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Carbazole phenylthiosemicarbazone-based ensemble of Hg^{2+} as selective fluorescence turn-on sensor toward cysteine in water

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ABSTRACT

A carbazole-phenylthiosemicarbazone-based open chemosensor has been designed and synthesized, which exhibits high selectivity toward Hg^{2+} by forming a 1:1 complex. In its Hg^{2+}ensemble, it is reported as a highly sensitive and selective probe for the detection of mercapto biomolecules in aqueous solution. The addition of Cys (Cysteine) to a 99% aqueous solution of carbazole–phenylthiosemicarbazone–Hg^{2+} ensemble resulted in a rapid and remarkable fluorescence OFF–ON (emission at 436 nm). The IMP logic gate has also been generated by choosing Hg^{2+} and Cys as input and by monitoring the output signal at 436 nm that originates from the emission spectra of chemosensor in the presence of Hg^{2+}.

Mercapto amino acids play several important roles in biological systems.[1,2] The important mercapto amino acids are cysteine (Cys), homocysteine (Hcy), and glutathione (GSH). These are essential biological materials required for the growth of cells and tissues in living systems. Specifically, Cys deficiency is involved in many syndromes for instances, slow growth in children, hair depigmentation, edema, lethargy, neurotoxicity, liver damage, loss of muscle and fat, skin lesions, and weakness.[3] Because of its important role in biological systems, the optical method for the detection and sensing of Cys, Hcy, and GSH with high sensitivity is of current interest in the chemosensor research field. Up to now, various biothiol chemosensors based on Michael addition,[4] cyclization reaction with aldehyde,[5] cleavage reaction by thiols,[6] or many analytical techniques, including UV–vis detection assays, mass spectrometry (MS),[7] gas chromatography,[8] high-performance liquid chromatography,[9] and electrochemical methods,[10] are the available techniques. Among these techniques, the fluorescent probes have been widely used to sense mercapto biomolecules[11] because of their simplicity, high selectivity, and sensitivity. However, there are relatively few examples of fluorescent chemosensors for Cys/Hcy/GSH, which are capable with dual monitoring of metal ions and amino acids in water. Among the thiophilic metal ions, Hg^{2+} has attracted much more attention because it could cause a variety of symptoms in vivo, including digestive, cardiac, kidney, and neurological diseases.[12,13] Therefore, detecting and sensing mercury and cysteine based on one fluorescent sensor with dual functionality is feasible. It is more economical, convenient and also of great significance to cell imaging as it is a reversible probe. Such detection may be achievable in such a way of Hg^{2+} coordinated thiosemicarbazone moiety followed by demetalization to generate fluorescence ON. Here, we designed a carbazole–thiosemicarbazone–Hg^{2+} ensemble based first displacement approach fluorescence sensor for detecting Cys in aqueous media.

In this work, we developed a new carbazole-based imine, **C1**, bearing two thiourea moieties with an extensively blue emission (Scheme 1). The thiourea unit was incorporated into **C1** to increase its water compatibility and emitting ability in the hydrophilic environment. As expected, **C1** gives rise to an extensive emission at 436 nm in aqueous solution with a quantum yield of 0.63. The emission can be completely quenched (over 97%) by 1 equiv of Hg^{2+} and recovered again (over 96%) with the addition of Cys. Both **C1** and its ensemble with Hg^{2+} exhibit good stabilities under a wide pH span from 6 to 10, covering physiological conditions. These properties of **C1** including high water solubility, longer emission wavelength (436 nm), and fluorescence OFF–ON to the guests are comparable with those of coumarin/anthracene based fluorescent probes.[14]

With admirable properties of **C1** in hand, herein, we further developed the ensemble complex carbazole–diphenyldithiosemic-

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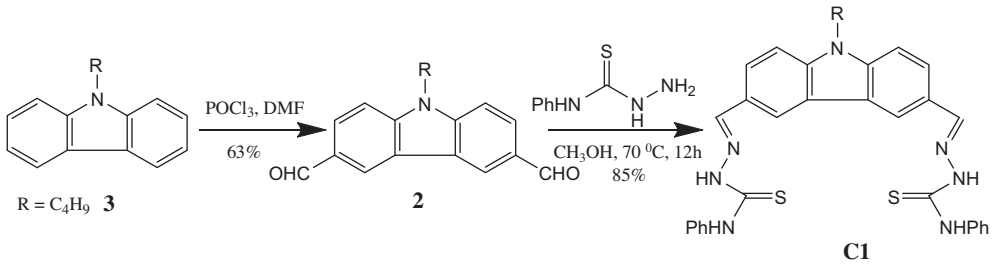


Scheme 1. Synthetic route to compound **C1**.

arbazone–Hg^{2+} as an exchangeable fluorescent probe, which exhibited high sensitivity and selectivity to mercapto biomolecules via a reversible decomplexation. Moreover, the complex of **C1** and Hg^{2+} was utilized as a chemosensing ensemble for Cys, Hcy and GSH detection, which showed highly sensitive and selective colorimetric response to thiols among the tested α-amino acids. Confocal fluorescence imaging reveals that **C1**/**C1**-Hg^{2+} can be applied to monitor the intracellular self-detoxification of foreign Hg^{2+} ions in candida cells.

