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PAPER

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*-butylphenoxymethyl group used in **L1–L3**, showed a binding stoichiometry toward only one Hg^{2+}. On the basis of the fluorescent sensing, IR, and ^1H 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*-butylphenoxymethyl of **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.[1] In particular, the detection of Hg^{2+} has attracted much attention, because it can cause serious health problems.[2] 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;[2a,3,4] 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.[5] 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[6] and chemodosimeters[7] for ions. Moreover, many studies have proven that metal complexed coumarins are useful as efficient agents in cancer therapy.[8] In designing a chemoselective fluoroionophore 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-Disubstitued-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.[9] As such, several chemosensors for metal ions have been synthesized by combining coumarins with 1,2,3-triazoles.[9,10] 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-tetradentate ligands and found that its complex with Cu^{2+} can be used in living cell detection of nitroxyl (HNO) with a turn-on fluorescence.[10a] Govindaraju and Maity reported that a conformationally constrained fluoroionophore conjugate (coumarin–pyrrolidinyl–triazolyl–bipyridyl) can serve as an Al^{3+} selective chemosensor based on internal charge transfer.[10b]

In this work, we synthesized a series of triazolyl coumarin derivatives **L1–L3**, by integrating a triazole unit into position 3 of

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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–$CHCl_3$ (v/v, 9 : 1), we found that only **L3** displayed a high selectivity toward Hg^{2+}. Furthermore, a 1 : 2 binding ratio between **L3** and Hg^{2+} was found in the presence of a high concentration (>12.5 mM) of Hg^{2+}. For comparison, a control compound **L4**, which replaces the 4-*tert*-butylphenoxymethyl group by a benzyl group, was also titrated with Hg^{2+} to elucidate whether the ether oxygen atom of the 4-*tert*-butylphenoxymethyl 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 ^{13}C 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 acetylcoumarin 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^+, Mg^{2+}, Ba^{2+}, Ca^{2+}, Cu^{2+}, Hg^{2+}, Cr^{3+}, Pb^{2+}, Ag^+, Mn^{2+}, Zn^{2+}, Cd^{2+}, and Ni^+) in a polar protic solution MeOH–$CHCl_3$ (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**

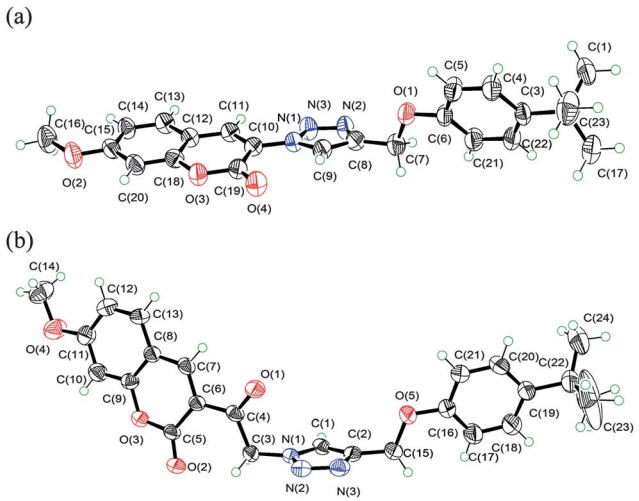


Fig. 1 X-ray crystal structures of coumarins (a) **L1** and (b) **L3**.

