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# Multi-branched triphenylamine–rhodamine derivatives: Synthesis and fluorescent sensing for $Cu^{2+}$ and $Hg^{2+}$ ions

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ABSTRACT

Three multi-branched rhodamine based fluorescent probes TPARH1-3 have been designed and synthesized by incorporating the rhodamine fluorophore with triphenylamine. The probe TPARH1 displayed high sensitivity to $Cu^{2+}$ in aqueous $CH_3CN$. The probes TPARH2 and TPARH3 showed high sensitivity towards $Hg^{2+}$ in aqueous EtOH medium as reflected by their signalling responses. The cooperative effects of multi-branched structures towards metal ions were carried out by UV–vis absorption titrations and time scanning fluorescence spectroscopic. In addition, the binding mode was proposed based on the job's plot. The absorption of probes 1–3 at 558 nm went through a maximum at a molar fraction of 0.5, indicating a 1:1 stoichiometry of the $Hg^{2+}$ to 1, 2 and 3 in the complex, respectively.



## 1. Introduction

The specific detection of transition metal ions is an active field of research because of their important roles in biological, industrial and environmental processes [1,2]. Among them, $Cu^{2+}$ is of particular interest due to its essential role in various physiological processes. Abnormal $Cu^{2+}$ levels in human body are toxic and can lead to major health concerns, especially in oxidative stress and neurological disorders [3]. As an environmental contaminant, $Hg^{2+}$ is considered to be dangerous as it can accumulate in human body and causes serious damage to the central nervous and endocrine systems even in a low concentration [4]. Therefore, it is of utmost interest to develop highly sensitive and selective optical chemosensors for $Hg^{2+}$ and $Cu^{2+}$ ions [5,6].

Since the pioneer work of Czarnik's group regarding a rhodamine-based $Cu^{2+}$ fluorescent chemodosimeter [7], rhodamine derivatives have attracted much attention in the construction of small-molecule fluorescence probes for the detection of heavy metal ions and protons in aqueous solutions [8,9]. These probes which are based on rhodamine derivatives can be potentially applied to biological and environmental materials [10]. However, the cooperative effect of multi-branched rhodamine derivatives in the presence of heavy metal ions has rarely been reported [11].

As the continuation of our study on the sensing of cations and anions of biological significance [12,13], we have synthesized three multi-branched triphenylamine–rhodamine probes 1–3, which can selectively recognize $Cu^{2+}$ and/or $Hg^{2+}$ in a mixed aqueous organic environment.

## 2. Experimental

### 2.1. Reagents and chemicals

Most of the metal salts were purchased from Sinopharm Chemicals Ltd. and used as received. Rhodamine B and triphenylamine were procured from Sinopharm Chemicals Ltd. and used without further purifications. Ethanol and acetonitrile (AR grade) were purchased from Beijing Chemical Reagent Plant and purified before use. Water used for the experiment was double distilled. Stock solutions of metal ions ($1.0 \times 10^{-2}$ mol/L) were prepared by dissolving 0.1 mmol of nitrate salts the following compounds in 10 mL of double distilled water. High concentration of the stock solutions TPARH1-3 ($1.0 \times 10^{-4}$ M) were prepared in $CH_3CN$ and EtOH. Before spectroscopic measurements, the solution was freshly prepared by diluting the high concentration stock solution to the corresponding solution.

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### 2.2. Instruments

$^{1}H$ and $^{13}C$ NMR spectra were recorded with a Bruker Avance 300 spectrometer using tetramethylsilane as the internal standard. IR spectra were recorded in diffuse reflection with a Magna 560 FT-IR spectrophotometer. Mass spectra were obtained from Bruker micro TOF-Q mass spectrometer. Fluorescence spectra were taken on a Hitachi F-4500 and Hitachi F-7000 fluorescence spectrophotometer. The UV/vis spectra were recorded on a Shimadzu UV-3101 spectrophotometer.

