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A selective thioxothiazolidin-coumarin probe for Hg^{2+} based on its desulfurization reaction. Exploring its potential for live cell imaging.

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Abstract

Sensing the most toxic heavy metal (mercury) has attracted a lot of attention in recent years due to its extreme harmfulness to both human health and the environment. Thus, we reported herein the synthesis, spectroscopic and kinetic characterization, and biological evaluation of a new thioxothiazolidin coumarin derivative (**ILA92**), which undergoes a desulfurization reaction induced by mercuric ions (Hg^{2+}). This process is the origin of a selective sensing of Hg^{2+} ions in aqueous solution by colorimetric and fluorescent methods. Furthermore, the probe showed great potential for imaging Hg^{2+} in living cells.

Keywords: Desulfurization reaction; Hg^{2+} ions; thioxothiazolidin coumarin derivative; naked eye sensor; colorimetric fluorescent dye.

1. Introduction

The detection and quantification of heavy metal ions have emerged as a research area due to the prominent impact of these ions in biologically and environmentally important matrices [1-8]. In particular, mercury (Hg) is one of the most highly toxic heavy metals [9,10]. Thus, sensing the metal has attracted a lot of attention in recent years [11,12] due to its extreme harmfulness to both human health and the ecosystem. In fact, according to the established agreements in the Minamata Convention [10], it is very important to perform measurements of mercury-compounds emissions to both land and water, and monitor their regulation.

Typically, traditional mercury species detection methods include atomic absorption/emission spectrometry [4], hyper Rayleigh scattering (HRS) [13], ultrasensitive stripping voltammetry, [14] inductively coupled plasma mass spectrometry (ICP-MS) [15], among others. However, all these methods have various limitations because they not only require sophisticated instrumentations but also are time-consuming. Therefore, it is still a great challenge to develop simple, fast, and low-cost methods to detect mercury species sensitively and selectively.

Thus, between various reported methods for the determination of such heavy-metal ions, colorimetric and fluorescent probes arise as optimal tools [1-3,16-24]. The latter is because of their inherent cheapness, operational simplicity and high stability and sensitivity. In this context, attractive sensors for the recognition of Hg^{2+} have been reported. For example, some fluorescent chemosensor for the detection of such ions are based on their coordination to heteroatom containing ligands [23a].

Other specific probes reported act *via* hydrolysis [23b] or desulfurization [24] reaction induced by the metal. Moreover, other derivatives have induced dual fluorogenic signalling responses toward Hg^{2+} and Fe^{3+} ions depending upon the nature of substituent, [25] as well as a function of solvent medium [26]. Prompted by these observations, we explored sensitive and selective chemosensors for the recognition of mercuric ions over other metal ions, in particular, iron. Thus, in this work we present a new sensor (*Z*)-2-(5-((7-(diethylamino)-2-oxo-2*H*-chromen-3-

yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)ethanesulfonic acid (**ILA92**) see Scheme 1, which has been designed to incorporate a diethylaminocoumarin fragment with a rhodanine unit, in which the thiocarbonyl group would be would be in particulary in charge of recognizing the Hg^{2+} ions. We speculate that mercuric ions may promote a reaction of the sulphur-containing group. The latter may influence the π-electron delocalization of the system and, consequently change the absorption properties of dye.

2. Material and Methods

All analytes were purchased from Sigma-Aldrich and Merck and were used as received. The metal salts used were: Hg^{2+} ($HgCl_2$); Na^+ (NaCl); K^+ (KCl); Ca^{2+} ($CaCl_2$), Mg^{2+} ($MgCl_2$); Zn^{2+} ($ZnCl_2$); Fe^{3+} ($FeNH_4(SO_4)_2 \cdot 12H_2O$); Fe^{2+} (Fe-$(NH4)_2(SO4)_2 \cdot 6H_2O$); Cu^{2+} ($CuCl_2$) and Ag^+ (AgCl). Unless indicated otherwise, all solutions employed in this study were prepared in HEPES-DMSO (99/1 v/v; pH 7.0).