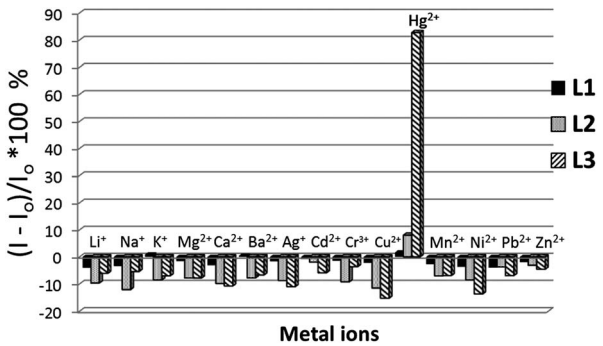
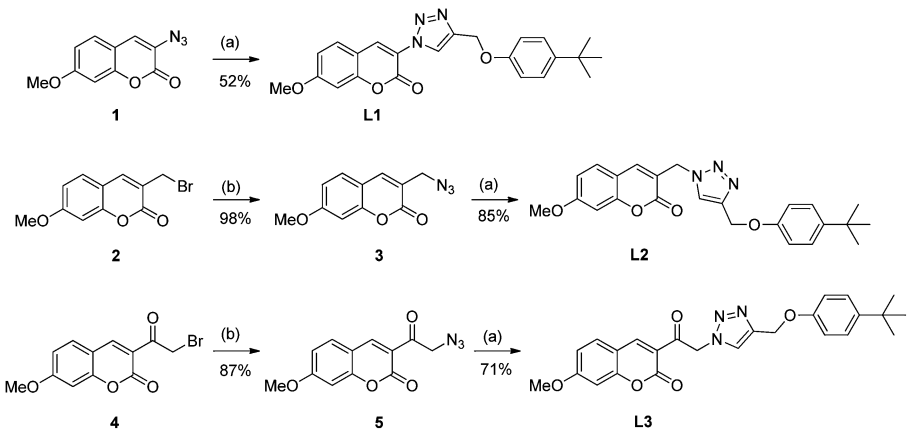


Fig. 2 Percentage fluorescence intensity changes of **L1**, **L2**, and **L3** by the addition of 10 equiv. of various metal perchlorates (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{3+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Ag^+, Cd^{2+}, and Pb^{2+}) in MeOH–$CHCl_3$ (9 : 1, v/v). Excitation wavelengths for **L1**, **L2**, and **L3** were 344, 328 and 367 nm, respectively. The fluorescence intensities of **L1**, **L2**, and **L3** were recorded at 416, 395 and 423 nm, respectively.

exhibited a high selectivity toward Hg^{2+} with a fluorescence intensity enhancement by 1.8-fold. Furthermore, **L3** retained its sensitivity toward Hg^{2+} even in the presence of other competing



Scheme 1 Synthesis of triazole based coumarins **L1**, **L2**, and **L3**. *Reagents and conditions*: (a) 4-*tert*-butylphenyl propargyl ether, Cu(I), THF–H_2O, 40 °C, overnight; and (b) NaN_3, acetone, reflux, 1 h.

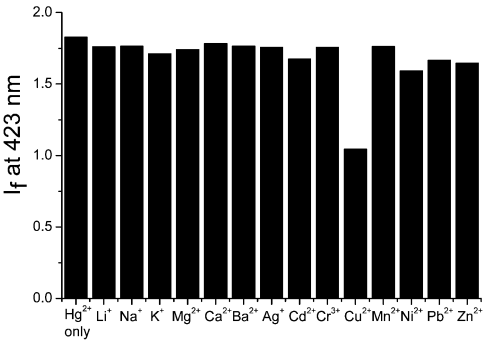


Fig. 3 The fluorescence intensities of **L3** (20 μM) at 423 nm upon the addition of Hg^{2+} (0.2 mM) in the presence of other metal ions (2 mM) in MeOH–$CHCl_3$ (9 : 1, v/v).

metal ions (Li^+, Na^+, K^+, Mg^{2+}, Ba^{2+}, Ca^{2+}, Cu^{2+}, Cr^{3+}, Pb^{2+}, Ag^+, Mn^{2+}, Zn^{2+}, Cd^{2+}, and Ni^{2+}) except Cu^{2+} (see Fig. 3). A slight quenching of fluorescence was observed when 100 equiv. of Cu^{2+} was added to the solution of a 1 : 10 mixture of **L3** with Hg^{2+}. Thus, **L3** can be used as a Hg^{2+} selective fluorescent chemosensor in the presence of most competing cations in polar protic solvents MeOH–$CHCl_3$ (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 Hg^{2+}.