The target compound **C1** was readily synthesized in two steps as shown in Scheme 1. Compound **C1** was synthesized by condensation of *N*-butylcarbazoledicarbaldehyde **2** and phenylthiosemicarbazide in high yield, and its structure has been proved by various spectroscopic characterizations. The detailed experimental procedures and ^{1}H and ^{13}C NMR spectra are summarized in Supporting Information.

The photophysical properties of **C1** were evaluated with physiologically important transition metal perchlorates/chlorides in 99% aqueous solution (containing 1% DMSO for complete dissolution of sample, 30 mM HEPES at pH 7.4). Generally, imine derived molecules are pH sensitive[15] upon irradiation. In this work, we found that carbazole-derived imine and its complex, **C1**/**C1**–Hg^{2+}, were stable enough even within a wide pH range (6–10). So, all the spectroscopic studies were performed in 99% aqueous solution (50 mM HEPES at pH 7.4) in which compound **C1** formed a colorless solution that was stable for more than three months. The ligand **C1** exhibited three absorption bands centered at 324, 365, and 382 nm; all of these bands showed a decrease in the absorbance upon addition of Hg^{2+}. Two clear isosbestic points were observed at 289 and 398 nm (Fig. 1), which are consistent with the presence of only two species, free ligand, and Hg^{2+}–ligand complex. The color of the solution changes from nearly colorless to yellow. This direct visualization of color change makes the detection straight forward. No obvious responses could be observed upon the addition of Na^{+}, K^{+}, Cu^{2+}, Co^{2+}, Pb^{2+}, Al^{3+}, Mg^{2+}, Ni^{2+}, Cd^{2+}, Fe^{3+}, and Zn^{2+}, respectively, (Fig. S1). These results clearly suggested that the metal complexation of **C1** shows a great preference for mercury ion over other cations. The binding constant was determined to be $1.98 \times 10^{4}\ M^{-1}$(Fig. S4).

Corresponding to its absorption spectra, the fluorescence spectrum of **C1** ($c = 1.0 \times 10^{-5}$(M), $\lambda_{ex} = 324$ nm) in 99% aqueous solution exhibited an intensive emission band at 436 nm and its fluorescence was completely quenched (over 98%) immediately upon the addition of 1 equiv of Hg^{2+} (Fig. 2).

As a result, excess Hg^{2+} cannot achieve further quenching of fluorescence. Based on the mole ratio method, mole ratio between **C1** and Hg^{2+} is 1:1, meanwhile, Job's plots highly indicated a 1:1 mol ratio. The detection limit of Hg^{2+} is about 2.54×10^{-10} M. Based on the 1:1 binding mode, the binding constant[16] derived from the fluorescence titration data was found to be $3.04 \times 10^{4}\ M^{-1}$ (Fig. S5). The 1:1 binding model of $Hg^{2+}$and **C1** can be further confirmed by mass spectra. The ESI mass spectrum of complex **C1**–Hg^{2+} has a major peak with m/z of 836.9674 [**C1**–Hg^{2+}+NaCl], which corresponds to 1:1 complex (Fig. S6).

Upon interaction with various metal ions, the fluorescence intensity at 436 nm was markedly quenched with Hg^{2+} ions, while other metal ions (Na^{+}, K^{+}, Cu^{2+}, Co^{2+}, Pb^{2+}, Al^{3+}, Mg^{2+}, Ni^{2+}, Cd^{2+}, Fe^{3+}, and Zn^{2+}) did not cause any noticeable responses(Fig. S7a). To find out whether **C1** can detect Hg^{2+} selectively even in the presence of other metal ions, competitive metal ion titrations were carried out. Of practical significance is that even 20 equiv each of these metal ions did not interfere in the sensing of Hg^{2+}, and the results of the competition experiments are shown in (Fig. S8). It is generally believed that, in most fluorescent molecules, the intro-

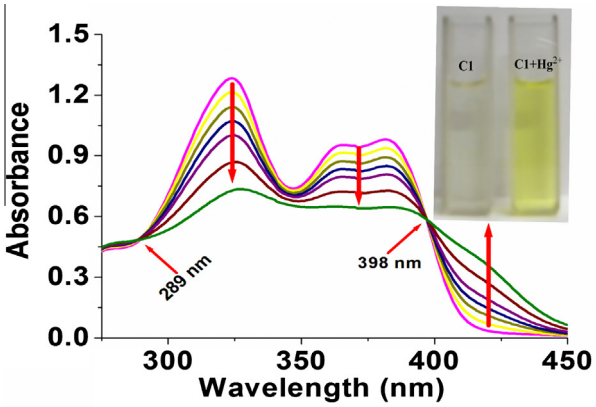


Fig. 1. UV–vis spectra of compound **C1**(10 μM) with the increasing concentrations of Hg^{2+} ions (0–2 equiv) in pH 7.4 HEPES buffer (25 mM, pH 7.4, containing 1.0% DMSO). Inset shows the change in color of compound **C1** (10 μM) upon addition of Hg^{2+} ions (10 μM).

