Design and synthesis of triazolyl coumarins as Hg$^{2+}$ selective fluorescent chemosensors‡

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A series of triazolyl coumarin derivatives L1–L4, with and without spacer groups between the coumarin and the triazole groups, were synthesized as fluorescent sensors to study their binding ability and selectivity toward metal ions. Ligand L3, which contains an acetyl linker between the triazole and the coumarin, exhibited a high selectivity toward Hg$^{2+}$ in polar protic solvents MeOH–CHCl$_3$ (9 : 1, v/v) with fluorescent enhancement, furthermore, it was found to bind two Hg$^{2+}$ at a high concentration (>12.5 mM) of Hg(ClO$_4$)$_2$. In contrast, L4, in which position 4 of the triazole unit was replaced by a benzyl group instead of the 4-tert-butyphenoxymethyl group used in L1–L3, showed a binding stoichiometry toward only one Hg$^{2+}$. On the basis of the fluorescent sensing, IR, and $^1$H NMR titration results of ligands L1–L4, we proposed that not only the acetyl C==O but also the ether group of the 4-tert-butylyphenoxymethyl group in L3 assisted the triazole nitrogen atoms in the complexation of Hg$^{2+}$ to form a 1 : 2 complex (L3–(Hg$^{2+}$)$_2$).

Introduction

In recent years, considerable efforts have been devoted to the development of chemosensors for the selective detection of heavy metal ions of environmental and biological importance. In particular, the detection of Hg$^{2+}$ has attracted much attention, because it can cause serious health problems. Accordingly, the design of a selective and sensitive chemosensor for Hg$^{2+}$ is highly desirable. To date, a number of small-molecule sensors designed for the selective detection of Hg$^{2+}$ have been reported, however, most of them exhibited fluorescence quenching upon complexation with Hg$^{2+}$. The latter phenomenon is a natural outcome because a heavy atom effect usually favours the spin–orbit coupling and therefore decreases the lifetime of the singlet states of the fluorophores. However, sensors that exhibit fluorescence enhancement upon complexation with metal ions are preferred because of their ease in detection and low interference background. Thus, there is still a strong demand for designing Hg$^{2+}$ selective fluorescent sensors with turn-on fluorescence.

The achievement of high selectivity in a chemosensor relies on how one assembles all binding moieties in a stereo fashion so that they may function cooperatively. In this study, 7-methoxycoumarin was chosen as the fluorophore for Hg$^{2+}$ because it is easy to be synthesized and derivatized; furthermore, it is strongly fluorescent and readily soluble in polar protic solvents making it very popular in the recent developments of chemosensors and chemodosimeters for ions. Moreover, many studies have proven that metal complexed coumarins are useful as efficient agents in cancer therapy. In designing a chemoselective fluorionophore for metal ions, we not only need to choose a good fluorophore but also need to design a stable and selective binding ligand close to the fluorophore. 1,4-Disubstituted-1,2,3-triazoles, obtained from the “click” chemistry of azides with terminal alkynes, are easy to prepare and are very stable, making them very popular in coordination chemistry. As such, several chemosensors for metal ions have been synthesized by combining coumarins with 1,2,3-triazoles. It should be noted that, besides the triazole binding unit, most chemosensors need other auxiliary groups, such as pyridinyl units, to assist its complexation with metal ions. For example, recently Yao and co-workers reported the synthesis of a coumarin probe containing triazole and dipicolylamine N4-tetradenate ligands and found that its complex with Cu$^{2+}$ can be used in living cell detection of nitroxy (HNO) with a turn-on fluorescence. Govindaraju and Maity reported that a conformationally constrained fluorionophore conjugate (coumarin–pyridinyl–triazolyl–bipyridyl) can serve as an Al$^{3+}$ selective chemosensor based on internal charge transfer.

In this work, we synthesized a series of triazolyl coumarin derivatives L1–L3, by integrating a triazole unit into position 3 of
7-methoxycoumarin through three different ways: (1) by direct conjugation (L1), (2) by inserting a methylene bridge (L2), and (3) by inserting a carbonyl methyl group (L3). Using fluorescence titration experiments to study L1–L3 toward metal ions in polar protic solvents MeOH–CHCl₃ (v/v, 9 : 1), we found that only L3 displayed a high selectivity toward Hg²⁺. Furthermore, a 1 : 2 binding ratio between L3 and Hg²⁺ was found in the presence of a high concentration (>12.5 mM) of Hg²⁺. For comparison, a control compound L4, which replaces the 4-tert-butylphenoxy-methyl group by a benzyl group, was also titrated with Hg²⁺ to elucidate whether the ether oxygen atom of the 4-tert-butylphenoxy-methyl group was involved in the coordination event.

