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ARTICLE TYPE

Imino−Phenolic−Azodye Appended Rhodamine as Primary Fluorescence "Off−On" Chemosensors for Tin (Sn^{4+}) in Solution and in Raw Cells and the Recognition of Sulphide by [AR-Sn]

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A new azo-rhodamine based, **AR** was developed to act as 'Off-On' reversible luminescent probe for Sn^{4+} detection. The chemosensing behavior of the **AR** has been demonstrated through fluorescence, absorption, visual fluorescence color changes, ESI MS and ^1H NMR titrations. This chemosensor **AR** shows a significant visible color change and displays a remarkable luminescent switch on (> 2300 fold) in the presence of Sn^{4+} ions. The chemosensor can be used as a 'naked eye' sensor. The roles of the fluorophore-photochrome (azodye) dyad platform as well as the iminophenolic binding core in **AR**'s selective recognition of tin have been demonstrated by studying appropriate control molecules. Importantly, **AR** can selectively recognize Sn^{4+} in organo-aquous media in the presence of other cations. The biological applications of **AR** were evaluated in RAW cells and it was found to exhibit low cytotoxicity and good membrane permeability for the detection of Sn^{4+}. The development of practically viable colorimetric test strips of the chemosensor **AR** to detect Sn^{4+} was also reported. It has been possible to build an INHIBIT logic gate for two binary inputs *viz.,* Sn^{4+} and S^{2-} by monitoring the fluorescence emission band at 582 nm as output.

Introduction

The development of highly sensitive and selective fluorescent chemosensors for heavy and transition-metal ions has attracted tremendous interest because of their importance in chemistry, biology, and environmental science.[1-4] The development of tin (Sn^{4+}) fluorescent chemosensors has attracted intense attention due to the concern over the adverse effect of tin on the environment and human health due to excess accumulation. Tin, widespread in the air, water and soil, is one of the most commonly used heavy metals in agricultural, industry,[5] including food container, food processing equipment, toothpaste, perfumes, soaps, food additives and dyes. Again organotin compounds are used to make plastics, plastic pipes, PVC stabilizer, pesticides, paints, and pest repellents.[6] Inorganic tin compounds are used as pigments in the ceramic and textile industry.[7] However , human and various animal studies show that excess accumulation of tin can cause eye and skin irritation, headaches, stomachaches, sickness and dizziness, breathlessness, urination problems, liver damage, malfunctioning of immune systems, chromosomal damage, gastrointestinal effects (abdominal cramps, nausea, diarrhoea, vomiting).[8] Thus, there is a strong need for tin selective chemosensors that rapidly detect Sn^{4+} in aqueous media by simple spectroanalysis.

A promising way is to develop optical chemosensors for detecting Sn^{4+} ions, which are based on an indicator that is capable of reporting on the selectivity of Sn^{4+} ions recognition through a variety of optical responses, mainly due to their distinct advantages in sensitivity, selectivity and fluorescence imaging in living cells. Among numerous indicators, rhodamine-based dyes are a kind of excellent candidate for the construction of an off/on-type fluorescent chemosensor due to their excellent spectroscopic properties of large molar extinction coefficients, high fluorescence quantum yields, and long absorption and emission wavelengths elongated to the visible region.[9] The metal ion sensing mechanism of these sensors is based on the change in structure between the spirocyclic and open-cycle forms. Typically, these sensor molecules prefer their spirolactam ring-closed state, which shows little absorption or fluorescence, whereas ring-opening of the corresponding spirolactam induced by metal ions gives rise to orange fluorescence and a clear color change from colorless to pink. Therefore, most of the reported rhodamine-based chemosensors for metal ions[10] are of colorigenic or fluorogenic type.

However, Sn^{4+} responsive fluorescent probes are barely explored. To date, very few Rhodamine B-based probe for Sn^{4+} has been reported, whose application is limited by its low sensitivity and water solubility.[11] Recently, our group developed a oxo-chromene–rhodamine B-derivative acts as a selective colorimetric

and fluorometric Sn^{4+} chemosensor in water/organic two phase detection system.[12] The colorless spirolactam form is converted into the pink colored ring opening form upon binding with Sn^{4+}, which shows a distinctive absorption band at 555 nm. The addition of Sn^{4+} resulted in a prominent enhancement (14 fold) of fluorescence at 580 nm, which allows detection limit of Sn^{4+} (2.58 μM) by a fluorescent analysis.

However, in the present case, we report the design and synthesis of a new Rhodamine B-based probe operating in turn-on mode for selective and sensitive detection of Sn^{4+} ions in a water-organic solvent mixture. Here, the change in spirocycle to open-ring form of the rhodamine fragment in **AR** result in the remarkable enhancement of emission intensities (> 2300 fold), and these offer us the possibility of studying the Sn^{4+} recognition process through the *switch-on* optical response-a criterion that is important for developing an in-field detection reagent. The chemosensor is composed of a rhodamine dye, a hydroxyl group, and an azo moiety. We reasoned that Sn^{4+} could coordinate to the novel receptor formed by the hydroxyl group and an imine unit due to its selective coordinating character.[13] This coordination may induce the transformation of the rhodamine dye from the non-fluorescent spirocyclic form to the highly fluorescent opened-ring form for a significant fluorescence enhancement signal. To achieve high selectivity, we designed a new reversible and selective chemosensor (**AR**) for tin ions by simple structural modifications of our previously reported receptors.[12] Obviously, the structural modifications should: (1) introduction of nonfluorescent azodye quencher; (2) significantly improve the sensitivity for tin (> 2300 fold) over other ions; (3) taking model compounds by the combination of azo and fluorophore moieties. It is noteworthy that this design concept has not been previously employed in the construction of Sn^{4+} fluorescent probes.