### 2.3. Synthesis of probe TPARH1

To a stirring solution of rhodamine B hydrazide (0.456 g, 1.0 mmol) in EtOH (30 mL), 4-(diphenylamino)benzaldehyde (0.273 g, 1.0 mmol) was added and the reaction mixture was heated to reflux for 12 h. When the reaction was finished the mixture was cooled to room temperature, poured into ice water and then the precipitate was collected through filtration. $CH_2Cl_2$ (50 mL) was added to the precipitate and washed thoroughly with water (300 mL). The organic phase was dried over $MgSO_4$, concentrated and column chromatographed on silica-gel (elution with petroleum ether:$CH_2Cl_2$=5:1) to obtain 1 as a yellow powder in 75% yield: $^{1}H$ NMR (300 MHz, $CDCl_3$) $\delta$ 8.70 (s, $^{1}H$), 7.97 (dd, $J$=5.6, 3.2 Hz, $^{1}H$), 7.47 (dd, $J$=8.9, 5.5 Hz, $^{2}H$), 7.39 (d, $J$=8.6 Hz, $^{2}H$), 7.21 (dd, $J$=6.8, 1.6 Hz, $^{3}H$), 7.11 (dd, $J$=5.6, 2.9 Hz, $^{2}H$), 7.07–6.97 (m, 6H), 6.91 (d, $J$=8.7 Hz, $^{2}H$), 6.52 (d, $J$=8.8 Hz, $^{2}H$), 6.41 (d, $J$=2.3 Hz, $^{2}H$), 6.29–6.19 (m, $^{2}H$), 3.31 (q, $J$=7.0 Hz, $^{8}H$), 1.15 (t, $J$=7.0 Hz, $^{12}H$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 164.59, 153.30, 149.25, 149.08, 147.89, 147.29, 132.98, 129.96, 129.28, 128.46, 128.18, 128.06, 124.90, 123.84, 123.30, 122.27, 108.08, 106.61, 98.08, 66.16, 44.32, 12.64; IR (KBr, $cm^{-1}$): $\nu$=2965, 2925, 1714, 1589, 1547, 1512, 1489, 1306, 1267, 1115, 822, 754, 695. ESI-MS: $m/z$=710.3.

### 2.4. Synthesis of probe TPARH2

To a stirring solution of rhodamine B hydrazide (0.456 g, 1.0 mmol) and 4,4′-(phenylazanediyl)dibenzaldehyde (0.150 g, 0.5 mmol) were used in accordance with the general procedure given above. The product 2 was obtained as a yellow powder in 62% yield: $^{1}H$ NMR (300 MHz, $CDCl_3$) $\delta$ 8.65 (s, $^{2}H$), 7.97 (d, $J$=6.3 Hz, $^{2}H$), 7.46 (s, 4H), 7.38 (d, $J$=8.2 Hz, $^{4}H$), 7.08 (ddd, $J$=24.0, 22.2, 8.0 Hz, $^{8}H$), 6.89 (d, $J$=8.2 Hz, $^{4}H$), 6.51 (d, $J$=8.7 Hz, $^{4}H$), 6.41 (s, $^{4}H$), 6.24 (d, $J$=7.8 Hz, $^{4}H$), 3.32 (dd, $J$=13.6, 6.7 Hz, $^{16}H$), 1.15 (t, $J$=6.7 Hz, $^{24}H$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 164.62, 153.28, 148.94, 148.60, 146.81, 133.06, 129.98, 129.40, 129.08, 127.47, 125.51, 123.94, 123.19, 108.12, 98.11, 66.15, 44.36, 12.62; IR (KBr, $cm^{-1}$): $\nu$=2967, 1684, 1617, 1549, 1512, 1462, 1367, 1309, 1268, 1225, 1116, 821, 790, 689. ESI-MS: $m/z$=1178.8.