Results and discussion

The synthesis of L1, L2, and L3, started from literature known coumarins 1,11 2,12 and 4,13 are depicted in Scheme 1. Under Cu(I)-mediated click reaction conditions, the azido coumarin 1 reacted with p-tert-butylphenyl propargyl ether in a mixed solution of THF and water to afford triazolyl coumarin L1 in 52% yield. By treating corresponding bromomethylcoumarins 2 and 4 with sodium azide, the azido coumarins 3 and 5 were obtained in 98% and 87% yields, respectively. Following similar methods used in the synthesis of L1, the methyl-triazolyl coumarin L2 and acetyl-triazolyl coumarin L3 were obtained in 85% and 71% yields, respectively. All coumarins L1, L2, and L3 were fully characterized by ¹H and ¹³C NMR, EI mass spectrometry, and HRMS (see ESI†). The structures and conformations of L1 and L3 were further confirmed by a single-crystal X-ray diffraction analysis (Fig. 1a and b). The triazolyl and the coumarin groups of L1 are almost coplanar with a dihedral angle of 10° (Fig. 1a), whereas the triazole ring and the acetyl-coumarin of L3 are almost orthogonal to each other with a dihedral angle of 94° (Fig. 1b).

The selectivity of coumarins L1, L2, and L3 toward 15 different perchlorate salts of metal ions (Li⁺, Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Cr³⁺, Mn²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Hg²⁺, Ag⁺, Cd²⁺, and Pb²⁺) in MeOH–CHCl₃ (9 : 1, v/v) was examined by UV-Vis spectroscopy (Fig. S13, ESI†) and fluorescence spectroscopy (Fig. 2). Among these three triazolyl coumarin chemosensors, only the acetyl-triazolyl coumarin L3 exhibited a high selectivity toward Hg²⁺ with a fluorescence intensity enhancement by 1.8-fold. Furthermore, L3 retained its sensitivity toward Hg²⁺ even in the presence of other competing metal ions.

Scheme 1 Synthesis of triazole based coumarins L1, L2, and L3. Reagents and conditions: (a) 4-tert-butylphenyl propargyl ether, Cu(i), THF–H₂O, 40 °C, overnight; and (b) NaN₃, acetone, reflux, 1 h.
metal ions (Li⁺, Na⁺, K⁺, Mg²⁺, Ba²⁺, Ca²⁺, Cu²⁺, Cr³⁺, Pb²⁺, Ag⁺, Mn²⁺, Zn²⁺, Cd²⁺, and Ni²⁺) except Cu²⁺ (see Fig. 3). A slight quenching of fluorescence was observed when 100 equiv. of Cu²⁺ was added to the solution of a 1 : 10 mixture of L₃ with Hg²⁺. Thus, L₃ can be used as a Hg²⁺ selective fluorescent chemosensor in the presence of most competing cations in polar protic solvents MeOH–CHCl₃ (9 : 1, v/v). The results imply that the acetyl linker between 7-methoxycoumarin and the triazole units played a crucial role in the complexation of L₃ with Hg²⁺.

To gain further insight into the chemosensing properties and the mechanism of L₃ towards Hg²⁺, we carried out UV-Vis, fluorescence, and ¹H NMR titration experiments. The UV-Vis spectrum of L₃ exhibits a main absorption band at 367 nm, which did not show any obvious change by the addition of excess Hg²⁺ (Fig. S14†). However, the fluorescence intensity of L₃ (Φ_F = 0.10, using the fluorescent quantum yield of anthracene¹⁴ in EtOH = 0.27 as a reference) at 423 nm was gradually enhanced by the addition of Hg²⁺, which gave a fluorescence quantum yield of 0.19 at the saturation level (Fig. 4). At low equiv. of Hg²⁺, the changes in the fluorescence intensity (ΔI at 423 nm) showed a delayed response to the addition of Hg²⁺ (Fig. 4b). Such a sigmoidal curve implied that the binding of Hg²⁺ by L₃ is cooperative,¹⁵ and the curvature data can be analyzed by the Hill plot¹⁶ (as shown in eqn (1)), where [M] is the concentration of Hg²⁺, K is the association constant, n is the Hill coefficient, and I₀ and I_max are the fluorescence intensities of the free ligand L₃ and its complex at the saturation level, respectively.

\[
\log \left( \frac{I_{\text{max}} - I}{I - I_0} \right) = n \times \log [M] - \log K
\] (1)

As shown in Fig. 5a, the Hill coefficient n was found to be 1.91 which indicated that the binding between L₃ and Hg²⁺ might be a 1 : 2 complex.¹⁶ The binding constant was calculated to be 6.94 × 10⁷ M⁻². Furthermore, the Job plot¹⁷ experiment showed...
a maximum as the mole fraction of L3 reached 0.4, suggesting that 1:1 and 1:2 complexes of L3 with Hg^{2+} co-existed (Fig. 5b). According to the definition by IUPAC (C_{DL} = 3 \times S_i/m), the detection limit of L3 toward Hg^{2+} was found to be 2.00 \times 10^{-7} M within the linear response range of Hg^{2+} from 1.6 \times 10^{-5} to 3.2 \times 10^{-7} M (ESI, p. S6†).