To gain further insight into the chemosensing properties and the mechanism of **L3** towards Hg^{2+}, 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 Hg^{2+} (Fig. S14†). However, the fluorescence intensity of **L3** ($\Phi_F = 0.10$, using the fluorescent quantum yield of anthracene[14] in EtOH = 0.27 as a reference) at 423 nm was gradually enhanced by the addition of Hg^{2+}, which gave a fluorescence quantum yield of 0.19 at the saturation level (Fig. 4). At low equiv. of Hg^{2+}, the changes in the fluorescence intensity (ΔI at 423 nm) showed a delayed response to the addition of Hg^{2+} (Fig. 4b). Such a sigmoidal curve implied that the binding of Hg^{2+} by **L3** is cooperative,[15] and the curvature data can be analyzed by the Hill plot[16] (as shown in eqn (1)), where [M] is the concentration of Hg^{2+}, K is the association constant, n is the Hill coefficient, and I_0 and I_{max} are the fluorescence intensities of the free ligand **L3** and its complex at the saturation level, respectively.

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

As shown in Fig. 5a, the Hill coefficient n was found to be 1.91 which indicated that the binding between **L3** and Hg^{2+} might be a 1 : 2 complex.[16c] The binding constant was calculated to be $6.94 \times 10^7\ M^{-2}$. Furthermore, the Job plot[17] experiment showed

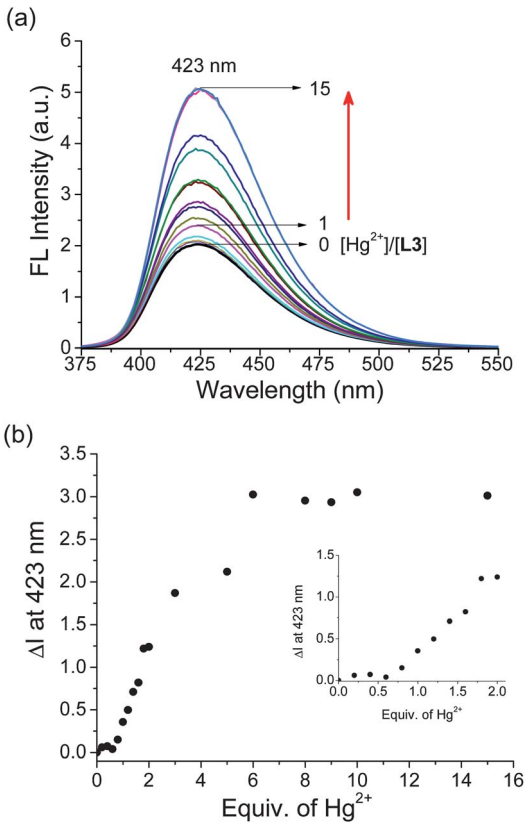


Fig. 4 (a) The fluorescence emission spectra of **L3** (20 μM) with various equiv. of $Hg(ClO_4)_2$ in polar protic cosolvent MeOH–$CHCl_3$ (9 : 1, v/v). The excitation wavelength was 367 nm. (b) The variation of fluorescence intensity at 423 nm of **L3** by adding different amounts of $Hg(ClO_4)_2$. The inset is the expanded area from 0 to 2.0 equiv. of Hg^{2+}.

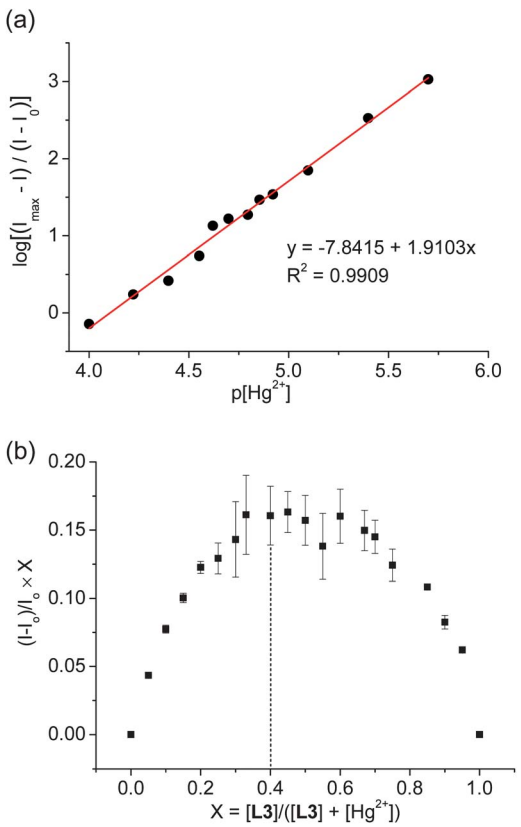


Fig. 5 (a) The Hill plot fitting and (b) the Job plot of **L3** with $Hg(ClO_4)_2$, where the difference in fluorescence intensity at 423 nm was plotted against the molar fraction of **L3** at an invariant total concentration of 20 μM in MeOH–$CHCl_3$ (9 : 1, v/v).