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker multidimensional 400 MHz spectrometer, using the solvent or the TMS (Trimethylsilane) signal as an internal standard. All chemical shifts are reported in the standard notation of parts per million.

HRMS-ESI experiments were executed using a Thermo Scientific Exactive Plus Orbitrap spectrometer with a constant nebulizer temperature of 250 °C. The experiments were carried out in negative ion mode, with a scan range of m/z 400.000-1000.000 with resolution 140.000. The samples were infused directly into the ESI source, via a syringe pump, at flow rates of 5 µL min^{-1}, through the instrument's injection valve.

Absorption spectra were obtained by means of an Agilent Cary 60. The emission spectra were recorded on an Agilent Cary Eclipse fluorometer. Zeiss 710 confocal microscopy was used. The confocal microscopy at 63X amplification.

2.1. Synthesis of 7-diethylaminocoumarin (2)

4-Diethylaminosalicylaldehyde (1) (1.93 g, 10 mmol), diethyl malonate (3.2 g, 20 mmol), piperidine (1.0 mL) and one drop of AcOH, were combined in absolute

EtOH (60 mL) and refluxed for 6 h. All volatiles were evaporated under reduced pressure, and then concentrated, HCl (20 mL) and glacial acetic acid (20 mL) were added to cyclize, hydrolyze and decarboxylate the intermediate ester while the reaction was continued at reflux temperature for 24 hours. This solution was cooled to room temperature and poured into ice water (100 mL). NaOH solution (40%) was added dropwise to adjust the pH to 5, and a grey precipitate formed immediately. After stirring for 1 h, the mixture was filtered, washed with water, and dried to yield product 7-(diethylamino)-2*H*-chromen-2-one (2) (1.78 g, 8.2 mmol) in 82.0 % yield. ^{1}H-NMR (DMSO-d_6) δ 7.50 (d, J=8.0 Hz, 1H), 7.21 (d, J=8.0 Hz, 1H), 6.53 (dd, J=12.0 Hz, J=2.0 Hz, 1H), 6.44 (d, J=2.0 Hz, 1H); 5.99 (d, J=8.0 Hz. 1H), 3.42 (q, J=8.0 Hz, 4H), 1.17 (t, J=8.0 Hz, 6H). ^{13}C NMR (DMSO-d_6): 162.7, 157.1, 151.1, 144.1, 129.2, 109.5, 109.1, 108.5, 97.9, 45.2, 12.8.

2.2. Synthesis of 7-diethylaminocoumarin-3-aldehyde (3)

Freshly distilled DMF (6.5 mL) was added dropwise to $POCl_3$ (6.5 mL) at 20-50 °C under N_2 atmosphere and stirred for 30 minutes yielding a red solution. The solution was added with 7-diethylaminocoumarin (2) (4.50 g, 20.7 mmol) in 30 mL DMF to afford a scarlet suspension. The mixture was stirred at 60 °C for 24 h and then poured into ice water (300 mL). NaOH solution was added to adjust the pH to 5.2, generating an abundant precipitate. The crude product was filtered thoroughly washed with water, dried and recrystallized in absolute ethanol to give 3 (2.80 g, 11.4 mmol) in 55.2% yield. ^{1}H NMR (DMSO-d_6) δ 10.05 (s, 1H), 8.18 (s, 1H), 7.36 (d, J=8.0 Hz, 1H), 6.59 (dd, J=8.0 Hz, J=2.0 Hz, 1H), 6.42 (d, J=2.0 Hz, 1H), 3.44 (q, J=8.0 Hz, 4H), 1.22 (t, J=8.0 Hz, 6H). ^{13}C NMR (DMSO-d_6): 188.2, 162.2, 159.3, 153.9, 145.7, 132.9, 114.6, 110.6, 108.6, 96.5, 45.7, 12.8 [27].