In order to gain structural information on the complexes, we further carried out 1H NMR titration experiments of L3 with Hg^{2+} (Fig. 6, the labeling of protons on L3 is shown in Scheme 2). In the presence of 1 equiv. of Hg^{2+}, the methylene protons H_6 (6.37 ppm) adjacent to the carbonyl and the triazole methine proton H_8 (8.28 ppm) were both significantly downfield shifted by ca. 0.23 and 0.64 ppm, respectively. Such downfield shifts of H_6 and H_8 in the metal complex of L3 indicated that Hg^{2+} was chelated by the N2 nitrogen atom of the triazole and the oxygen atom of the carbonyl group. To our surprise, all of the protons seem to be split into two sets of signals when more than 5.0 equiv. of Hg^{2+} was added.

In contrast, L1 and L2 showed basically no fluorescent changes toward Hg^{2+}. The direct conjugation of triazole to the coumarin of L1 presumably made it hard to rotate freely so as to help chelate the Hg^{2+} with the lactone group. For L2, even though it has a flexible methylene linker, there is not enough space between the triazole group and the lactone ring to accommodate a Hg^{2+} ion. In addition, L1 and L2 might be capable of binding with Hg^{2+} by the N3 nitrogen atom of triazole and the ether oxygen atom of the tert-butylphenoxypy group, but the unbound coumarin part may remain flexible and therefore their fluorescence emission intensities may be reduced. Thus, L1 and L2 exhibited relatively low fluorescent sensitivity toward Hg^{2+}.

In order to confirm that the chelation of the second equiv. of Hg^{2+} by L3 was indeed through the binding of the triazole and the phenoxy ether groups, we synthesized coumarin L4, where position 4 of the triazole unit was replaced by a benzyl group instead of the 4-tert-butylphenoxypy group. Coumarin L4 was obtained in 50% yield using a similar synthetic method to that used for L3 (Scheme 3). The selectivity of L4 toward 15 perchlorate salts of metal ions was also studied by the UV-Vis and fluorescence spectroscopy (Fig. S-16†). Similar to L3, coumarin L4 showed a high selectivity toward Hg^{2+}. The binding abilities of L4 toward Hg^{2+} were studied by the UV-Vis (Fig. S-18†) and fluorescence titration experiments (Fig. 7). Surprisingly, the fluorescence intensity of L4 was enhanced by 4.5-fold after addition of Hg^{2+} ions (\phi_{free} = 0.07 and \phi_{complex} = 0.32 based on \phi_{anthracene} = 0.27 in EtOH) as compared to that of Hg^{2+} at position 3 of the coumarin and the N2 nitrogen atom of the triazole. After the conformational reorganization of the complex L3-Hg^{2+}, it was facile to bind the second equiv. of Hg^{2+} by the N3 nitrogen atom of triazole and the ether oxygen atom of the tert-butylphenoxypy group. A similar binding mode was reported by Liu’s group, in which a semi-rigid molecule that combined the 1,3,4-oxadiazole subunit with two 8-hydroxy quinolines was able to accommodate two Cd^{2+} ions and resulted in a fluorescence enhancement. Furthermore, small molecular sensors that can accommodate two Hg^{2+} ions are also well documented. This binding model can also rationalize the fluorescence enhancement of L3 by Hg^{2+}, because the conformation of L3 should be locked firmly when its flexible parts were coordinated to Hg^{2+}.

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1.8-fold enhancement by L3 under similar conditions. The titration of L4 by Hg$^{2+}$ did not show a sigmoidal curve as that in L3 (inset in Fig. 7a), indicating that the complexation between L4 and Hg$^{2+}$ was not cooperative. In addition, the Hill plot analysis of the titration data gave a coefficient ($n$) of 1.17 implying a 1 : 1 binding stoichiometry between the L4 and Hg$^{2+}$ in the complex, and the binding constant of 4 $\text{Hg}^{2+}$ was calculated to be 7.44 $\times$ 10$^4$ M$^{-1}$ (Fig. 7b). The detection limit was determined to be 3.15 $\times$ 10$^{-7}$ M (ESI, S6†). Why Hg$^{2+}$ induced the larger fluorescence enhancement factors on L4 than that on L3 might be due to two major factors: (1) in general, Hg$^{2+}$ tends to quench the fluorescence of a fluorophore by spin–orbit coupling, thus the complexation of L3 with two Hg$^{2+}$ may reduce part of the fluorescence intensity; (2) the t-butylphenoxy side chain in L3 is more flexible compared to the benzyl side chain in L4, therefore, the former is more efficient in radiationless decay processes through coupling with “loose” stretching vibrational modes of the t-butyl group.24