### 2.5. Synthesis of probe TPARH3

Rhodamine B hydrazide (0.456 g, 1.0 mmol) and 4,4′,4″-nitrilotribenzaldehyde (0.110 g, 0.33 mmol) were used in accordance with the general procedure given above. The product 3 was obtained as a yellow powder in 57% yield: $^{1}H$ NMR (300 MHz, $CDCl_3$) $\delta$ 8.65 (s, $^{3}H$), 8.01–7.90 (m, $^{3}H$), 7.51–7.41 (m, $^{7}H$), 7.37 (d, $J$=8.7 Hz, $^{6}H$), 7.24–7.15 (m, $^{4}H$), 7.15–7.07 (m, $^{4}H$), 7.06–6.95 (m, $^{6}H$), 6.88 (d, $J$=8.7 Hz, $^{5}H$), 6.51 (d, $J$=8.8 Hz, $^{6}H$), 6.40 (d, $J$=2.4 Hz, $^{6}H$), 6.24 (d, $J$=8.9 Hz, $^{6}H$), 3.31 (dd, $J$=14.2, 7.1 Hz, $^{24}H$), 1.14 (t, $J$=7.0 Hz, 36H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 164.52, 153.37, 153.30, 151.66, 147.08, 133.27, 133.17, 131.66, 131.23, 129.00, 128.74, 128.27, 128.03, 126.11, 125.20, 123.89, 123.28, 123,18, 121.27, 108.11, 98.10, 66.17, 44.29, 12.55; IR (KBr, $cm^{-1}$): $\nu$=2906, 2676, 1793, 1694, 1613, 1590, 1510, 1376, 1307, 1218, 1147, 1074, 821, 689. ESI-MS: $m/z$=1644.8.

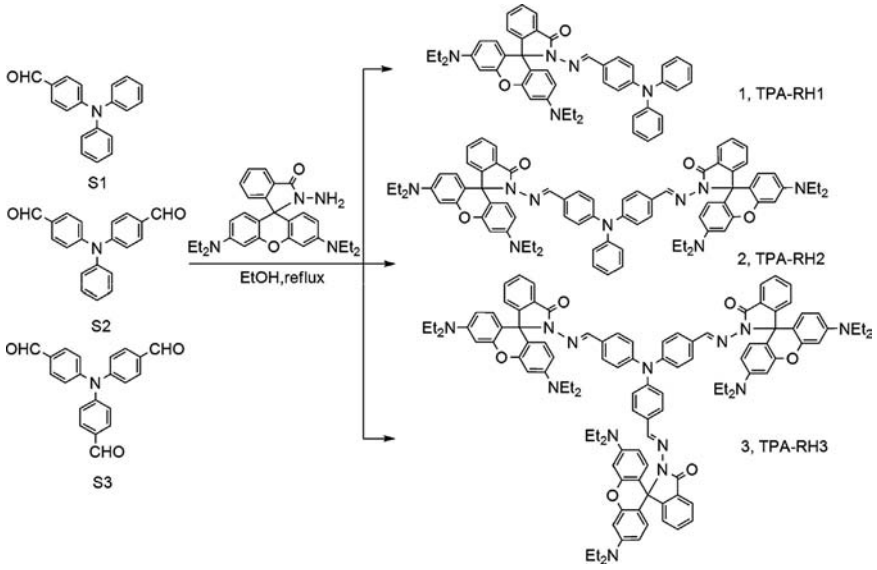
## 3. Results and discussion

### 3.1. Synthesis

The synthesized compounds (TPARH$n$, $n$=1, 2, 3) had triphenylamine (TPA) as the core and rhodamine hydrazide as branch substitutes. Probes 1–3 were facilely synthesized from rhodamine B hydrazide and substituted triphenylamine aldehyde (S1–S3) on the basis of the route shown in Scheme 1.

### 3.2. Fluorescence and absorbance spectra

In order to investigate the metal ion induced signaling responses in these probes, various metal ions such as $Na^{+}$, $K^{+}$, $Ca^{2+}$, $Mg^{2+}$, $Hg^{2+}$, $Cu^{2+}$, $Cd^{2+}$, $Fe^{2+}$, $Fe^{3+}$, $Co^{2+}$, $Ni^{2+}$, $Zn^{2+}$, $Ag^{+}$ and $Pb^{2+}$ (taken as their nitrate salts) were investigated in $CH_3CN$ and EtOH solutions. In contrast, when water was added to organic




**Scheme 1.** Synthesis of rhodamine based probes TPARH1-3 .

solvents, especially in 80% aqueous solutions, the fluorescence signal reached its maximum value. These results indicated that 80% aqueous $CH_3CN$ and EtOH media is favorable for fluorescent measurement (Fig. S1). In the presence of 10 equiv excess of metal ions, the fluorescence intensity at 580 nm for most metal ions was found to be almost the same in magnitude and there was no distinguishable difference. In the case of $Cu^{2+}$ and $Hg^{2+}$, however, a nonstructured emission at 580 nm increased to a significant extent [14].