2.3. Synthesis of (Z)-2-(5-((7-(diethylamino)-2-oxo-2H-chromen-3-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)ethanesulfonic acid

2-(4-oxo-2-thioxotetrahydrothiophen-3-yl)ethanesulfonic acid (1 mmol) and appropriate 7-diethylaminocoumarin-3-aldehyde (1 mmol) were dissolved in methanol (5 mL). The solution was refluxed for 2-18 h in the presence of a small amount of piperidine as a catalyst [28-29]. After the reaction was completed

(monitored by TLC), the mixture was cooled, the precipitate was filtered and crystallized from ethanol to produce corresponding products (**ILA92**). (0.350 g, 75 %). ^{1}H NMR (DMSO-d_6) δ 8.13 (s, 1H), 7.59 (d, J=8.0 Hz, 1H), 7.57 (s, 1H), 6.79 (d, J=8.0 Hz, 1H), 6.58 (s, 1H), 4.27 (m, 2H), 3.49 (q, J=8.0 Hz, 4H), 2.77 (m, 2H), 1.15 (t, J=8.0 Hz, 6H). ^{13}C NMR (DMSO-d_6): 194.2, 167.3, 160.6, 157.1, 152.9, 147.4, 131.9, 129.2, 120.1, 111.9, 110.8, 109.1, 96.7, 47.7, 44.9, 41.5, 12.8.

2.4. High-resolution mass spectrometry (HRMS-ESI) studies

High-resolution mass spectra (HRMS-ESI) were extracted from high resolution mass spectrometer Exative™ Plus Orbitrap, Thermo Fisher Scientific. The analysis for the reaction products was performed with the following scan parameters: resolution 140.000; AGC target 1e6; inject time 200. HESI source was heath gas flow 8; auxiliary gas flow rate 3; sweep gas flow rate 0; capillary temperature 250°C, S-lens RF 100; Heater temperature 100°C, at mode positive with spray voltage 2.8 kV.

2.5. Nuclear magnetic resonance (NMR) studies

^{13}C NMR spectra were obtained at 25°C on a Bruker Advance 400 MHz spectrometer using TMS as an internal standard. The NMR spectra were processed with MestreNova software v9.0. All solutions were prepared by mixing appropriate volumes of stock solutions of **ILA92** in DMSO-d_6 and D_2O.

2.6. Kinetic measurements

These measurements were performed spectrophotometrically (diode array) in the 300-700 nm range, by following the disappearance of **ILA92** (at 530 nm) or the formation of product at 485 nm, by means of a Hewlett-Packard 8453 instrument. The reactions were carried out in water, at 25 ºC. At least a 10-fold excess of mercuric ions over the substrate (**ILA92**) was employed. Pseudo-first-order rate coefficients (k_{obsd}) were found for all reactions. They were obtained by means of the kinetic software of the spectrophotometer at the wavelength where the greatest absorbance changes were observed.

2.7. Determining the quantum yield of emission

Fluorescence quantum yield of ILA92 was measured using a solution of Rhodamine 101 in ethanol as standard (Φs = 1). All values were corrected considering the solvent refraction index. Quantum yield was calculated using Eq. (1), where the subscripts x and s denote sample and standard, respectively, Φ is the quantum yield, η is the refractive index, and Grad is the slope from the plot of integrated fluorescence intensity vs. absorbance.

$$\Phi_x = \Phi_s \left(\frac{Gr}{Gr}\right. \qquad \text{Eq. (1)}$$

2.8. Detection limit

The detection of limit (LOD) was calculated based on absorbance and fluorescence titrations. To determine the S/N ratio, the absorbance (at 525 nm) or emission intensity of **ILA92** with Hg^{2+} was measured three times and the standard deviation of calibration curve was determined. The detection limit was calculated with the equation: LOD = $3\sigma_b/m$, where σ_b is the standard deviation of calibration curve and m is the slope of the plot of absorbance or fluorescence intensity versus analyte concentration.