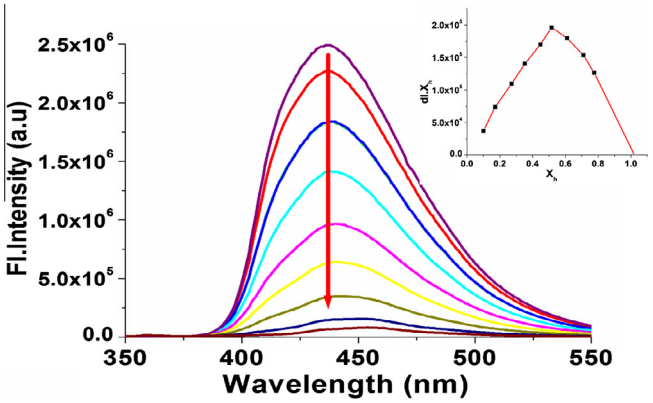


Fig. 2. Fluorescence spectra of compound **C1** (5 μM) with the increasing concentrations of Hg^{2+} ions (0–1.0 equiv) in pH 7.4 HEPES buffer (25 mM, pH 7.4, containing 1.0% DMSO). The inset shows Fluorescence Job plot of **C1** with Hg^{2+} at 436 nm.

duction of an extended conjugation structure to the rigid aryl ring will result in a red shift of the emission wavelength, as well as a drastic decrease of the fluorescent quantum yield because of its weaker coplanar effect.[17] Here, the origin of the fluorescence quenching may result from the electron or energy transfer from the excited carbazole fluorescence to the Hg^{2+} ion or the fluorescence quenching of **C1** at 436 nm (Fig. S7b) bound with Hg^{2+} may arise due to the MLCT-based[18] heavy metal ion effect. Alkali and alkaline earth metal ions showed no interaction with **C1**, which may be due to their hard acid properties. Thus, Hg^{2+} ion can be detected quantitatively in the presence of a number of biologically relevant metal ions.

As the stoichiometry of the complex formed between Hg^{2+} and **C1** was found to be 1:1 based on emission, absorption, and ESI MS studies, the nature of Hg^{2+} coordination with **C1** was studied by computational studies using the Gaussian 2003(B3LYP/6-31G(*d,p*)) software package.[19] The geometry optimizations for **C1** and **C1**–Hg^{2+} complex were done in a cascade fashion starting from semiempirical PM2 followed by ab initio HF to DFT B3LYP by using various basis sets, viz., PM2 → HF/STO-3G → HF/3-21G → HF/6-31G → B3LYP/6-31G(*d,p*). For **C1**–Hg^{2+} complex, a starting model was generated by taking the DFT optimized **C1** and placing the Hg^{2+} ion in between the diphenyldithiosemicarbazone moieties at a non-interacting distance. This model was then optimized initially using HF/3-21G level of calculations, and the output structure from this was taken as input for DFT calculations performed using B3LYP with SDDAll basis set for Hg^{2+} and 6-31+G(*d,p*) for all other atoms in the complex. The optimized complex of Hg^{2+} with **C1** (Fig. S19) showed that a distinct complexation occurs between Hg^{2+} and thiosemicarbazone sulfurs where two thiosemicarbazone sulfurs are bonded to the Hg^{2+} ion with a symmetrical Hg–S distance of 2.36 Å. These Hg–S distances are comparable with the experimental ones reported in the literature.[20]The spatial distributions and orbital energies of HOMO and LUMO of **C1** and **C1**–Hg^{2+} were also determined (Fig. S20). The π electrons on the HOMO of **C1**–Hg^{2+} complex are mainly located on the whole π-conjugated carbazole framework (excluding the *n*-butyl group), but the LUMO is mostly positioned at the center of the guest Hg^{2+} ion. Moreover, the HOMO–LUMO energy gap of complex becomes smaller relative to that of probe **C1**.

The energy gaps between HOMO and LUMO in the probe **C1** and **C1**–Hg^{2+} complex were 84.45 kcal mol^{-1} and 53.02 kcal mol^{-1}, respectively (see the supplementary data). The result clearly suggests that the two thiosemicarbazone moieties undergo conformational changes to bind to the Hg^{2+} in a symmetrical fashion.