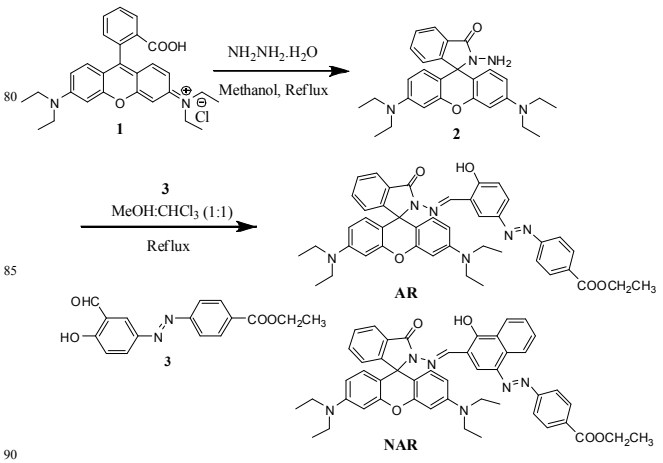
Experimental Section

Experimental details corresponding to the materials and methods used, synthesis, characterization, solution preparation for absorption and fluorescence titrations, determination of association constant, calculation of detection limit, effect of p^H, cellular and computational studies are given in the Supporting Information (pp S3−S8).

Synthesis of AR. A 326 mg portion of 5-(4-carboethoxyphenylazo)salicylaldehyde (1.1 mmol) was dissolved in 15 ml dry chloroform with continuous stirring and then 500 mg (1.1 mmol) of Rhodamine B hydrazide in 15 ml dry methanol was added to the solution and heated to reflux for 12 hours. A yellow precipitate was appeared. After that the reaction mixture was cooled to room temperature and then the precipitate was collected through filtration. The residue was washed several times with ethanol to isolate **AR** in pure form with 90% yield. M.P. > 250˚C. ^{1}H NMR (400 MHz, $CDCl_3$, $Si(CH_3)_4$, J (Hz), δ(ppm)): 11.57(1H, s, -OH), 9.14(1H, s, -CH=N), 8.15 (2H, d, J=8.44 Hz), 7.99 (1H, d, J=6.92 Hz), 7.84 (3H,d, J=8.24 Hz), 7.76 (1H, d, J=2.08 Hz), 7.53(2H, m), 7.18 (1H, d, J=7.04 Hz), 6.98 (1H, d, J=8.84 Hz), 6.49 (4H, dd, J= 3.36 & 9.36 Hz), 6.27(2H, dd, J= 2.24 & 2.28 Hz), 4.39 (2H, q, J=7.16 Hz), 3.32 (8H, q, J=7.04 Hz, -N$\underline{CH_2}CH_3$), 1.41(3H, t, J= 7.12 Hz), 1.15 (12H, t, J= 7.00 Hz, -$NCH_2\underline{CH_3}$).[13] C NMR ($CDCl_3$, 400 MHz) δ(ppm): = 12.58, 14.10, 44.35, 61.17, 66.34, 98.01, 105.01, 108.21, 118.00, 118.76, 122.27, 123.48, 124.13, 125.26, 127.93, 128.02, 128.63, 129.12, 129.34, 129.85, 130.52, 131.54, 133.74, 145.59, 149.17, 153.43, 155.19, 162.03, 164.43, 166.14. TOF MS ES^+, m/z = 736.9504, calc. for $C_{44}H_{44}N_6O_5$ =736.8686.

Results and discussion

The receptor **AR** was prepared according to Scheme 1. Initially the intermediate azo compound 5-(4-carboethoxyphenylazo)salicylaldehyde was synthesized according to our reported procedure.[14] The Schiff base chemosensor **AR** was prepared in 90% yield as yellow solid from the condensation of 5-(4-carboethoxyphenylazo)salicylaldehyde with rhodamine B hydrazide in an equal molar ratio in methanol and chloroform mixture under refluxing condition. A control molecule contain naphthylazo platform, **NAR** has been synthesized by similar diazocoupling and condensation reactions starting from 2-formyl-1-naphthol instead of salicylaldehyde. **AR** and **NAR** were well characterized by ^{1}H NMR, ^{13}C, FTIR and mass analyses (Figure S1-S3, S9A and S5-S6 Supporting Information).



Scheme 1. Synthetic approach for the chemosensor **AR** and control molecule **NAR**

Fluorescence titrations of **AR** with Sn^{4+} in aqueous EtOH (HEPES, 10 mM, pH = 7.4, 1: 4, v/v) were performed. Probe **AR** exhibited almost no fluorescence in aqueous EtOH (HEPES, 10 mM, pH = 7.4, 1:4, v/v), indicating that the spirocyclic form is retained in solution. However, with the addition of increasing concentrations of Sn^{4+}, a excellent enhancement (up to 2428-fold) in fluorescence intensity at 582 nm was noticed, which was much higher than the result obtained with the early reported Sn^{4+} sensors.[15] The large fluorescence turn-on was corroborated by the observation that the emission color of the sensor solution turned from dark to orange (Figure 1a, inset), which indicates that probe **AR** is an excellent turn-on sensor for Sn^{4+}.

A more than thousand-fold increase observed in the quantum yield (QY) of [**AR**+ Sn^{4+}] (0.216±0.004) as compared to that of **AR** (1.16×10^{-4}) supports the fluorescence enhancement of **AR**

observed in presence of Sn^{4+}, suggesting a sensitive and selective detection of Sn^{4+} by **AR** compared to other metal ions. The recognition interaction was completed immediately after the addition of Sn^{4+} within 2 mins and hence, **AR** could be used in real-time determination of Sn^{4+} in environmental and biological conditions. The formation of an intense absorption peak at 563 nm in the absorption profile of probe **AR** upon the addition of Sn^{4+} is in good agreement with the fluorescence enhancement (Figure 1a).

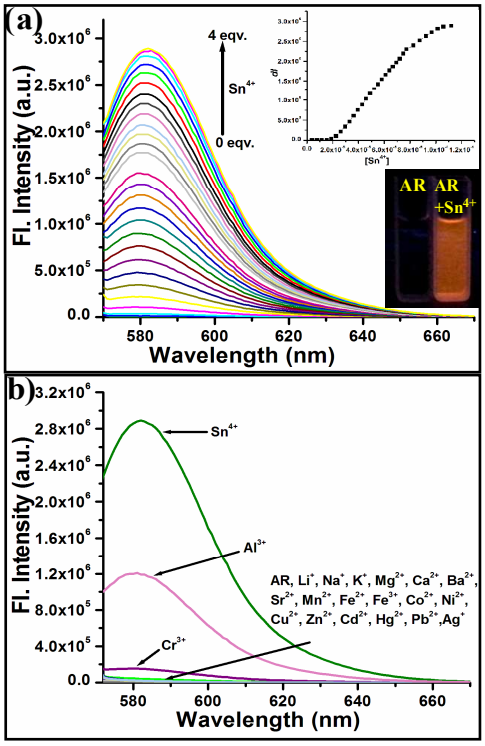
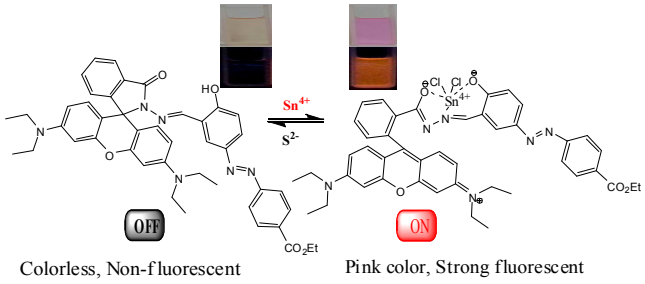


