \( M^{2+} \). These results indicated that \( L1 \) could act as a selective fluorescent sensor for \( M^{2+} \) in the presence of most competing metal ions.

**Conclusions**

A novel quinoline-hydrazone-crown ether N-(8'-hydroxy-5'-quinoline aldehyde acetylhydrazone)-benzoaza-15-crown-5 (\( L1 \)) have been prepared. The spectroscopic properties of \( L1 \) were investigated in the presence of alkali and alkaline earth metal ions (Li\(^{+}\), Na\(^{+}\), K\(^{+}\), Mg\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\) and Ba\(^{2+}\)) by means of UV-Vis and fluorescence spectroscopy. In ethanol, \( L1 \) displayed a high selectivity for \( M^{2+} \) compared to biologically interfering Li\(^{+}\), Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\), Sr\(^{2+}\) and Ba\(^{2+}\). Upon the addition of \( M^{2+} \), the solution color of \( L1 \) changed remarkably from colorless to yellow, so \( L1 \) could act as a colorimetric chemosensor for the detection of \( M^{2+} \) in ethanol with naked eye. In the presence of \( M^{2+} \), \( L1 \) showed a large fluorescence enhancement owing to the formation of 1:1 ligand-metal complex inhibiting PET process, showing that \( L1 \) could be utilized as an ion-selective fluorescence chemosensor for \( M^{2+} \) in acetonitrile. In a word, \( L1 \) should find potential applications of being used as a selective and sensitive colorimetric as well as a fluorometric sensor to detect \( M^{2+} \) in biological systems.

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**Supplementary Information**

Supplementary information associated with this article, *i.e.*, Tables S1–S4 and Figures S1–S7, is available in the website http://nopr.niscair.res.in/handle/123456789/60.

**References**