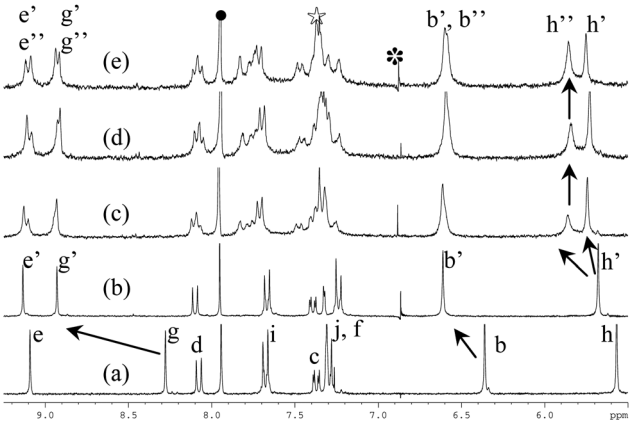
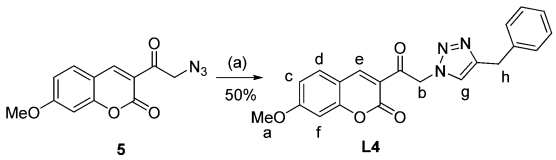


Fig. 6 The ^{1}H NMR titration of **L3** (2.5 mM) in the presence of different amounts of $Hg(ClO_4)_2$ in CD_3OD–$CDCl_3$ (1 : 1, v/v). (a) 0, (b) 1.0, (c) 5.0, (d) 6.0, and (e) 8.0. ☆: external $CHCl_3$, ●: internal $CHCl_3$, *: instrumental noise. Some yellow precipitate was formed when more than 5.0 equiv. of Hg^{2+} was added.

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 ($C_{DL} = 3 \times S_b/m$), the detection limit of **L3** toward Hg^{2+} was found to be 2.00×10^{-7} M within the linear response range of Hg^{2+} from 1.6×10^{-5} to 3.2×10^{-5} M (ESI, p. S6†).[18]

In order to gain structural information on the complexes, we further carried out ^{1}H 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_b (6.37 ppm) adjacent to the carbonyl and the triazole methine proton H_g (8.28 ppm) were both significantly downfield shifted by *ca.* 0.23 and 0.64 ppm, respectively. Such downfield shifts of H_b and H_g 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, especially the methylene protons H_h adjacent to the *tert*-butylphenoxy group were clearly split into two peaks (5.72 and 5.83 ppm). Since both the Hill Plot fitting and Job plot analysis implied that **L3** might have a 1 : 2 binding stoichiometry with Hg^{2+}, we suggest that **L3** chelates with two equiv. of Hg^{2+} by a stepwise binding mechanism and a possible binding mode is depicted in Scheme 2.

Two of the three nitrogen atoms in the 1,2,3-triazole ring, namely N2 and N3, are known to be Lewis basic, therefore, they are capable of binding with metal ions. Based on the big chemical shift changes of protons H_b and H_g of **L3** by Hg^{2+}, we inferred that the first equiv. of Hg^{2+} was coordinated with the aid of

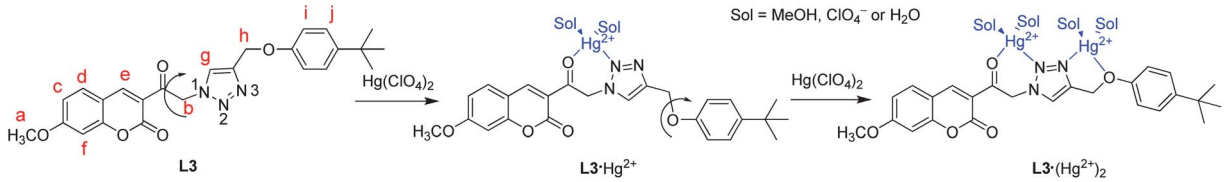


Scheme 3 Synthesis of **L4**. *Reagents and conditions*: (a) prop-2-ynylbenzene, Cu(I), THF–H_2O, 40 °C, overnight.