The UV–vis spectra of 1–3 solutions each exhibited only a very weak band above 558 nm, which was ascribed to the spirolactam form of molecule TPARH1-3. Upon adding 10 equiv of $Cu^{2+}$ and $Hg^{2+}$, the absorption intensity at 558 nm was significantly enhanced, which induced a clear and gradual change from colorless to pink. This suggested the formation of the ring-opened amide form of TPARH1-3 upon binding. Compared with $CH_3CN/H_2O$, the absorption enhancement impelled by $Hg^{2+}$ was more obvious in $EtOH/H_2O$ solution. The addition of $Fe^{3+}$ into the solution of probes 1–3, under the same conditions, could induce a small but significant enhancement of the absorption at 558 nm (Fig. 1).

We first decided to examine the selectivity of the probes 1–3 in mixed aqueous–organic environment. For all the following titration experiments, absorption spectra were recorded 5 min after the addition of $Cu^{2+}$ to ensure the equilibrium of sensing procedure. As shown in Fig. 2a, probes 1–3 displayed only minimal absorption response to $Cu^{2+}$ within 2 equiv in aqueous acetonitrile. By contrast, gradually increasing addition of $Cu^{2+}$ elicited intense absorption within 2 equiv in ethanol–water (Fig. 2b). The absorption of probes 1–3 remarkably increased to their maximum values within 5 min. The significant absorption change in Fig. 2a and b was assigned to the chemical reaction between $Cu^{2+}$ and the spirolactam ring in aqueous ethanol and the complexation equilibrium took place in aqueous acetonitrile [15].

The absorption behavior of probes 1–3 towards $Cu^{2+}$ in ethanol was different from that towards $Hg^{2+}$ in the mixed aqueous media. In Fig. 2b, the $Cu^{2+}$ titration of the absorption intensities increased and reached the maximum values at about 5 equiv of $Cu^{2+}$ ions with the order: $3 < 2 < 1$. While addition of $Hg^{2+}$ ions within 10 equiv, the intensity of absorption band at 558 nm was in the order of $3 < 2 < 1$, but when the concentration of $Hg^{2+}$ ions increased more than 10 equiv the intensity of absorption band shifted to $3 > 2 > 1$ (Fig. 2c).

Furthermore, the time scanning absorption and fluorescence spectrum of probes 1–3 were evaluated in the presence of 10 equiv

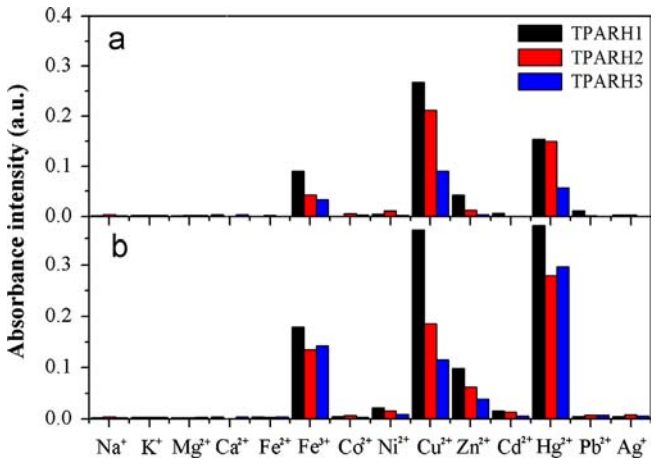



**Fig. 1.** Change in the absorption at 558 nm of 1–3 (10 μM) in presence of 10 equiv of various metal ions in (a) $CH_3CN/H_2O$ and (b) $EtOH/H_2O$ (8:2, v/v). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