Figure 1. (a) Change in the emission spectra of ligand **AR** [c = 4× 10^{-5} M, EtOH / H_2O = 4: 1, v/v, 10 mM HEPES buffer, pH = 7.4, λ_{ext}=563 nm) upon addition of Sn^{4+} ions (c = 4× 10^{-4} M). Inset: Change of emission intensity at 582 nm with incremental addition of Sn^{4+} [λ_{ext}=563nm] and fluorescence photographs of **AR** before and after addition of Sn^{4+} ions. (b) Change in the fluorescence emission of **AR** in presence of different metal ions such as Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Sn^{4+}, Cr^{3+}, Al^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Sn^{4+} and Ag^+ in aq. EtOH (EtOH : H_2O = 4 : 1, v/v, 10 mM HEPES buffer, pH = 7.4) (λ_{ext}= 563 nm).

Importantly, the sensor showed a nice linear relationship between the fluorescence intensity at 582 nm and the concentrations of Sn^{4+} from 3.96 to 112 μM (Figure 1a, inset), suggesting that sensor **AR** is potentially useful for quantitative determination of Sn^{4+} with a large dynamic range. Similar fluorescence studies were carried out with the control molecule such as **NAR** and found very small amount of change (only 3.7 fold) in the emission intensity compare to **AR**, suggesting that fluorophore (naphthyl moiety)-photochrome (azodye part) dyad activates intercomponent electron or energy transfer pathways and weakens the emission of the fluorophore (Figure S14 B, Supporting Information).[16]

The Job plot [17] shows that sensor **AR** forms a 1: 1 complex with Sn^{4+} ions (Figure S8A, Supporting Information). The 1:1 binding mode of the sensor with Sn^{4+} was also confirmed by the ESI MS mass spectrum of the complex (Figure S4, Supporting Information), which showed an intense peak at m/z = 943.3710 (calc. 943.5153), assigned to the 1 : 1 complex [**AR** + Sn^{4+} + $2Cl^-$ + NH_4^+]$^+$. Therefore, we suggest that probe **AR** coordinates with Sn^{4+} with 1: 1 stoichiometry. Based on a 1:1 binding mode, apparent association constant (Ka) of the **AR**-Sn^{4+} interaction was calculated to be (1.414±0.41) × 10^4 M^{-1} (Figure S8B, Supporting Information) from the data of the fluorescence titration experiments using nonlinear curve fitting procedure.[18, 19d] The detection limit for Sn^{4+} ion with **AR** was estimated to be 7.1 μM. Figure S9 (Supporting Information) shows the partial IR spectra of sensor **AR** in the absence or presence of 1 equiv. of Sn^{4+}. The peak at 1617.32 cm^{-1}, assigned to the characteristic amide carbonyl absorption, was shifted to 1592.61 cm^{-1} in the presence of Sn^{4+}, indicating that the amide carbonyl group is involved in the interactions with Sn^{4+}. This is key to the spiro ring-opening and fluorescence turn-on of the rhodamine dye. Taken together, a likely sensing mechanism based on the Sn^{4+}-triggered spiro ring-opening process is proposed in Scheme 2. We then proceeded to examine the selectivity of the sensor. The selectivity of compound **AR** to the various metal ions was tested as selectivity is an important characteristic feature of an ion-selective chemosensor. We tested our chemosensor with possible interferences including metal ion salts of Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Cr^{3+}, Al^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+} and Ag^+ in aqueous EtOH (HEPES, 10 mM, pH = 7.4, 1:4, v/v) (Figure 1b). Remarkably, only Sn^{4+} elicited a large fluorescence enhancement. By contrast, alkali metals ions (Li^+, Na^+, K^+) and alkali-earth metals ions (Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}) even in the presence of large excess (100 equiv.) have no observable fluorescence response.



Scheme 2. Schematic Presentation Showing the Possible Binding Mechanism of **AR** with Sn^{4+}

Furthermore, sensor **AR** gave only a minimal response to transition metal ions such as Al^{3+} and Cr^{3+}, indicating that the sensor is highly selective. To explore the utility of **AR** as an ion-selective chemsensor, the competition experiments was carried out by adding Sn^{4+} to **AR** solution in presence of other competitive metal ions (Figure S10, Supporting Information). As shown in Figure 1b, Sn^{4+} induced fluorescent responses were not hardly influenced by these common coexistent metal ions (Figure S12, Supporting Information). Thereby, sensor **AR** appears to be

useful for selectively sensing Sn^{4+} even in the presence of other relevant metal ions.

UV−vis spectra recorded for **AR** in EtOH/H_2O HEPES buffer (10 mM, pH 7.4; 4:1, v/v) indicated three spectral bands at 267 nm, 276 nm and 320 nm respectively, which may possibly be attributed to intraligand charge transfer (CT) transition.

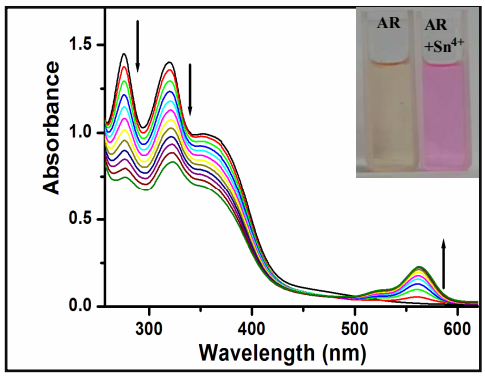
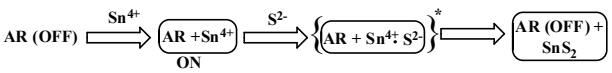


Figure 2 : Change in the absorption spectrum of receptor **AR** [c = 4× 10^{-5} M, EtOH / H_2O = 4 : 1, v/v, 10 mM HEPES buffer, pH = 7.4) upon addition of increasing amounts of Sn^{4+} ions (c = 4× 10^{-4} M). Inset showing the change in color of **AR** solution before and after addition of Sn^{4+}.