C=O 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*-butylphenoxy group.[19] 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.[20] Furthermore, small molecular sensors that can accommodate two Hg^{2+} ions are also well documented.[3a–c] 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+}.

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*-butylphenoxy 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*-butylphenoxymethyl. 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



Scheme 2 Possible binding modes of **L3** with $Hg(ClO_4)_2$.

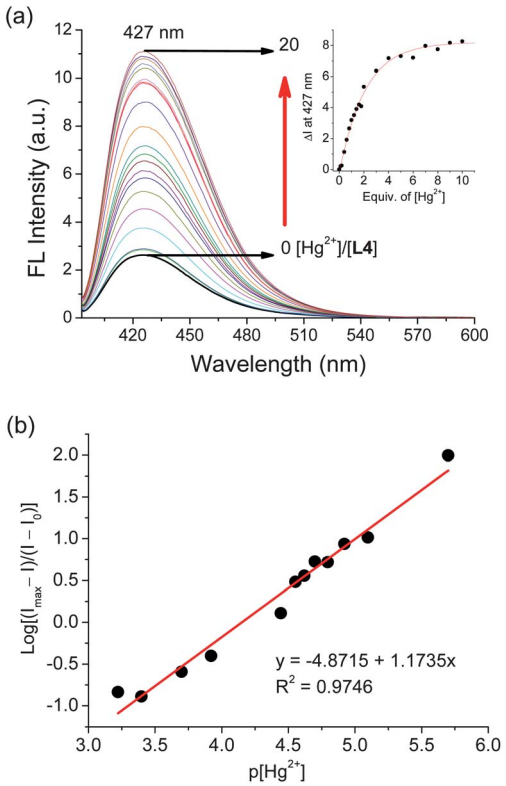


Fig. 7 (a) The fluorescence emission spectra of **L4** (20 μM) with various equiv. of $Hg(ClO_4)_2$ in cosolvent MeOH–$CHCl_3$ (9 : 1, v/v). The excitation wavelength was 367 nm. The inset is the variation of fluorescence intensity at 427 nm of **L4** by adding different amounts of $Hg(ClO_4)_2$. (b) The Hill plot fitting of **L4** with $Hg(ClO_4)_2$ using the fluorescence intensity data from (a).

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**·Hg^{2+} was calculated to be 7.44×10^4 M^{-1} (Fig. 7b). The detection limit was determined to be 3.15×10^{-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.[21]

The ^1H 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_g (7.77 ppm) was significantly downfield shifted by *ca.* 0.72 ppm and the methylene protons H_b (6.20 ppm), adjacent to the carbonyl group, were also downfield shifted by 0.27 ppm. The methylene protons H_h (4.39 ppm) at the benzylic position were also downfield shifted by 0.22 ppm, which

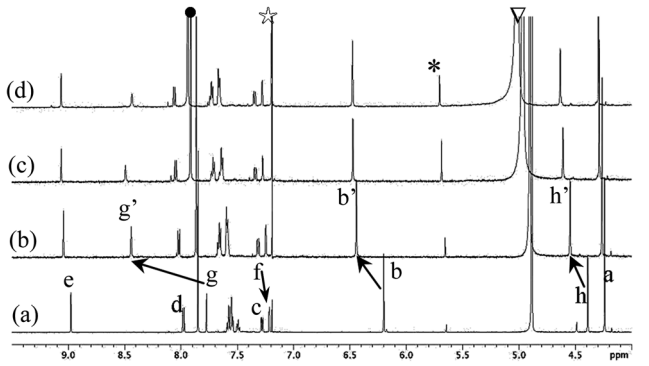


Fig. 8 The ^1H NMR titration of **L4** (2.5 mM) in the presence of different amounts of $Hg(ClO_4)_2$ in CD_3OD–$CDCl_3$ (1 : 1, v/v): (a) 0, (b) 0.5, (c) 1.0, and (d) 5.0. ☆: external $CHCl_3$, ●: internal $CHCl_3$, *: CH_2Cl_2, and ▽: H_2O in CD_3OD. Some yellow precipitate was formed when more than 1.0 equiv. of Hg^{2+} was added. For the labeling of protons on **L4**, please see Scheme 3.