Based on the results of emission, absorption titration experiments and ESI MS suggest that the probe **C1** clearly recognizes Hg^{2+}, the utility of [**C1**+Hg^{2+}] complex toward selective recognition of amino acid has been studied so that this complex can act as a secondary sensor for specific amino acid. To test this idea, the probe **C1**–Hg^{2+} ensemble based sensor was prepared by mixing equal equivalents of probe **C1** and mercuric chloride in the solution of HEPES buffer (50 mM, pH 7.4, containing 1% DMSO). We found that **C1**–Hg^{2+} ensemble is initially nonfluorescent (Φ_{fl} = 0.03), the addition of mercapto biomolecules such as Cys, Hcy, or GSH to the aqueous solution of **C1**–Hg^{2+} ensemble resulted in obvious spectral changes.

The fluorescence increase (about 40-fold) was observed, and the fluorescence quantum yield could be revived to 0.61, indicating that **C1**–Hg^{2+} ensemble could be applied as a fluorescent OFF–ON probe for Cys in aqueous solution. This is exactly reverse to what happens when probe **C1** is titrated with Hg^{2+}, as reported in this Letter, the Hg^{2+} is being removed from the complex by cysteine to release free **C1** and thus the recognition of cysteine by [**C1**+Hg^{2+}] ensemble as a secondary sensor.

Fluorescence titration of [**C1**+Hg^{2+}] ensemble was conducted in the solution of HEPES buffer (50 mM, pH 7.4, containing 1% DMSO) by the addition of cysteine from 0 to 1.0 × 10^{-5} M. About 2.1 equiv

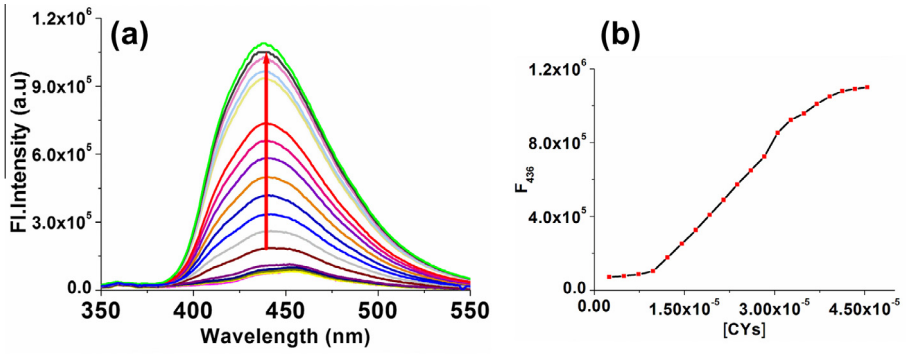
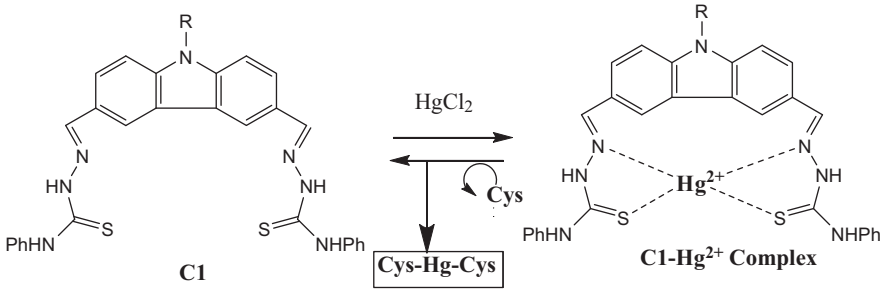


Fig. 3. (a) Fluorescence spectra of the **C1**-Hg^{2+} ensemble (5 μM) in pH 7.0 HEPES buffer (25 mM, pH 7.4, containing 1.0% DMSO) in the presence of Cysteine (0–2 equiv). (b) Fluorescence intensity changes at 436 nm of the ensemble (5 μM) in the presence of increasing concentrations of cysteine .



Scheme 2. Proposed displacement mechanism for sensing of cysteine.

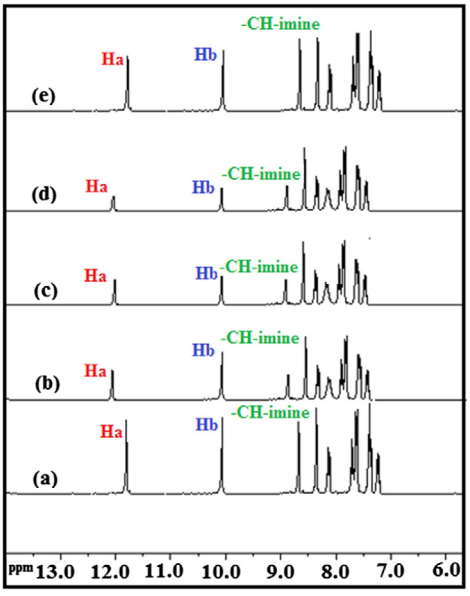
of cysteine makes the quenched fluorescence restore to the largest at 436 nm. Excess cysteine cannot achieve further enhancing of fluorescence. It is found that cysteine increases the FL intensity of [**C1**+Hg^{2+}] ensemble in a concentration dependence (Fig. 3b). The release of probe **C1** in the titration of cysteine is followed by the formation of [Cys–Hg–Cys] complex in Scheme 2 (Fig. S11), as it can form a stable complex with the thiol functionality.[21]