Upon addition of increasing concentrations of Sn^{4+} ions to the solution, a new absorption band centered at 563 nm appeared with increasing intensity and other three bands decrease gradually resulted in an isosbestic point at 473 nm (Figure 2). Switch on responses for the absorption spectral band at 563 nm and the luminescence band at 582 nm on binding to Sn^{4+} suggest opening of the spirolactam ring in **AR** on metal ion coordination. This type of enhancement was not observed when the analogous experiments were conducted with other cations (Figure S11, Supporting Information). On the other hand, the control molecule, **NAR** did not show any significant change in the absorbance as well as necked eye response when titrated with Sn^{4+}, suggesting that **AR** is sensitive and selective toward Sn^{4+} (Figure S14A, Supporting Information). Interestingly, a solution of **AR** in optimized EtOH/H_2O solution (4: 1, v/v, HEPES buffer, pH = 7.4) is colorless and emits no orange fluorescent light, but during the fluorometric titration of **AR** with Sn^{4+} ions the colorless solution of the receptor became deep orange (Figure 1a, inset). This orange fluorescent color is attributed to the opening of the spirolactam ring and generation of the delocalized xanthenes moiety.[19] Whereas compound **AR** shows obvious pink color in buffered ethanol /HEPES solution upon addition of Sn^{4+} under visible light (Figure 2, inset). This was not observed with other metal ions except Cu^{2+}, Cr^{3+} and Al^{3+} ions. In case of Cu^{2+}, Cr^{3+} and Al^{3+} ions, the colorless solution of **AR** turned into a red and faint pink color respectively (Figure S12, Supporting Information). The results support our expectation that **AR** could serve as a sensitive naked-eye probe for Sn^{4+}.



Scheme 3. Schematic Presentation for Fluorescence Quenching

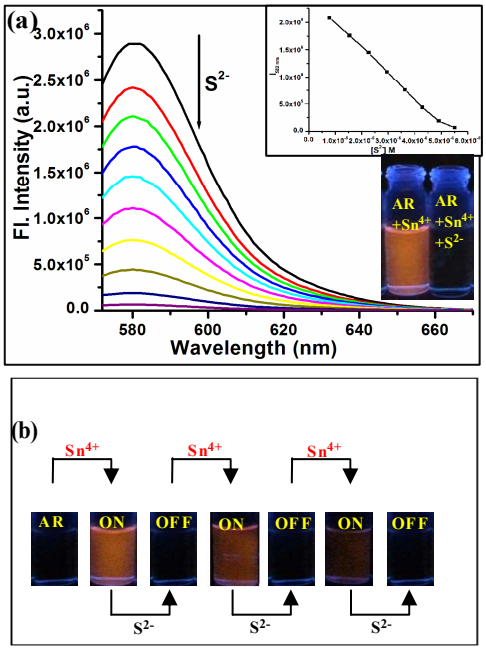


Figure 3: (a) Change in the emission spectra of ligand **AR** [c = 4× 10^{-5} M, EtOH / H_2O = 4 : 1, v/v, 10 mM HEPES buffer, pH = 7.4, λ_{ext}=563 nm) with 4 equiv of Sn^{4+} upon addition of sodium sulfide (c = 4× 10^{-4} M). Inset: fluorescence photographs of **AR**-Sn complex after addition of S^{2-} ions. (b) Fluorescence experiment showing on-off reversible visual fluorescent color changes after each addition of Sn^{4+} and S^{2-} sequentially.

The reversibility is an important aspect of any receptor to be employed as a chemical sensor for detection of specific metal ions. To examine whether the process is reversible, an excess amount of Na_2S was added into the solution of sensor **AR** pre-incubated with Sn^{4+}. The bright fluorescence immediately turned off (Inset, Figure 3a). We also studied fluorometric titration experiments with a series of different anions such as F^-, Br^-, I^-, NO_3^-, NO_3^-, SCN^-, SO_4^{2-}, SO_3^{2-}, ClO_4^-, CH_3COO^-, HSO_4^-, and $H_2PO_4^-$. Among these only addition of S^{2-} to the complex solution of **AR** brought the reverse change in the emission spectra due to the regeneration of **AR** (Figure S13A, Supporting Information).This is attributable to the binding of S^{2-} ions to the [AR + Sn^{4+}] followed by the removal of Sn^{4+} in the form of SnS_2, thus releasing the free AR (Scheme 3). Interestingly, while Na_2S diminished emission significantly, further addition of excess Sn^{4+} ions could recover emission signals with less intense color in a reversible manner (Figure 3b). This result implies the reversible character of the binding of sensor **AR** with Sn^{4+} (Figure 3). Furthermore, like Na_2S, addition of aq. solutions of Na_2EDTA brought about similar change in emission spectra (Figure S13B, Supporting Information) and further confirmed the reversibility in the binding process. Thus, fluorescent chemosensors are reversible in general.[20]

To get insight into the binding mode, the ^1H NMR titration experiments were carried out. As shown in Figure 4, the ring protons of rhodamine and salicylaldehyde moieties were moved to apparent downfield region ($\Delta\delta_c$ = 0.60, $\Delta\delta_{d,e}$ = 0.37, $\Delta\delta_f$ = 0.19, $\Delta\delta_{g,h}$= 0.05 and $\Delta\delta_i$= 0.13 ppm) in the presence of Sn^{4+}.

