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A Rhodamine Chemodosimeter Bearing Thiourea Moiety for Mercury(II) and Its Bioimaging Application

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Abstract:
A selective rhodamine chemodosimeter **RTP** was developed for Hg^{2+} detection based on the Hg^{2+}-promoted intramolecular cyclic guanylation of thiourea linked with *o*-phenylenediamine as a linker. Upon the addition of Hg^{2+}, a colorless solution of **RTP** turned pink with a maximum absorption band at 555 nm and with a 62-fold fluorescence enhancement at 578 nm (Φ = 0.34). **RTP** is significantly selective to Hg^{2+} among other metal ions with a detection limit of 1.6 nM (0.3 ppb). The membrane-permeable **RTP** probe was successfully demonstrated in Hg^{2+} monitoring in cultured HeLa cells.

Key words: bioimaging, fluorescence detection, Hg^{2+}-selective chemodosimeter, rhodamine, thiourea

Introduction

A number of Hg^{2+}-selective chemosensors have been reported allowing the sensitive detection of mercury ions [1]. However, various downsides that may limit the practical use of chemosensors are low aqueous solubility, low quantum yield in aqueous media, and cross-sensitivity to other metal cations. Thus, the development of novel Hg^{2+}-selective chemosensors is still a challenge. Herein, we report the thiourea-appended fluorescent chemodosimeter **RTP** for Hg^{2+} detection with *o*-phenylenediamine as a linker. Binding between Hg^{2+} and thiourea would promote desulfurization of thiourea leading to the spirolactam ring opening and intrinsic fluorescence emission.

Synthesis

The production of rhodamine-phenylthiourea conjugate **RTP** was synthesized in 2 steps with 51% yield (Fig. 1).

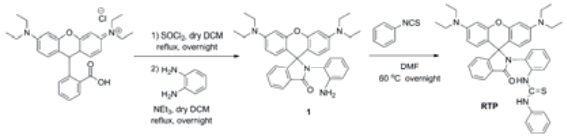

*Fig. 1. Synthesis of **RTP**.*

The structures of **RTP** and **1** were spectroscopically established by ^{1}H NMR, ^{13}C NMR, FT-IR and HR-ESI-MS analysis. In addition, the structure of **RTP** was also confirmed by a single-crystal X-ray crystallographic analysis showing spirolactam ring is in the closed form (Fig. 2).

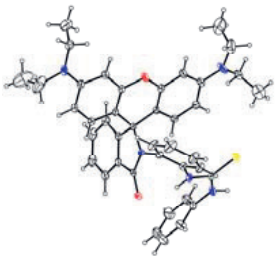

*Fig. 2. ORTEP plot of the chemodosimeter **RTP**.*

Optical and Fluorescence Responses

A colorless solution of **RTP** responded to Hg^{2+} by turning pink with a strong fluorescence emission (Φ = 0.37, using rhodamine B as a standard in 1:9 CH_3CN-HEPES) which the optical change can be visualized by naked eyes. Moreover, solutions of **RTP** (10 μM) was also examined with various metal ions (10 μM) in CH_3CN-HEPES buffer (1:9, v/v, 50 mM pH 7.2) including competitive experiment using fluorometric technique. After Hg^{2+} addition, the fluorescent intensity was clearly visible while other metals did not cause any significant change in the intensity (Blue bar, Fig. 3). The results suggested that the spirolactam ring was only opened upon binding of Hg^{2+}. In the presence of miscellaneous competitive cations, Hg^{2+} still caused the higher fluorescence change (Red bar, Fig. 3). Obviously, the increase of the fluorescence intensity by the

addition of Hg^{2+} was not influenced by the subsequent addition of those cations.

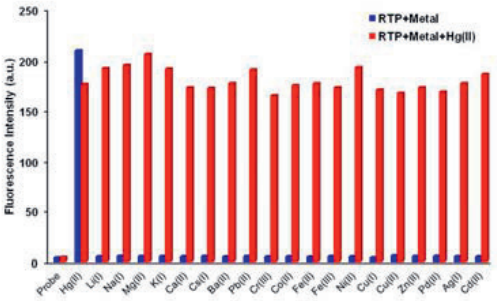

*Fig. 3. Fluorescence response of **RTP** (10 μM) in the selectivity and the competitive experiments with various metal ions (λ_{ex} = 550 nm. λ_{em} = 578 nm).*

Fluorescent titration

Fluorescence titration of **RTP** (10 μM) with Hg^{2+} (0-30 μM) revealed that fluorescence intensity of **RTP** was linearly proportional to the concentration of Hg^{2+} in the range of 0-8 μM (Fig. 4). The limit of detection (LOD) for Hg^{2+} of **RTP** is 1.6 nM (0.3 ppb).