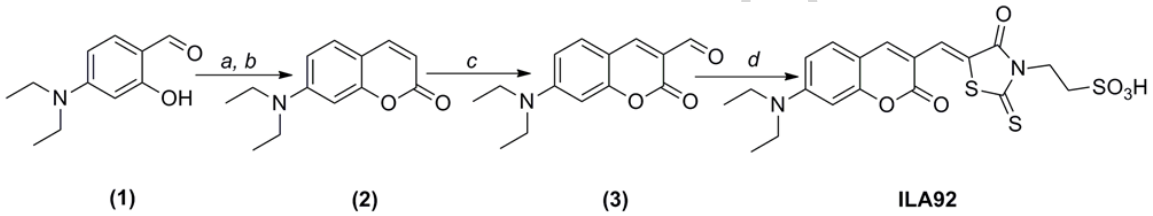
2.9. Cell culture and fluorescence imaging

Human neuroblastoma SH-SY5Y cells (CRL-2266, American Type Culture Collection, Rockville, MD) were cultured in MEM-F12 medium supplemented with 10% FBS, non-essential amino acids, antibiotic-antimycotic mixture, and 20 mM, pH 7.0 HEPES buffer. The medium was replaced every 2 days. Cells were exposed to 2 μM **ILA92** for 30 min and washed. Then, the basal fluorescence was taken after the cells were treated with 30 μM $HgCl_2$ for 120 min. Changes were observed by epifluorescence microscopy, 63× objective [23b].

3. Results and discussion

The proposed compound **ILA92** was synthesized in four steps, as shown in Scheme 1. Firstly, 4-(diethylamino)-2-hydroxybenzaldehyde (**1**) was condensed with diethyl malonate in a Knoevenagel condensation, cyclized and decarboxylated in one step to afford 7-(diethylamino)-2*H*-chromen-2-one (**2**). Subsequently, the

compound was formylated (Vilsmeier-Haack) to obtain 7-(diethylamino)-2-oxo-2*H*-chromene-3-carbaldehyde (**3**) [27], which was condensed with 2-(4-oxo-2-thioxotetrahydrothiophen-3-yl) ethanesulfonic acid to yield the proposed compound (*Z*)-2-(5-((7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)ethanesulfonic acid (**ILA92**). The chemical structure of precursors and the final product **ILA92** were well characterized by using ^{1}H and ^{13}C NMR spectroscopy (see Supporting Information Figures S1-S4). In addition, for the characterization of **ILA92**, high-resolution mass spectrometry (HRMS-ESI) was employed (Figure S5).



Scheme 1. Synthetic route to **ILA92**. Reagents and conditions: a) Diethyl malonate, piperidine, reflux, 6h; b) HCl, AcOH, reflux, 24 h; c) $POCl_3$, DMF, 60 °C, 24 h; d) 3, 2-(4-oxo-2-thioxotetrahydrothiophen-3-yl)ethanesulfonic acid, MeOH, piperidine, 10-12 h.

Compound **ILA92** was also examined by UV−Vis absorption, in HEPES-DMSO (99/1 v/v; pH 7.0). The absorption spectrum of compound **ILA92** (5 µM) in such a medium exhibited a strong band at 530 nm (Figure S6; SI) and a molar extinction coefficient (ε) of 148518 M^{-1} cm^{-1} (Figure S7; SI). Regarding the fluorescent properties of **ILA92**, its emission spectrum shows an emission band at 645 nm (Figure S8A; SI). Compound **ILA92** has a quantum yield of 0.049 (using Rhodamine 101 as standard, see Figure S9) and a Stokes shift of 110 nm.