Both **C1** and [**C1**−Hg^{2+}] ensemble exhibit water solubility and biocompatibility, as well as good stability under a wide pH span from 6 to 10 covering physiological conditions (Fig. S12). These features of **C1** and [**C1**−Hg^{2+}] ensemble facilitate their practical applications for the determination of Cys. [**C1**−Hg^{2+}] ensemble displays a high sensitivity to Cys (the reaction can be completed within several seconds). Cys was added at different concentrations from 0 to 30 μM, and the fluorescence intensity of [**C1**−Hg^{2+}] ensemble (5.0 μM) at 436 nm was recorded to generate a calibration curve. A good linearity ($R = 0.995$) was found between the fluorescence intensity of the solution and the Cys concentration (Fig. 3b). This linear fitting analysis reveals that [**C1**−Hg^{2+}] is suitable for determining Cys from 0.5 to 30 μM. The detection limit of cysteine is about 9.6×10^{-11} M.

NMR experiments were performed to explore the coordination sites and sensing mechanism between probe **C1** and Hg^{2+}. The proton chemical shifts of the dithiosemicarbazone moieties in probe **C1** were distinctly shifted downfield upon the addition of 1.5 equiv of $HgCl_2$ in DMSO-d_6 and returned to the original values with the subsequent addition of 1.0 equiv of Cys. The titration of [**C1**+Hg^{2+}] ensemble with Cys could not be continued beyond 1 equiv owing to precipitation of [Cys+Hg^{2+}] complex. The family of ^{1}H-NMR spectra of probe **1** obtained by the titration of Hg^{2+} is shown in Fig. 4. The continuous addition of Hg^{2+} (from 0.5 to 1.5 equiv.) to the solution of probe **C1** caused minimal to marginal changes of δ values, a downfield shift by 0.46 ppm in the signal corresponding to –NHa (–CH═N–NHa–), whereas imine-H (–CH═N–) is shifted downfield by 0.19 ppm. These ^{1}H NMR changes, especially the move of thiosemicarbazone proton Ha, suggest that the Hg^{2+} ion of the [**C1**+Hg^{2+}] ensemble may be coordinated to the lone pair electrons on the sulfur atom and imine nitrogen. It is well-known that Hg^{2+} ions (a soft acid) can preferentially interact with sulfur (a soft base) according to Pearson's hard and soft acids and bases theory.[22] These results are also fully in line with the fluorescence spectrum changes (Fig. 2), which were observed for the [**C1**+Hg^{2+}] ensemble.

With the exception of Cys, other mercapto biomolecules such as Hcy and GSH also induced similar variations in the absorption and fluorescence spectra of [**C1**+Hg^{2+}]. Among these, Cys led to the largest fluorescence increase, whereas Hcy and GSH gave relatively smaller increases in fluorescence (Fig. S9).

For practical applications, an important consideration is its selective detection in the presence of other amino acids without



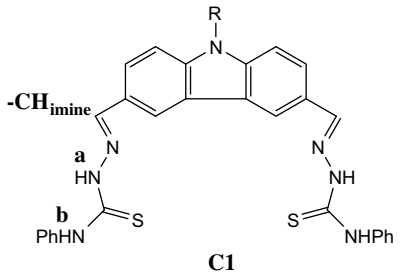


Fig. 4. ^{1}H NMR (400 MHz) spectra of Compound **C1** (a), **C1**+0.5 equiv Hg^{2+} (b), **C1**+1.0 equiv Hg^{2+} (c), and **C1**+1.5 equiv Hg^{2+} (d), **C1**+Hg^{2+} ensamble+2.0 equiv cysteine and (e) in d_6 DMSO-$D_2$0, it is appeared same as (a).

Entry	In_A Hg^{+2}	In_B Cys	Output Emission at 436nm
1.	0	0	1
2.	1	0	0
3.	0	1	1
4.	1	1	1

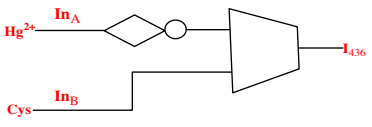


Fig. 5. Truth table and molecular circuit generated upon the addition of Hg^{2+} and cysteine to **C1**.