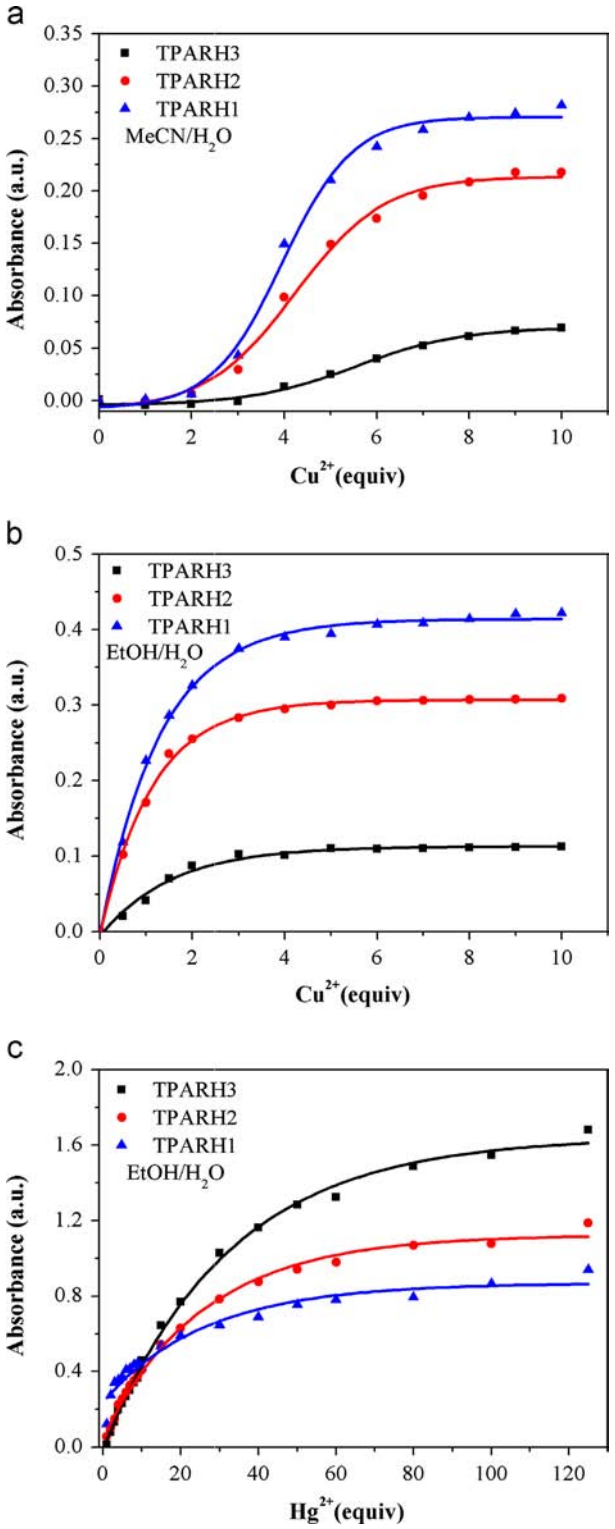



**Fig. 2.** Change in absorption spectra (558 nm) of 1–3 (10 μM) upon addition of different amounts of $Cu^{2+}$ and $Hg^{2+}$ in $CH_3CN/H_2O$ and $EtOH/H_2O$ (8:2, v/v) solutions.

concentrations of $Hg^{2+}$ ions. In Fig. 3a, there was a linear relationship between the absorption intensity at 558 nm and the reaction time. The gradients of 1–3 were corresponded to 0.00692, 0.01116 and 0.00616 in the order of $1 < 3 < 2$. By the way, the graphs in Fig. 3a also seem curved, when the reaction time prolonged more than 1 h. The maximum fluorescence emission enhancement at 580 nm was observed after the addition of 10 equiv of $Hg^{2+}$ ions which clearly indicated the highly reactive nature of the $Hg^{2+}$