The selectivity of compound **ILA92** was tested in the presence of a series of different metal ions of biological and environmental interest, such as Hg^{2+}, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, Fe^{2+}, Cu^{2+} and Ag^{+} (Figure S8B; SI). As shown in Figure 1, relevant absorbance changes at 530 nm were observed for this compound only in the presence of solutions containing Hg^{2+} ions. It is important to mention that ferric ions, a typical interferent [25], produced slight variations only in spectral properties

of **ILA92.** Moreover, the variation of absorbance (ratio A/A_0) associated with the **ILA92-**Hg^{2+} system at 530 nm in the presence and absence of coexisting metal ions under competitive conditions ([I**LA92**]:[Hg^{2+}]:[M^{n+}] = 1:10:20) exhibited no significant differences in their responses by being compared between them. Results show a ratio that is in the range between 0.23 and 0.34 for [Hg^{2+}]:[Fe^{3+}] and [Hg^{2+}]:[Ag^{+}], respectively, which implies that **ILA92** depicts a high selectivity for mercuric ions even in the presence of other competitive metal ions.

Thus, we can establish that the absorption changes of **ILA92** are highly specific for Hg^{2+} and minor interference from this metal ion occurs, in the same experimental conditions.

Fig. 1. Selectivity and competition assays. The ratio of the absorption at 530 nm (A/A_0) of **ILA92** (2 μM) upon addition of 10 equiv. of different metal ions (including 1: Hg^{2+}, 2: Na^{+}, 3: K^{+}, 4: Ca^{2+}, 5: Mg^{2+}, 6: Zn^{2+}, 7: Fe^{3+}, 8: Fe^{2+}, 9: Cu^{2+}, 10: Ag^{+}). In the case of competition experiments (orange line dashes) the absorbance ratio was assessed under competitive conditions ([I**LA92**]:[Hg^{2+}]:[M^{n+}] = 1:10:20). Each set of data was obtained 2h after the addition of different metal ions in in HEPES-DMSO (99/1 v/v; pH 7.0) at 25 °C.

In fact, upon addition of five equivalent of Hg^{2+} ions to the solution of **ILA92**, the absorption band at 530 nm slightly decreased and a new band (shifted 50 nm) time-dependent was formed at 485 nm (Figure S10; SI), with a colour change from dark to light pink, evident through the naked eye (Insert Figure S10; SI). Furthermore, more marked changes were observed in the fluorescence spectra of the dye after 120 minutes of incubation in presence of 10 eq. of mercuric ions (Figure 2). As shown in the figure, the fluorescence associated to the dye increases and the maximum emissive wavelength shifts from 630 nm to 580 nm (0-120 minutes).

Fig. 2. Changes in the emission spectra of **ILA92** (2×10^{-6} M) upon addition of $HgCl_2$ (1×10^{-4} M) during 120 minutes. Each spectrum was acquired in HEPES-DMSO (99/1 v/v; pH 7.0) at 25 °C.

To view the ability of mercuric ions to modify the fluorescence spectra of dye, Insert to Figure 2 depicts the effect of increasing concentrations of these ions on the emission bands associated with the dye.

Regarding the disappearance of the UV-vis band at 530 nm and the formation of a new band at 485 nm, it could be attributed to the interaction of mercuric ions (soft acid) with the thiocarbonyl group (sulphur atom, as a soft base) leading to a chemical reaction. In fact, as mentioned above, it is well known that thiocarbonyl containing compounds can undergo desulfurization reaction in the presence of mercury salts [30-31]. Recently, some authors have reported that a blue-shift was observed when a specific mercury-promoted desulfurization reaction of thiocoumarin to coumarin occurs [31]. Considering these pieces of evidence and the chemical structure of compound **ILA92**, we propose that it is thiocarbonyl moiety, in the presence of mercuric ions, that turns more electrophile. Thus, **ILA92** would be more easily attacked by water, leading to its conversion into the carbonyl analogue. As a result, the spectroscopic changes described in the UV-Vis of the probe could be associated with conversion. Thus, the clear isosbestic point at 505 nm observed in the absorption spectra of **ILA92** (Figure S8; SI). The latter indicates the reaction of **ILA92** with Hg^{2+} produces a single new component.