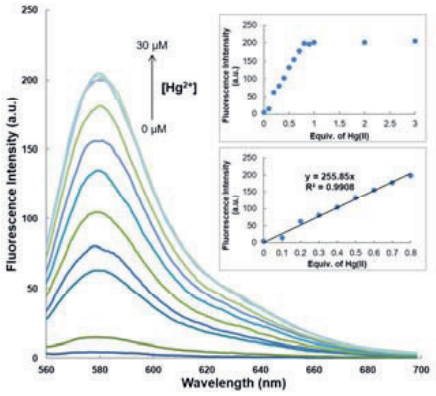

*Fig. 4. Fluorescence titration spectra of **RTP** (10 μM) upon the addition of increasing Hg^{2+} concentrations (0-30 μM) in CH_3CN-HEPES buffer (1:9, v/v, pH 7.2) by excitation at 550 nm.*

Sensing mechanism

An ESI-MS analysis of the mixing of **RTP** and excess Hg^{2+} disclosed three distinct molecular ions at m/z 634.3239, 457.2557 and 210.1029. Upon binding of Hg^{2+} with the thiourea sulfur atom, the N atom of the spirolactam was induced to react with the thiourea carbon and hence caused the ring opening of the spirolactam. After HgS removal and intramolecular guanylation, the benzimidazole appended rhodamine intermediate **2** (calcd for $C_{41}H_{40}N_5O_2$ *m*/*z* = 634.3182) was observed [2]. The benzimidazole moiety, as a good leaving group, underwent a hydrolysis reaction to yield the corresponding rhodamine methyl ester **3** (calcd for $C_{29}H_{33}N_2O_3$ *m*/*z* = 457.2491) and 2-(4-aminophenyl) benzimidazole **4** (calcd for $C_{13}H_{12}N_3$ *m*/*z* = 210.1031), respectively (Fig. 5).

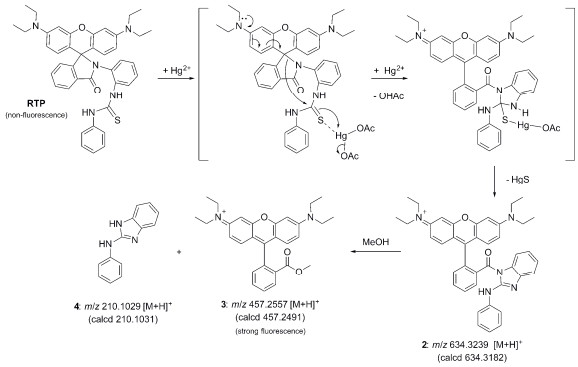

*Fig. 5. Proposed mechanism of Hg^{2+}-promoted ring opening of **RTP**.*

Fluorescent imaging

Bioimaging of HeLa cells incubated with only **RTP** probe revealed the cell morphology was not harmed in this event (a-e, Fig. 6). Evidently, the cells incubated with **RTP** and then Hg^{2+} exhibited an intense fluorescence emission (e-h, Fig. 6). The result clearly demonstrated that the **RTP** probe could permeate the cell membrane and showed a specific fluorescence emission only in presence of Hg^{2+}.

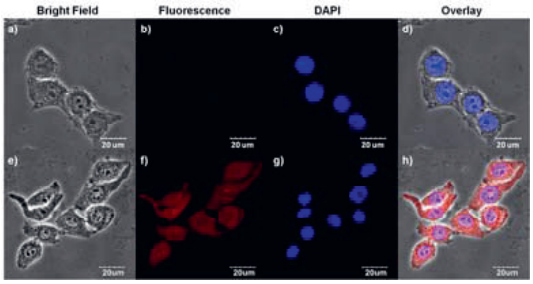

*Fig. 6. Confocal fluorescence images. The HeLa cells were incubated with Hg^{2+} (30 μM) in DPBS buffer, 1 h (e-h) and then incubated with **RTP** (10 μM), 1 h (a-h). (a, e) Bright-field images; (b, f) fluorescence images; (c, g) fluorescence images stained with DAPI; (d, h) merged images.*

Conclusion

RTP was a highly selective fluorogenic chemodosimeter to Hg^{2+} with a detection limit of 1.6 nM (0.3 ppb). Hg^{2+} promoted desulfurization of the probe and caused the ring-opening of the spirolactam in an irreversible fashion. Furthermore, **RTP** could be a beneficial fluorescence probe for bioimaging of Hg^{2+}.

References

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