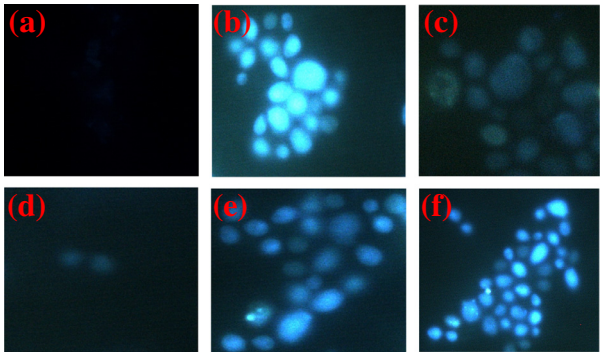


Fig. 6. (a) Fluorescence microscope images of *Candida albicans* cells only, (b) images of cells+**C1,** (c) images of cells+**C1**+Hg^{2+}(5 μM), (d) images of cells+**C1**+Hg^{2+} (25 μM) (e) images of cells+**C1**-Hg^{2+} ensemble+Cys and (f) same as (e) after 12 min.

thiol groups, such as glycine, alanine, valine, leucine, isoleucine, methionine, proline, tyrosine, lysine, histidine, serine, and tryptophan. As shown in Figure S10, the addition of other competitive amino acids to the solution of [**C1**+Hg^{2+}] did not result in any notable fluorescent increases. However, when Cys was added to the aqueous solution of [**C1**+Hg^{2+}] containing other competitive amino acids, an obvious increase in fluorescence was observed. This result indicates that [**C1**+Hg^{2+}] can detect Cys with a high selectivity over other coexisting amino acids.

Since fluorescence changes occur only in the presence of Cys and not with the other amino acids, the role of the –SH function in Hg^{2+} binding was further confirmed by studying the fluorescence response of [**C1**+Hg^{2+}] with different sulfur-containing systems, viz., Cys, GSH, and methionine (Met) (Fig. S9). Cys and GSH exhibited changes in the fluorescence owing to the presence of the –SH functionality, but Met does not show changes in the fluorescence owing to the presence of R–S–Me and not the –SH function. This experiment clearly suggests that Hg^{2+} mainly interacts through the –SH function of cysteine, and hence the [**C1**+Hg^{2+}] chemoensemble acts as a sensor for the thiol functionality.

In the present case, the logic gate properties of **C1** have been studied using two chemical input signals as Hg^{2+} and Cys, respectively, by monitoring the emission of **C1** at 436 nm (Fig. 3a). The titration of **C1** with a solution of Hg^{2+} ions (5.0×10^{-4} M), the emission band at 436 nm gets quenched.

Upon addition of aqueous solution of Cys (2.0×10^{-4} M) the original band at 436 nm gets recovered. Under the conditions where Hg^{2+} is absent and Cys is present, no characteristic change of emission is observed in the 436 nm band of **C1** (Fig. S13) and no significant output signal was observed. From these studies, it has been found that the perspective of logic functions of **C1** with respect to emission band at 436 nm can be used as a rarely reported IMPLICATION (IMP) logic gate toward Hg^{2+} (represented as an OR gate) in the absence of Cys by observing the emission at 436 nm. The mechanism by which IMPLICATION gate functions is suggested to be based on the formation of a stable mercury–Cys complex that causes the displacement of Hg^{2+} from [**C1**+Hg^{2+}] complex formed in solution. The truth table and the pictorial representation for the corresponding IMP are given in Figure 5.

To test the capability of [**C1**+Hg^{2+}] ensemble to image thiols in living cells,[23] *Candida* cells preloaded with [**C1**+Hg^{2+}] ensemble were treated with various amounts of Cys (Fig. 6). Initially, the *Candida* cells were incubated with probe **C1** (10 μM; Fig. 6b) in PBS buffer containing 1/500 DMSO for 30 min at 30 °C and then it was treated with $HgCl_2$ (100 μM) for12 min. Their fluorescence images became dim (Fig. 6c and d), implying that the intracellular uptake of Hg^{2+} ions complexed with probe **C1** yielded nonfluorescent [**C1**+Hg^{2+}] ensemble. Upon further incubation of these cells with various amounts of Cys (upto 200 μM) for 12 min, blue fluorescence imaging was recovered (Fig. 6e and f). The recurrent imaging indicated that the uptake of Cys resulted in the decomplexation of intracellular [**C1**+Hg^{2+}] ensemble to fluorescent **C1**. Through reversible fluorescence imaging, intracellular interconversion of [**C1**+Hg^{2+}] ensemble was explicitly illustrated. Therefore, the ON–OFF–ON fluorescence imaging of probe **C1** was accomplished in *Candida* cell lines by the intracellular complexation/decomplexation interaction modulated by Hg^{2+}/Cys. These results also indicate that probe **C1** is cell membrane permeable and able to response to Cys in the living cells.

In summary, we have synthesized a new carbazole-derived imine probe **C1**, which exhibits high selectivity toward Hg^{2+}. The selectivity has been demonstrated by fluorescence, absorption, and ESI MS spectroscopy. Most significantly, the in situ prepared mercury ensemble of **C1**, viz., [**C1**+Hg^{2+}], was able to detect selectively Cys among the naturally occurring amino acids to a lowest concentration of 9.6×10^{-11} M and it is exactly reverse to what happens when Hg^{2+} is added to probe **C1** in fluorescence spectroscopy. Hence probe **C1** acts as secondary sensor toward Cys.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.03.111.