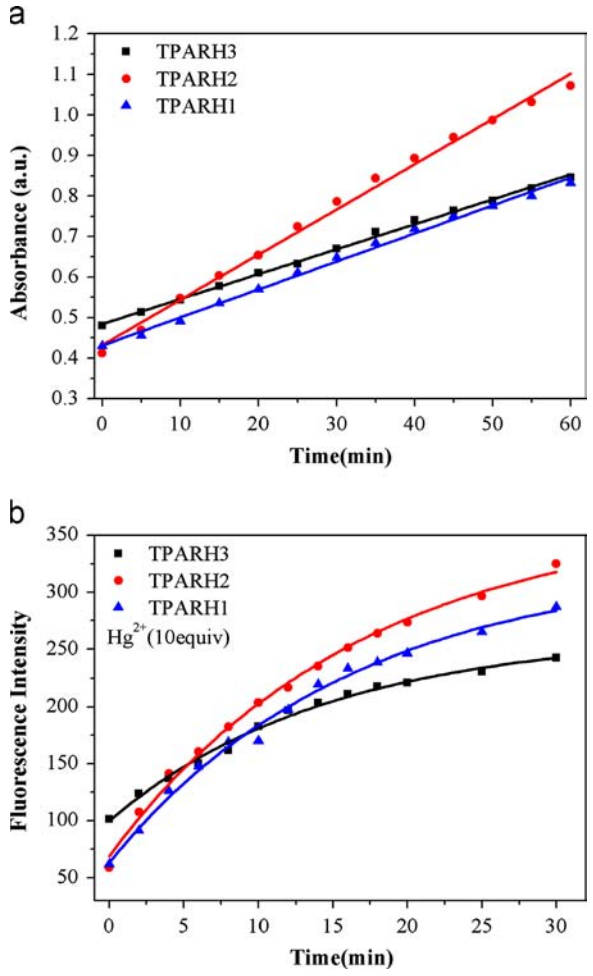



**Fig. 3.** Time scanning increase of absorption (558 nm) and fluorescence intensity (580 nm) of probes 1–3 to $Hg^{2+}$ (10 equiv) in ethanol–water (8:2, v/v), $\lambda_{ex}$=552 nm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mediated hydrolysis of the spirolactam ring of the rhodamine moiety. The fluorescence intensity at 580 nm increased as the reaction time was prolonged with the order of 3 < 1 < 2. Careful scrutiny showed the intensity of the pink colours arising from adding $Cu^{2+}$ and $Hg^{2+}$ ions was a little different. It was mentionable that $Cu(NO_3)_2$ instead of $Hg(NO_3)_2$ opened the spirolactam ring weakly as confirmed from the appearance of the faint pink colour of the solution as well as a weakly intense emission peak at 580 nm [16].

### 3.3. Sensing mechanism

To find out the stoichiometry of the $Cu^{2+}$ and $Hg^{2+}$-ligand complex, Job's method for absorption measurement was applied [17]. The absorption of 1–3 in the absence ($A_0$) and presence ($A$) of $Cu^{2+}$ and $Hg^{2+}$ were determined at about 558 nm in 5 min (Fig. S5). A plot of ($A$–$A_0$) versus the molar fraction of $Hg^{2+}$ was provided in Fig. 4. It showed that the ($A$–$A_0$) value went through a maximum at a molar fraction of 0.5, indicating a 1:1 stoichiometry of the $Hg^{2+}$ to 1, 2 and 3 in the complex, respectively. More direct evidence was obtained by comparing the ESI mass spectra of $Cu^{2+}$ and $Hg^{2+}$ complex with chemosensors TPARH1-3. The signal at $m/z$ 1296 corresponded to $[TPARH2+Cu^{2+}+3H_2O]^+$ and the signal at $m/z$ 1891 corresponding to $[TPARH3+Hg^{2+}+3H_2O]^+$ was clearly observed. These results indicated a 1:1 binding ratio between TPARH1-3 and $Cu^{2+}/Hg^{2+}$ existed in solvent.