The $^1$H NMR titration of L4 with Hg$^{2+}$ also supported that Hg$^{2+}$ was coordinated by the acetyl C—O and the N2 nitrogen of the triazole group (Fig. 8). At one equiv. of Hg$^{2+}$ versus L4, the triazole methine proton $H_6$ (7.77 ppm) was significantly downfield shifted by ca. 0.72 ppm and the methylene protons $H_5$ (6.20 ppm), adjacent to the carbonyl group, were also downfield shifted by 0.27 ppm. The methylene protons $H_5$ (4.39 ppm) at the benzylic position were also downfield shifted by 0.22 ppm, which
of L3 and L4 in the absence and presence of various equiv. of Hg^{2+} and the results are shown in Fig. 9 and Table 1. All fluorescence decay spectra and their fitting curves are shown in Fig. S19 and S20 (ESI†). In the absence of Hg^{2+}, the fluorescence lifetime of free L3 and L4 calculated by single exponential decay was found to be 0.25 and 0.20 ns, respectively. When Hg^{2+} was added to the solution of L3, a longer decay component in the range of 1.06 ± 0.03 ns emerged and resulted in a bi-exponential decay. The percentage of the longer decay component increased gradually upon increasing the amount of Hg^{2+}, while the percentage of the shorter decay component decreased accordingly. The species with a longer lifetime (1.06 ± 0.03 ns) was attributed to the complex of L3 with Hg^{2+}. The other species with a shorter lifetime (0.25 ns) was suggested to be the un-complexed L3 or the free ligand resulting from cation release. Unfortunately, we were unable to determine whether the longer lifetime of L3 came from the 1 : 1 or 1 : 2 complex of L3 with Hg^{2+} due to the resolution limit of our instrument. Similar results were observed in the case of L4, where the lifetime of the complex L4-Hg^{2+} was determined to be 0.94 ± 0.03 ns.

The possibility of chelation of lactone (C(=O)O) to Hg^{2+} by L3 or L4 was excluded for two reasons. First, coumarin L2, with a methylene linker instead of a carboxyl, did not exhibit any binding ability toward Hg^{2+}, which means that L2 cannot coordinate with Hg^{2+} by only the lactone (C(=O)O) of a coumarin and the triazole group. Second, there would be a severe steric hindrance in the complex L3-Hg^{2+} if the triazole group were to bend toward the carbonyl of the lactone to chelate Hg^{2+} as shown in Fig. 10. Data from infrared spectra of L3 and its complex with Hg^{2+} (Fig. S21†) corroborated with our inference above (vide infra). The strong IR bands at 1725 and 1691 cm⁻¹ for the free L3 could be assigned to the stretching frequency of the lactone carbonyl and the acetyl group, respectively. For comparison, a broad band around 1712 cm⁻¹ with a shoulder at 1675 cm⁻¹ was observed in the IR spectrum of the complex L3-Hg^{2+}. Because the acetyl group is in conjugation with the coumarin in L3, the coordination of Hg^{2+} with the acetyl group could induce a polarization in the lactone ring and affect the stretching frequency of the lactone carbonyl, therefore, both the lactone and the acetyl carbonyl groups were shifted to lower wavenumbers.

### Conclusions

We have synthesized a series of triazolyl-coumarin ligands L1–L4 and studied their binding ability toward metal ions. Among these four ligands, only L3 and L4 exhibited good binding affinities toward Hg^{2+} accompanied by a fluorescence enhancement by 1.8- and 4.5-fold, respectively. The fluorescence decay measurements showed that the lifetime of free L3 and L4 was 0.25 and 0.20 ns, respectively. After complexation with Hg^{2+} ions, longer lifetimes (1.06 ± 0.03 and 0.94 ± 0.03 ns, for L3 and L4, respectively) were observed besides the short lived free ligands. On the basis of the fluorescent sensing, IR, and ¹H NMR titration results, L3 was found to bind two equiv. of Hg^{2+} through a stepwise binding mechanism (Scheme 2), where the first Hg^{2+} was complexed by the acetyl C=O and the triazole group; whereas the second Hg^{2+} was complexed through the triazole and the ether group of the 4-tert-butylphenoxymethyl in L3 instead of the benzyl in L4) helped to coordinate a second Hg^{2+}, which showed quite different fluorescence response curves. Work on developing water-soluble coumarins using this protocol and click chemistry is in progress in our group.

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### Notes and references


The nitrogen atom (N3) of triazole and the nearby oxygen atom were involved in the coordination of Hg$^{2+}$, please see ref. 3d for a related work.