References

1. (a) Seshadri, S.; Beiser, A.; Selhub, J. P.; Jacques, F. I.; Rosenberg, H.; D'Agostino, R. B.; Wilson, P. W.; Wolf, P. A. N. *Engl. J. Med.* **2002**, *346*, 476–483; (b) Ueland, P. M.; Vollset, S. E. *Clin. Chem.* **2004**, *50*, 1293–1295.
2. (a) Hassan, S. S. M.; Rechnitz, G. A. *Anal. Chem.* **1982**, *54*, 1972–1976; Hwang, C. A.; Sinskey, J.; Lodish, H. F. *Science* **1992**, *257*, 1496–1502; (c) Hong, R.; Han, G.; Fernandez, J. M.; Kim, B.-J.; Forbes, N. S.; Rotello, V. M. *J. Am. Chem. Soc.* **2006**, *128*, 1078–1079.
3. Shahrokhian, S. *Anal. Chem.* **2001**, *73*, 5972–5978.
4. (a) Yi, L.; Li, H.; Sun, L.; Liu, L.; Zhang, C.; Xi, Z. *Angew. Chem., Int. Ed.* **2009**, *48*, 4034–4037; Girouard, S.; Houle, M.-H.; Grandbois, A.; Keillor, J.; Michnick, S. W. *J. Am. Chem. Soc.* **2005**, *127*, 559–566; (c) Sreejith, S.; Divya, K. P.; Ajayaghosh, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 7883–7887; (d) Matsumoto, T.; Urano, Y.; Shoda, T.; Kojima, H.; Nagano, T. *Org. Lett.* **2007**, *9*, 3375–3377; (e) Hewage, H. S.; Anslyn, E. V. *J. Am. Chem. Soc.* **2009**, *131*, 13099–13106; (f) Guy, J.; Caron, K.; Dufresne, S.; Michnick, S. W.; Skene, W. G.; Keillor, J. W. *J. Am. Chem. Soc.* **2007**, *129*, 11969–11977; (g) Hong, V.; Kislukhin, A. A.; Finn, M. G. *J. Am. Chem. Soc.* **2009**, *131*, 9986–9994; (h) Lin, W.; Yuan, L.; Cao, Z.; Feng, Y.; Long, L. *Chem. Eur. J.* **2009**, *15*, 5096–5103.
5. (a) Tanaka, F.; Mase, N.; Barbas, C. F., III *Chem. Commun.* **2004**, 1762–1763; (b) Duan, L.; Xu, Y.; Qian, X.; Wang, F.; Liu, J.; Cheng, T. *Tetrahedron Lett.* **2008**, *49*, 6624–6627; (c) Kim, T.-K.; Lee, D.-N.; Kim, H.-J. *Tetrahedron Lett.* **2008**, *49*. 4879–4781; (d) Lee, K.-S.; Kim, T. K.; Lee, J. H.; Kim, H.-J.; Hong, J.-I. *Chem. Commun.* **2008**. 6173-6139; (e) Zhang, X.; Ren, X.; Xu, Q.-H.; Loh, K. P.; Chen, Z.-K. *Org. Lett.* **2009**, *11*, 1257–1260; (f) Lin, W.; Long, L.; Yuan, L.; Cao, Z.; Chen, B.; Tan, W. *Org. Lett.* **2008**, *10*, 5577–5580; (g) Li, H.; Fan, J.; Wang, J.; Tian, M.; Du, J.; Sun, S.; Sun, P.; Peng, X. *Chem. Commun.* **2009**, 5904–5906.
6. (a) Pires, M. M.; Chmielewski, J. *Org. Lett.* **2008**, *10*, 837–840; (b) Tang, B.; Xing, Y.; Li, P.; Zhang, N.; Yu, F.; Yang, G. *J. Am. Chem. Soc.* **2007**, *129*, 11666–11667; (c) Maeda, H.; Matsuno, H.; Ushida, M.; Katayama, K.; Saeki, K.; Itoh, N. *Angew. Chem., Int. Ed.* **2005**, *44*, 2922–2925; (d) Ji, S.; Yang, J.; Yang, Q.; Liu, S.; Chen, M.; Zhao, J. *J. Org. Chem.* **2009**, *74*, 4855–4865; (e) Bouffard, J.; Kim, Y.; Swager, T. M.; Weissleder, R.; Hilderbrand, S. A. *Org. Lett.* **2008**, *10*, 37–40.
7. Guan, X.; Hoffman, B.; Dwivedi, C.; Matthees, D. P. *J. Pharm. Biomed. Anal.* **2003**, *31*, 251–261.
8. Capitan, P.; Malmezat, T.; Breuille, D.; Obled, C. J. *Chromatogr., B Biomed. Sci. Appl.* **1999**, *732*, 127–135.