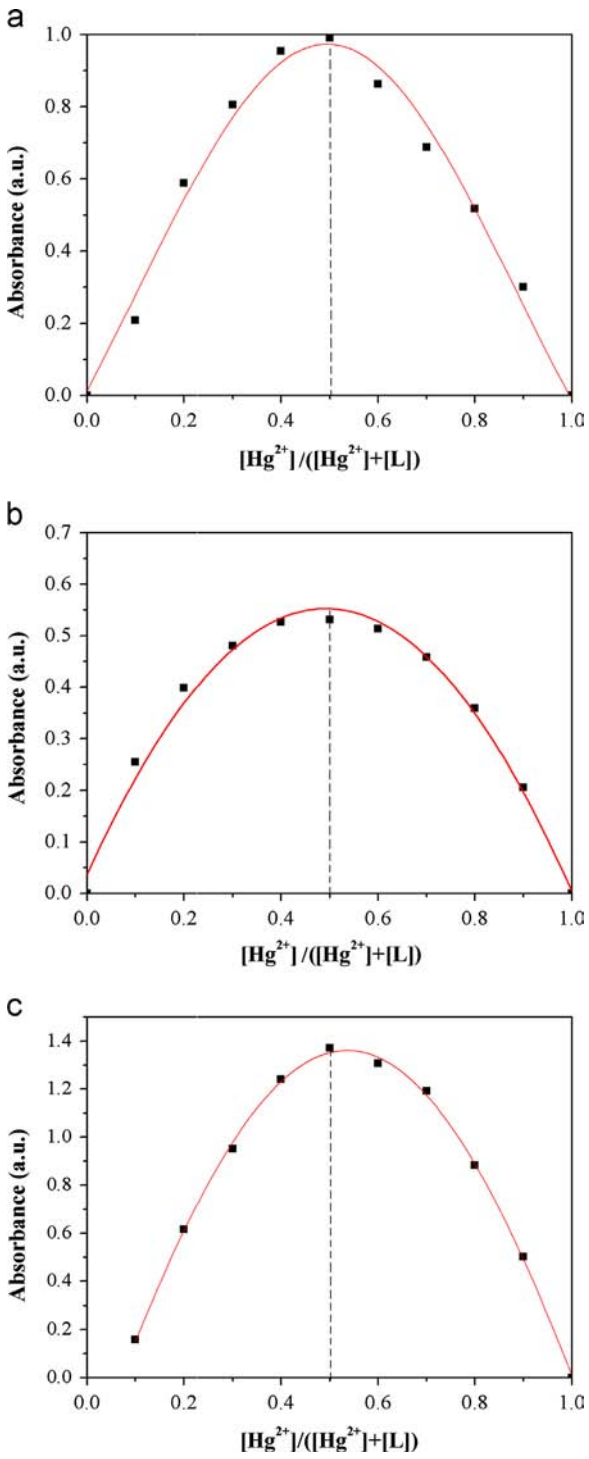
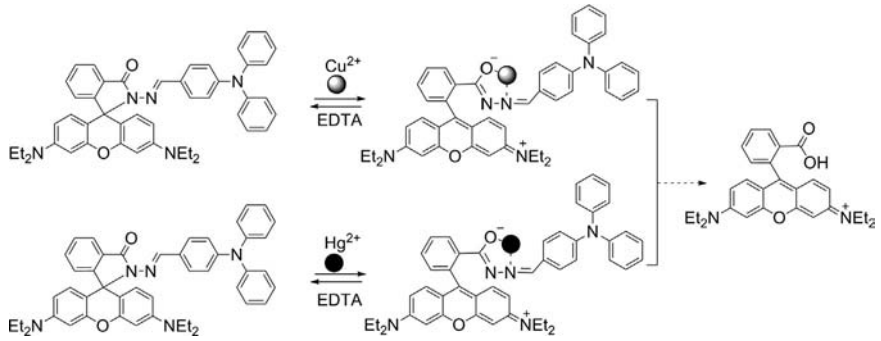



**Fig. 4.** The Job's plot analysis between probes 1–3 and $Hg^{2+}$ in EtOH–$H_2O$ (8:2, v/v) at room temperature. The total concentration of probes 1–3 and $Hg^{2+}$ was kept constant at 100 μM. The wavelength of absorbance was 558 nm.