This downfield movement is ascribed to the decrease in electron density arising from an intramolecular charge transfer from NEt_2

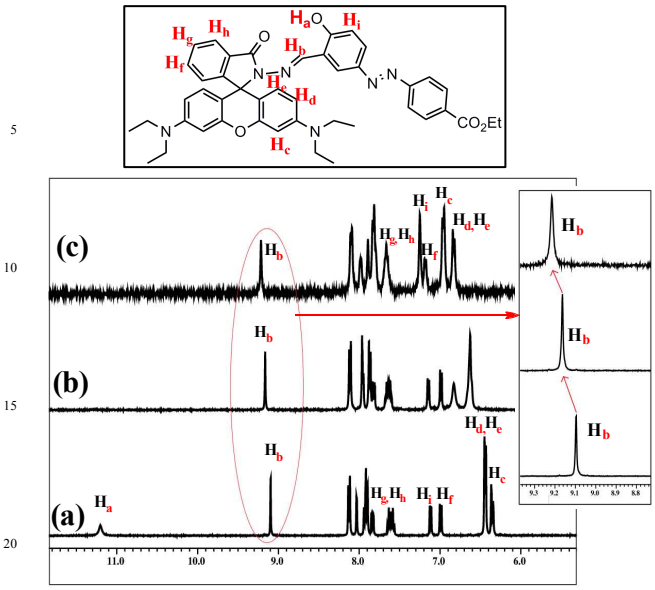
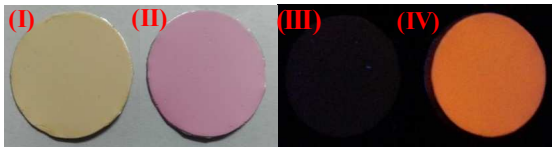


Figure 4. Partial ^{1}H NMR of (a) **AR** (3×10^{-3} M) and with (b) 0.5 equiv. (c) 1 equiv. amounts of Sn^{4+} in D_2O .

group to spirolactam ring opening during metal ion complexation. Specially, the imine proton (H_b) at around δ 9.11 ppm was considerably shifted downfield toward δ 9.22 ppm upon Sn^{4+} addition, indicating that a decrease in electron density at imine nitrogen resulting from direct coordination with Sn^{4+}.

To explore potential and analytical applications of the chemosensor **AR** for Sn^{4+} ions tested, the test strips was carried out.[21] The strips was prepared by using a TLC plates which were further immersed into the solution of AR (4×10^{-4} M) in ethanol and then drying it by two way (i) exposure to air and (ii) in vacuum.



Under ambient light **Under fluorescent light**

Figure 5. Colorimetric and fluorometric test kit. Photographs of the TLC plate coated with **AR** used for the detection of Sn^{4+} ions in solution: **AR** (I & III); **AR**+ Sn^{4+} (II & IV).

After that, we immersed the TLC plate in Sn^{4+} (3×10^{-3} M) solution and then exposed to air and in vacuum to evaporate the solvent. The TLC plates turned pink from light yellow color and also give orange fluorescent in fluorescence light. This experiment exhibits steady colorimetric and fluorimetric changes in presence of Sn^{4+} by showing different colors and fluorescent changes which can be detect by our naked eye (Figure 5). Therefore, this experiment gives a real time monitoring without using any sophisticated instrumentation.

To better understand the photophysical properties of chemosensor **AR** and the complex **AR**-Sn^{4+}, they were examined by density function theory (DFT) calculations at the B3LYP[22] level of the Gaussian 09 program. The 6-31G (d) basis set was used for the H, C, N, and O atoms; the exception was for the Sn atom, where the LanL2DZ basis set with effective core potential was employed. The optimized structure is shown in Figure 6, which shows that the Sn^{4+} ion binds to **AR** very well through five coordination sites. The molecular orbital plots of compound **AR** and **AR**-Sn^{4+} are shown in Figure S16 and Figure S17 (Supporting Information) respectively. For chemosensor **AR**, the π electrons on both the HOMO and HOMO-1 are essentially distributed in the entire rhodamine backbone, but the LUMO and LUMO-1 are mostly positioned at the electron-withdrawing phenylazo-salicylaldehyde part. This indicates that **AR** bears efficient electron transfer from the rhodamine dye part to the phenylazo group, thus rendering the fluorescence relatively weak.

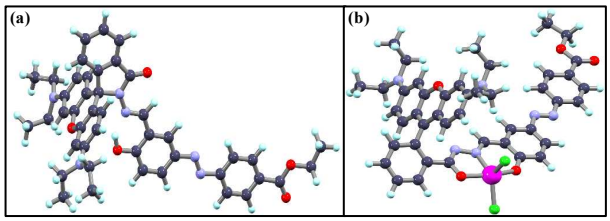


Figure 6: B3LYP optimized geometries of rhodamine derivative **AR** (a) and its complexes with Sn^{4+} ion (b).

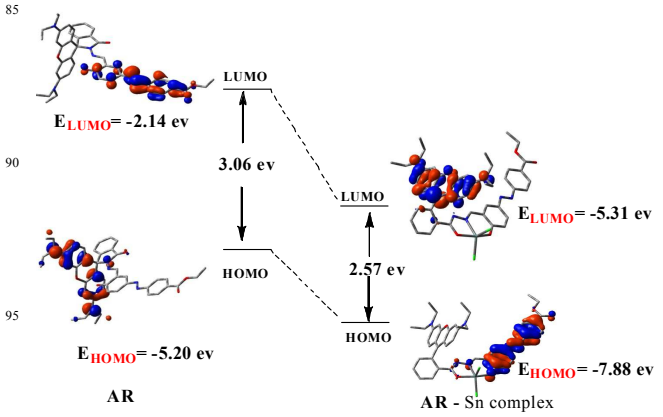


Figure 7. HOMO and LUMO orbitals energy diagram of **AR** and **AR**-Sn complex calculated by DFT/B3LYP/LANL2DZ/6-31+G(d) method.

In contrast, the π electrons on the HOMO of **AR**−Sn complex are mainly located on the phenylazo-salicylaldehyde part, but the LUMO is mostly positioned at the rhodamine framework. Moreover, the HOMO−LUMO energy gap of complex becomes much smaller relative to that of probe **AR**. The energy gaps between HOMO and LUMO in the probe **AR** and **AR**−Sn complex are 3.06 eV and 2.57 eV respectively (Figure 7). The interaction of the salicylaldehyde OH group with Sn^{4+} can change the orbital energy level, realizing the optical detection. The optimized complex of Sn^{4+} with **AR** at this stage showed a nearly planar pentacoordinated [23] geometry around Sn^{4+} where all five bonds (2×Sn- O, Sn-N and 2×Sn-Cl) are bonded to the central ion with their distances of 1.99, 2.04, 2.14, 2.32 and 2.33 Å

respectively. The result clearly suggest that the Sn^{4+} ion binds to **AR** very well through five coordination sites, and the whole molecular system forms a nearly planar structure. To get detailed insight into the changes in electronic spectra of **AR** and **AR**-Sn complex TDDFT calculations have been carried out by TDDFT/CPCM method in methanol solvent. The calculated vertical electronic excitations well matched with the experimentally observed bands both for the **AR** and **AR**-Sn complex and have intra-ligand charge transfer (ILCT) origin (Table S1 and S2, Supporting Information).