9. Qian, X.-X.; Nagashima, K.; Hobo, T.; Guo, Y.-Y.; Yamaguchi, C. *J. Chromatogr., A* **1990**, *515*, 257–264.
10. Hiraku, Y.; Murata, M.; Kawanishi, S. *Biochim. Biophys. Acta* **2002**, *1570*, 47–52.
11. (a) Jung, H. S.; Ko, K. C.; Kim, G.-H.; Lee, A.-R.; Na, Y.-C.; Kang, C.; Lee, J. Y.; Kim, J. S. *Org. Lett.* **2011**, *35*, 1498–1501; (b) Yue, Y.; Guo, Y.; Xua, J.; Shao, S. J. *New. J. Chem.* **2011**, *35*, 61–64; (c) Zhang, R.; Yu, X. J.; Ye, Z. Q.; Wang, G. L.; Zhangand, W. Z.; Yuan, J. L. *Inorg. Chem.* **2010**, *49*, 7898–7903; (d) Yang, Y. K.; Shim, S. Y.; Tae, J. S. *Chem. Commun.* **2010**, *46*, 7766–7768; (e) Long, L. L.; Lin, W. Y.; Chen, B.; Gao, W. S.; Yuan, L. *Chem. Commun.* **2011**, *47*, 893–895; (f) Shiu, H.-Y.; Wong, M.-K.; Che, C.-M. *Chem. Commun.* **2011**, *47*, 4367–4369; (g) Xu, L.; Xu, Y. F.; Zhu, W. P.; Zeng, B. B.; Yang, C. M.; Wu, B.; Qian, X. H. *Org. Biomol. Chem.* **2011**, *9*, 8284–8287; (h) Huang, X. M.; Guo, Z. Q.; Zhu, W. H.; Xie, Y. S.; Tian, H. *Chem. Commum.* **2008**, *41*, 5143–5145; (i) Zhu, W. H.; Huang, X. M.; Guo, Z. Q.; Wu, X. M.; Yu, H. H.; Tian, H. *Chem. Commun.* **2012**, *48*, 1784–1786; (j) Zhang, J. F.; Park, M.; Ren, W. X.; Kim, Y. S.; Kim, J.; Jung, J. H.; Kim, J. S. *Chem. Commun.* **2011**, *47*, 3568–3570; (k) Zhang, J. F.; Kim, S.; Han, J. H.; Lee, S.-J.; Pradhan, T.; Cao, Q. Y.; Lee, S. J.; Kang, C.; Kim, J. S. *Org. Lett.* **2011**, *13*, 5294–5297; (l) Zhou, Y.; Yoon, J. *Chem. Soc. Rev.* **2012**, *41*, 52–67.
12. Tchounwou, P. B.; Ayensu, W. K.; Ninashvili, N.; Sutton, D. *Environ. Toxicol.* **2003**, *18*, 149–175.
13. (a) Jalilehvand, F. B.; Leung, O.; Izadifard, M.; Damian, E. *Inorg. Chem.* **2006**, *45*, 66–73; (b) Pfohl-Leszkowicz, A.; Molinie, A.; Tozlovanu, M.; Manderville, R. A. *Food Contam.* **2008**, 56–79.
14. (a) Kim, H. N.; Lee, M. H.; Kim, H. J.; Kim, J. S.; Yoon, J. *Chem. Soc. Rev.* **2008**, *37*, 1465–1472; (b) Liu, Z.; He, W.; Guo, Z. *Chem. Soc. Rev.* **2013**, *42*, 1568–1600.
15. Nolan, E. M.; Burdette, S. C.; Harvey, J. H.; Hilderbrand, S. A.; Lippard, S. J. *Inorg. Chem.* **2004**, *43*, 2624–2635.
16. Bourson, J.; Pouget, J.; Valeur, B. *J. Phys. Chem.* **1993**, *97*, 4552–4557.
17. Coskun, A.; Akkaya, E. U. *J. Am. Chem. Soc.* **2006**, *128*, 14474–14475.
18. Jung, H. S.; Kwon, P. S.; Lee, J. W.; Kim, J. I.; Hong, C. S.; Kim, J. W.; Yan, S.; Lee, J. Y.; Lee, J. H.; Joo, T.; Kim, J. S. *J. Am. Chem. Soc.* **2009**, *131*, 2008–2012.
19. (a) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789; (b) Miehlich, B.; Savin, A.; Stoll, H.; Preuss, H. *Chem. Phys. Lett.* **1989**, *157*, 200–206.
20. Ahamed, B. N.; Arunachalam, M.; Ghosh, P. *Inorg. Chem.* **2010**, *49*, 943–951.
21. Wang, Z. G.; Elbaz, J.; Willner, I. *Nano Lett.* **2011**, *11*, 304–309.
22. Pearson, R. G. *J. Am. Chem. Soc.* **1963**, *85*, 3533–3539.
23. Chung, T. K.; Funk, M. A.; Baker, D. H. *J. Nutr.* **1990**, *120*, 158–165.