Considering the behaviors of fluorescence and absorption spectra, the turn-on response of probes 1–3 may be explained by the spirocycle open–close mechanism [18,19]. It was concluded that the free probes are in the spirocyclic form, which is colorless and non fluorescent, whereas the coordination of $Cu^{2+}$ or $Hg^{2+}$ induced the N atom of spirolactam to attack the C atom of carbonyl, and thus a ring opening of the spirolactam of rhodamine took place (Scheme 2). This reaction procedure was not complete quickly like $Cu^{2+}$, as the absorption and fluorescence intensity increased until the reaction time prolonged over 5 h. The




**Scheme 2.** Proposed mechanism for the fluorescence enhancement of TPARH1 upon the addition of $Cu^{2+}$ and $Hg^{2+}$ .

observation inferred a process between metal and ligand interaction and metal mediated hydrolysis of the spirolactam ring at the same time [20]. However, the mechanistic approach for understanding this process properly in rhodamine based signaling probes remains elusive.

## 4. Conclusion

In summary, we have successfully synthesized three multi-branched TPARH1, TPARH2 and TPARH3 compounds containing triphenylamine core and rhodamine hydrazide arms. The excellent colorimetric and fluorescent response to $Cu^{2+}$ in aqueous $CH_3CN$ can be conveniently detected even by the naked eye, which provides a facile method for visual detection of $Cu^{2+}$. In addition, the mixed aqueous EtOH medium had shown here to promote $Hg^{2+}$ selectivity in these probes as reflected by their signaling responses. For fluorescent sensory rhodamine derivatives, the cooperative effects of multi-branched structures could amplify the signal compared with its monobranched counterparts.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.06.041.

## References

[1] E.L. Que, D.W. Domaille, C.J. Chang, Chem. Rev. 108 (2008) 1517–1549.
[2] A.A. Mosa, A. El-Ghamry, P. Trüby, Water Air Soil Pollut. 217 (2011) 637–647.
[3] E. Gaggelli, H. Kozlowski, D. Valensin, G. Valensin, Chem. Rev. 106 (2006) 1995–2044.
[4] P.B. Tchounwou, W.K. Ayensu, N. Ninashvili, D. Sutton, Environ. Toxicol. 18 (2003) 149–175.
[5] D.T. Quang, J.S. Kim, Chem. Rev. 110 (2010) 6280–6301.
[6] H.N. Kim, M.H. Lee, H.J. Kim, J.S. Kim, J. Yoon, Chem. Soc. Rev. 37 (2008) 1465–1472.
[7] V. Dujols, F. Ford, A.W. Czarnik, J. Am. Chem. Soc. 119 (1997) 7386–7387.
[8] M. Beija, C.A.M. Afonso, J.M.G. Martinho, Chem. Soc. Rev. 38 (2009) 2410–2433.
[9] X. Chen, T. Pradhan, F. Wang, J.S. Kim, J. Yoon, Chem. Rev. 112 (2012) 1910–1956.
[10] R.P. Haugland, The molecular probes handbook: a guide to fluorescent probes and labeling technologies, Life Technologies (2010).
[11] K. Ghosh, T. Sarkar, A. Samadder, A.R. Khuda-Bukhsh, New J. Chem. 36 (2012) 2121–2127.
[12] J. Zhang, B. Li, L. Zhang, H. Jiang, Chem. Commun. 48 (2012) 4860–4862.
[13] L. Wang, B. Li, L. Zhang, L. Zhang, H. Zhao, Sens. Actuators, B 171–172 (2012) 946–953.
[14] W. Huang, C. Song, C. He, G. Lv, X. Hu, X. Zhu, C. Duan, Inorg. Chem. 48 (2009) 5061–5072.
[15] V. Bhalla, R. Tejpal, M. Kumar, Sens. Actuators, B 151 (2010) 180–185.
[16] J. Huang, Y. Xu, X. Qian, J. Org. Chem. 74 (2009) 2167–2170.
[17] W.C. Vosburgh, G.R. Cooper, J. Am. Chem. Soc. 63 (1941) 437–442.
[18] Y. Yang, Q. Zhao, W. Feng, F. Li, Chem. Rev. 113 (2013) 192–270.
[19] H.Y. Lee, K.M.K. Swamy, J.Y. Jung, G. Kima, J. Yoon, Sens. Actuators, B 182 (2013) 530–537.
[20] M. Kumar, N. Kumar, V. Bhalla, P.R. Sharma, T. Kaur, Org. Lett. 14 (2012) 406–409.