Chemosensor **AR** exhibited favorable features including fast response, reversibility, high sensitivity with a large fluorescence (up to 2384-fold) enhancement and a low detection limit of 7.1 μM, high selectivity for Sn^{4+}, and working well at physiological pH. These desirable attributes render the sensor suitable for fluorescent imaging of tin ions in living cells. Relying on the promising properties of chemosensor **AR**, we next questioned whether chemosensor **AR** could be used for monitoring the accumulation of tin ions in living cells. RAW cells were incubated with chemosensor **AR** (10 μM) for 30 min, followed by the addition of Sn^{4+} and incubation for another 30 min. The fluorescence images were recorded before and after the addition of Sn^{4+} (20 μM) (Figure 8).

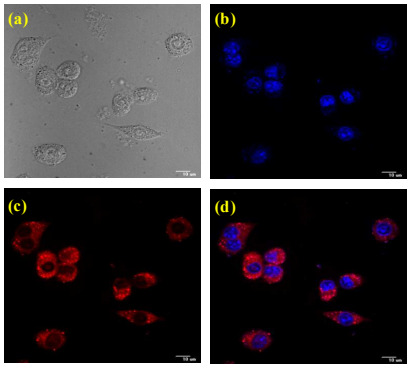


Figure 8. Confocal microscopic images (Andor Spinning Disk Confocal microscope, 40× objective lens) of chemosensor in Raw 264.7 cells. Cells pretreated with $SnCl_4$ (a) Bright field image of the Raw 264.7 cells. (b) After addition of Sn^{4+} (20 μM), nuclei counterstained with DAPI (1μg/mL) (c) Stained with chemosensor **AR** (10 μM) (in green filter) (d) Overlay image of Raw 264.7 cells in dark field.

RAW cells incubated with chemosensor **AR** exhibited no fluorescence, whereas a bright fluorescence signal was observed in the cells stained with chemosensor **AR** and Sn^{4+}, which in good agreement with the fluorescence turn-on profile of the sensor in the presence of Sn^{4+} in the solution.

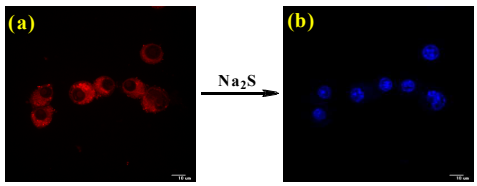
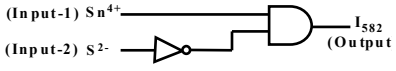


Figure 9. Confocal microscopic images of Raw 264.7 cells. (a) after treating with probe **AR** in presence of $SnCl_4$.(b) after adding Na_2S (30μM) solution to the (**AR**+Sn^{4+}) treated cells.

Moreover, bright red colored fluorescence cells obtained from the incubation of the receptor **AR** followed by treatment with Sn^{4+} became invisible in fluorescence upon addition of Na_2S (30 μM) (Figure 9). The results establish that chemosensor **AR** is cell membrane permeable and can be efficiently used for in vitro imaging of tin ions in living cells. Moreover, there were no indications of cell damage. Cells were intact and showed healthy spread and adherent morphology during and after the labeling process with chemosensor **AR**, indicating an absence of cytotoxic effects (Figure S18, Supporting Information).

The properties exhibited by **AR** as reported in this paper allows the design of molecular logic gate[24] using two binary input signals, while one is Sn^{4+} and the other is S^{2-}, and output monitored as a fluorescence emission of **AR** at 582 nm. From the logic gate functions, the emission of **AR** is observed only in the presence of single input, *viz*., Sn^{4+} and not with S^{2-} anion is insensitive to **AR**. Inputs of Sn^{4+} and S^{2-} have been considered as zero when they are not present and one when they are added. When both the inputs are zero, the output signal is zero and the gate is OFF.



Sn^{4+} Input-1	S^{2-} Input-2	I_{582} Output
0	0	0
0	1	0
1	0	1
1	1	0

Figure 10. Logic diagram and Truth table of INH logic circuit.

The **AR** treated with S^{2-} alone do not show any fluorescence change and thus upon adding S^{2-} the output signal is zero. On the other hand if **AR** is treated with Sn^{4+} alone as input, then the fluorescence enhances significantly (2368 fold at 582 nm), and the output signal is read out as one and the gate is ON. However, if all these two inputs (Sn^{4+} and S^{2-}) are present together, then the fluorescence of **AR** is quenched with the output read out as zero and the gate is OFF. From these studies, it has been clearly demonstrate that **AR** can be used as INHIBIT (INH) logic gate using Sn^{4+} and S^{2-} as inputs and the fluorescence emission at 582 nm as output. The truth table and the pictorial representation for the corresponding INH logic gate are given in Figure 10.

Conclusion

In conclusion, we have synthesized and characterized 5-(4-carboethoxyphenylazo) salicylaldehyde–rhodamine B -derivative of **AR** exhibits high selectivity towards Sn^{4+}. Its utility as a highly selective fluorescence switch ON (>2300-fold) chemosensor that responds stoichiometrically and rapidly to Sn^{4+} under physiological conditions. This azo appended chemosensor for Sn^{4+} ions is a new example among the few reports in the literature. Interaction of Sn^{4+} with **AR** enhances the fluorescence emission at 582 nm and induces a turn on response in electronic and fluorescence spectra in the visible region. **AR** is sensitive and selective toward Sn^{4+} over other biologically important ions studied, *viz*., Li^{+}, Na^{+}, K^{+}, Ca^{2+} , Mg^{2+}, Sr^{2+}, Ba^{2+}, Cr^{3+}, Al^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+} and Ag^{+}