might be due to a conformational change that occurred when **L4** chelated with Hg^{2+}. Fig. 8 also shows that the changes in chemical shifts reached a saturation level when one equiv. of Hg^{2+} was added to the polar protic solution of **L4** in CD_3OD–$CDCl_3$ (1 : 1, v/v).

In order to gain further information on the mercury complexes, we also carried out fluorescence decay measurements

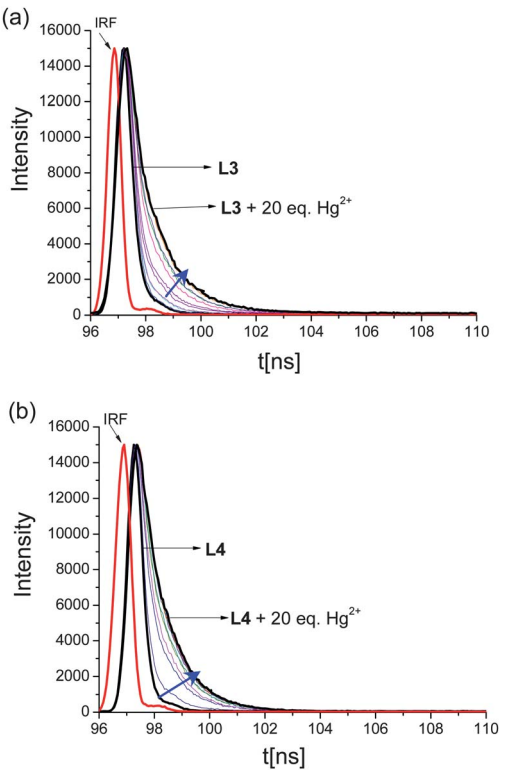


Fig. 9 Time-resolved fluorescence decay of (a) **L3** (monitored at 423 nm) and (b) **L4** (monitored at 427 nm) each was 20 μM with various amounts of $Hg(ClO_4)_2$ in cosolvent MeOH–$CHCl_3$ (9 : 1, v/v). The excitation wavelength for both **L3** and **L4** was 375 nm using a pulsed LED light. IRF means the instrument response function, which was obtained by the detection of Rayleigh scattered light.

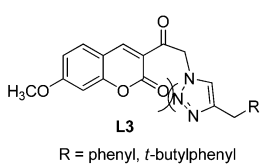
Table 1 Fluorescence decay time constants of **L3** and **L4** in the presence of Hg^{2+} ions

Ligand	Hg^{2+} (equiv.)	τ_1[a] (ns)	A_1	τ_2 (ns)	A_2	χ^2
L3	0	0.25	100%			1.24
	1	0.25	95%	1.09	5%	1.03
	2	0.25	72%	1.06	28%	1.06
	5	0.25	55%	1.03	45%	1.06
	10	0.25	52%	1.05	48%	1.03
	20	0.25	43%	1.03	57%	1.09
L4	0	0.20	100%			1.08
	1	0.20	67%	0.91	33%	1.14
	2	0.20	51%	0.94	49%	1.07
	5	0.20	40%	0.96	60%	1.12
	10	0.20	35%	0.96	65%	1.06
	20	0.20	32%	0.97	68%	1.15

[a] When fitted by a bi-exponential decay function, the τ_1 value was fixed for all measurements.

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.[22] 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 carbonyl, 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;[23] therefore, both the lactone and the acetyl carbonyl groups were shifted to lower wavenumbers.



L3

R = phenyl, *t*-butylphenyl

Fig. 10 Schematic of the bent structure of **L3**.

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*-butylphenoxy substituent. Thus, the carbonyl in **L3** not only acted as a bridge but also as a ligand in the complexation of Hg^{2+}. It is also found that an appropriate ligand at position 4 of the triazole group (for example, 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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