ions, as demonstrated by individual as well as competitive metal ion titrations. Thus, these chemosensors could be used as a dual probe for visual detection through change in color and fluorescence *via* Sn^{4+}-promoted reversible ring opening *i.e.* coordination/disconnection reactions. Comparison of the fluorescence results of **AR** with those of the control molecule, **NAR** clearly suggest that covalently bonded fluorophore-photochrome dyad (**NAR**) is less sensitive towards selective sensing of Sn^{4+} ion. It has to be mentioned that the in situ prepared tin complex **AR**−Sn^{4+}, was able to detect S^{2-} exactly reverse manner and this experiment could serve as experimental evidence to support this reversible spiro ring-opening mechanism. Additionally, the chemosensor is also noted to be efficient probe for intracellular detection of Sn^{4+} by confocal microscope. Preliminary confocal microscopy experiments showed that because of the nature of its good membrane permeability, and its low toxicity, chemosensor **AR** performed well as a sensing probe for the detection of intracellular Sn^{4+} and S^{2-} in RAW cells. The displacement of Sn^{4+} from the binding core of the [**AR** + Sn^{4+}] complex upon addition of S^{2-} has been demonstrated by molecular logic gate operation. Thus the results obtained in the present studies help to construct the molecular level INH logic gate.

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Notes and references

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1 (a) B. Valeur and I. Leray, *Coord. Chem. Rev.,* 2000, **205**, 3; (b) E. M. Nolan and S. J. Lippard, *Chem. Rev.,* 2008, **108**, 3443; (*c*) E. L. Que, D. W. Domaille and C. J. Chang, *Chem. Rev.,* 2008, **108**, 1517; (d) M. Suresh, A. Ghosh and A. Das, *Chem. Commun.,* 2008, 3906; (e) S. Yoon, A. E. Albers, A. P. Wong and C. J. Chang, *J. Am. Chem. Soc.,* 2005, **127**, 16030; (f) H. N. Kim, W. X. Ren, J. S. Kim and J. Yoon, *Chem. Rev.,* 2012, **41** , 3210.

2 (a) S. Yoon, E. W. Miller, Q. He, P. K. Do and C. J. Chang, *Angew. Chem. Int. Ed.,* 2007, **46**, 6658; (b) G. Zhang, D. Zhang, S. Yin, X. Yang, Z. Shuai and D. Zhu, *Chem. Commun.,* 2005, 2161; (c) E. M. Nolan and S. J. Lippard, *J. Am. Chem. Soc.,* 2003, **125**, 14270; (d) G. Hennrich, W. Walther, U. Resch-Genger and H. Sonnenschein, *Inorg. Chem.,* 2001, **40**, 641.

3 (a) G. Hennrich, H. Sonnenschein and U. Resch-Genger, *J. Am. Chem. Soc.,* 1999, **121**, 5073; (b) V. Dujols, F. Ford and A. W. Czarnik, *J. Am. Chem. Soc.,* 1997, **119**, 7386; (c) J. Y. Kwon, Y. J. Jang, Y. J. Lee, K. M. Kim, M. S. Seo, W. Nam and J. Yoon, *J. Am. Chem. Soc.,* 2005, **127**, 10107; (d) M. Suresh, S. K. Mishra, S. Mishra and A. Das, *Chem. Commun.,* 2009, 2496; (e) A. W. Czarnik, ed. Fluorescent Chemosensors for Ions and Molecule Recognition, American Chemical Society, Washington, D. C., 1992.

4 (a) A. P. de Silva, D. B. Fox, A. J. M. Huxley and T. S. Moody, *Coord. Chem. Rev.,* 2000, **205**, 41; (b) X. He, H. Liu, Y. Li, S. Wang, Y. Li, N. Wang, J. Xiao, X. Xu and D. Zhu, *Adv. Mater.,* 2005, **17**, 2811; (c) J. R. Miller, J. Rowland, P. J. Lechler, M. Desilers, and L. C. Hsu, *Water, Air, Soil Pollut.,* 1996, **86**, 373; (d) P. B. Tchounwou, W. K. Ayensu, N. Ninashvili and D. Sutton, *Environ. Toxicol. Chem.,* 2003, **18**, 149; (e) A. J. Weerasinghe, C. Schmiesing and E. Sinn, *Tetrahedron Letters,* 2009, **50**, 6407; (f) R. Kagit, M. Yildirim, O. Ozay, S. Yesilot and H. Ozay, *Inorg. Chem.* 2014, **53**, 2144; (g) F. Hou, L. Huang, P. Xi, J. Cheng, X. Zhao, G. Xie, Y. Shi, F. Cheng, X. Yao, D. Bai and Z. Zeng, *Inorg. Chem.,* 2012, **51**, 2454.

5 (a) A.-M. Florea and D. Bu¨sselber, *BioMetals* 2006, **19**, 419. (b) Concise International Chemical Assessment Document 65 by WHO, Geneva, 2006.

6 (a) Y. Arakawa and S. E. Zasshi, 1997, **39**, 1; (b) ILO. Tin. In: Stellman J, ed. *Encyclopedia of Occupational Health and Safety.* 4th ed. Geneva: International Labour Organization (ILO), 1998, 63:41 pp.

7 JECFA (1989) *Toxicological evaluation of certain food additives and contaminants.* Joint FAO/WHO Expert Committee on Food Additives. Cambridge, Cambridge University Press, pp. 329–336 (WHO Food Additives Series No. 24).

8 (a) Agency for Toxic substances and Disease Registry (ATSDR) 2005. (b) L. R. Herman, J. Masters, R. Peterson, S. Levine, *J Anal. Toxicol.,* 1986, **10**, 6; (c) H. C. Dreef-van der Meulen, V. J. Feron and H. P. Til *Pathologia Europaea* 1974, **9**, 185.

9 (a) J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd Ed.; Springer: New York, 2006; p 67; (b) R. P. Haugland, *The Handbook: A Guide to Fluorescent Probes and Labeling Technologies*, the 10th Edition, Molecular Probes; Invitrogen Corp., Karlsbad, CA, 2005; (c) H. Yang, Z. Zhou, K. Huang, M. Yu, F. Li, Yi. T , C. Huang, *Org. Lett.,* 2007, **9**, 4729.

10 (a) X. Chen, T. Pradhan, F. Wang, J. S. Kim and J. Yoon, *Chem. Rev.,* 2012, **112** , 1910; (b) Y. Yang, Q. Zhao, W. Feng and F.Li, *Chem. Rev.,* 2013, **113**, 192; (c) D. T. Quang, and J. S. Kim, *Chem. Rev.,* 2010, **110**, 6280; (d) P. Mahato, S. Saha, E. Suresh, R. D. Liddo, P. P. Parnigotto, M. T. Conconi, M. K. Kesharwani, B. Ganguly and A. Das, *Inorg. Chem.,* 2012, **51**, 1769; (e) Z. Yang, M. She, B. Yin, J. Cui, Y. Zhang, W. Sun, J. Li and Z. Shi, *J. Org. Chem.,* 2012, **77**, 1143; (f) H. N. Kim, M. H. Lee, H. J. Kim, J. S. Kim and J. Yoon, *Chem. Rev.,* 2012, **37** , 1465; (g) S. Goswami, A. Manna, A. K. Maity, S. Paul, A. K. Das, M. K. Das, P. Saha, C. K. Quah and H.-K. Fun, *Dalton Trans.,* 2013, **42**, 12844.

11 Q. Wang, C. Li, Y. Zou, H. Wang, T. Yi and C. Huang, *Org. Biomol. Chem.,* 2012, **10**, 6740.

12 A. K. Mahapatra, S. K. Manna, D. Mandal and C. D. Mukhopadhyay, *Inorg. Chem.,* 2013, **52**, 10825.

13 (a) F. Caruso, M. Rossi, F. Marchetti and Pettinari, C. *Organometallics* 1999**, 18**, 2398; (b) R. R .Holmes, S. Shafieezad, V. Chandrasekhar, J. M. Holmes and R. O. Day, *J. Am. Chem. Soc.,* 1988, **11**, 1174; (c) C. Camacho-Camacho, E. Mijangos, M. E. Castillo-Ramos, A. Esparza-Ruiz, A. Vásquez –Badillo, H. Nöth , A. Flores-Parra and R. Contreras, *J. Organomet. Chem.,* 2010, **695**, 833.

14 A. K. Mahapatra, S. K. Manna and P. Sahoo, Talanta 2011, **85**, 2673.

15 J. Liu, K. Wu, X. Li, Y. Han and M. Xia, *RSC Adv.,* 2013, **3**, 8924.

16 I. Yildiz, E. Deniz and F. M. Raymo, *Chem. Soc. Rev.,* 2009, **38**, 1859.

17 P. Job, *Ann. Chim.,* 1928, **9**, 113.

18 B. Valeur, J. Pouget and J. Bouson, *J. Phys. Chem.,* 1992, **96**, 6545.

19 (a) S. Saha, M. U. Chhatbar, P. Mahato, L. Praveen, A. K. Siddhanta and A. Das, *Chem. Commun.*, 2012, **48**, 1659; (b) J. Luo, S. Jiang, S. Qin, H. Wu, Y. Wang, J. Jiang and X. Liu, Sens. Actuators B 2011, **160**, 1191; (c) M. Kumar, N. Kumar and V. Bhalla, *Sens. Actuators B* 2012, **161**, 311; (d) K. Ghosh, T. Sarkar and A. Samadder, *Org. Biomol. Chem.,* 2012, **10**, 3236; (e) S. Sun, B. Qiao, N. Jiang, J. Wang, S. Zhang and X. Peng, *Org. Lett.,* 2014, **16***,* 1132.

20 (a) X. Li, X. Gao, W. Shi and H. Ma, *Chem. Rev.,* 2014, **114,** 590; (b) R. K. Pathak, V. K. Hinge, K. Mahesh, A. Rai, D. Panda and C. P. Rao, *Anal. Chem.,* 2012, **84**, 6907. (c) S. Goswami, S. Paul and A. Manna, *RSC Adv.,* 2013, **3**, 10639.

21 (a) D. Maity and T. Govindaraju, *Inorg. Chem.,* 2011, ***50***, 11282; (b) P. Ashokkumar, H. Weißhoff, W. Kraus and K. Rurack, *Angew. Chem. Int. Ed.,* 2014, **53**, 1; (c) R. Sheng, P. Wang, Y. Gao, Y. Wu, W. Liu, J. Ma, H. Li and S. Wu, *Org. Lett.*, 2008, **10**, 5015.

22 (a) A. D. Becke, *J. Chem. Phys.* 1993, **98**, 5648; (b) C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B,* 1988, **37**, 785; (c) D. Andrae, U. Haeussermann, M. Dolg, H. Stoll and H. Preuss, *Theor. Chim. Acta,* 1990, **77**, 123.

23 (a) A. Ramírez, E. Gomez and S. N. Hernandez, *J. Organomet. Chem.,* 2009, **694**, 2965; (b) S. Basu, G. Gupta, B. Das and K. M. Rao, *J. Organomet. Chem.,* 2010, **695**, 2098; (c) S. M .Crawford, A. Al-Sheikh Ali, T. S. Cameron and A. Thompson, *Inorg. Chem.,* 2011, **50**, 8207; (d) T. Zöller, C. Dietz, L. Iovkova-Berends, O. Karsten, G. Bradtmöller, A. -K. Wiegand, Y. Wang, V. Jouikov and K. Jurkschat , *Inorg. Chem.,* 2012, **51**, 1041.

24 (a) A. P. de Silva, H. Q. N. Gunaratne and C. P. McCoy, *Nature,* 1993, **364** , 42; (b) A. P. de Silva, N. D. McClenaghan and C. P. McCoy, *Molecular Switches*, Wiley-VCH, Weinheim, Germany, 2001; (c) J. Dessingou, A. Mitra, K. Tabbasum, G. S. Baghel and C. P. Rao, *J. Org. Chem.,* 2012, **77**, 371; (d) A. P. de Silva, I. M. Dixon, H. Q. N. Gunaratne, T. Gunnlaugsson, P. R. S. Maxwell and T. E. Rice, *J. Am. Chem. Soc.,* 1999, **121**, 1393.

Graphical Abstract

Imino−Phenolic–Azodye Appended Rhodamine as Primary Fluorescence "Off−On" Chemosensors for Tin (Sn^{4+}) in Solution and in Raw Cells and the Recognition of Sulphide by [AR-Sn]

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A new azo-rhodamine based, **AR** was developed for fluorescent 'turn-on'sensing of Sn^{4+} with remarkable enhancement of fluorescent (> 2300 fold).