Concerning the kinetic studies, mercuric ions solutions were used in excess (10 equiv.) over the dye **ILA92** to ensure first-order conditions, (details see Supporting Information Table S1; SI). The first-order rate constants k_{obsd} were obtained from the exponential decays of the absorbance of the dye or the absorption associated with the product formation (Insert Fig. 3).

Fig. 3. Dependence of k_{obsd} against [Hg^{2+}], in HEPES-DMSO (99/1 v/v; pH 7.0), for the reaction of **ILA92** at 25 °C. Inset: Kinetic profile of disappearance of **ILA92** (2×10^{-6} M) at 530nm and formation of product at 485nm.

As depicted in Figure 3, plot of k_{obsd} in contrast to [Hg^{2+}] at constant pH is non-linear (saturation kinetic). The kinetic behavior of the desulfurization reaction is consistent with the mechanism described in Scheme 2.

Scheme 2. Proposed mechanism for the studied reaction between mercuric ions and ILA92.

Applying the steady-state conditions to the intermediate in Scheme 2, yields to eq. 2:

$$k_{obsd} = \frac{k_1 \times k_2[Hg^{2+}]}{k_{-1} + k_2[Hg^{2+}]} \qquad \text{Eq. (2)}$$

Two limiting situations can arise: at low concentrations of the metal a first-order kinetic model is established, $k_{-1} >> k_2$ [Hg^{2+}]; In this case, eq. 2 reduces to eq. 3, where $K_1 = k_1/k_{-1}$.

$$k_{obsd} = K_1 \times k_2[Hg^{2+}] \qquad \text{Eq. (3)}$$

At high concentrations of the metal, they do not affect the kinetic rate, thus a zero order occurs due to that. $k_{-1} << k_2$ [Hg^{2+}]; therefore, eq. 2 simplifies to eq. 4.

$$k_{obsd} = k_1 \qquad \text{Eq. (4)}$$

Thus, the dye is able to interact with mercuric ions and produce a different kinetic response until concentration reaches 1×10^{-4} M.

On the other hand, the quantitative signaling behavior of **ILA92** for Hg^{2+} ions was assessed in aqueous solution using UV-vis titration (Figure S11; SI). In fact, the absorbance at 530 nm decreased as the concentration of Hg^{2+} increased, up to 500 µM; By using this concentration, dependent absorption change the detection limit for the determination of Hg^{2} as it was estimated to be 15.6×10^{-6} M (Absorbance) and 15.1×10^{-6} M (Fluorescence) (Figure S11; SI). These results demonstrated that **ILA92** can detect the submillimolar concentration range of the analyte, being the LOD value comparable with other recognized sensitives mercury probes [18].

Evidence for the proposed desulfurization reaction [30-33] was supported by ^{13}C NMR spectra of **ILA92** after its reaction with Hg^{2+}. As depicted in the partial ^{13}C NMR spectrum in Figure 4, upon addition of Hg^{2+}, the NC=S carbon atom **C2'** (rhodanine fragment) was clearly shifted from δ=194.7 to 166.0 ppm (NHC=O carbon of product).

Fig 4. ^{13}C NMR (DMSO-d_6) spectrum of probe **ILA92** alone (**A**) and in the presence of mercuric ions (**B**).

In addition, to confirm the mechanism for the reaction between **ILA92** and Hg^{2+} HRMS-ESI analyses were carried out. As shown in Figure 5, peaks of *m/z* 467.04 and 451.06 were observed after the addition of one equivalent of $HgCl_2$ to the solution containing **ILA92**. These peaks are attributed to the presence of reagent (probe **ILA92**) and of the desulfurization reaction product **ILO92**, respectively.

Fig. 5. HRMS-ES of probe **ILA92** in the presence of $HgCl_2$ (1 eq.).

Therefore, we confirm that the thiocarbonyl group of **ILA92** is readily transformed to its carbonyl function by reaction with Hg^{2+} ions and the liberated sulphur atom form a stable species of HgS with Hg^{2+} ions (Scheme 3).

Scheme 3. Desulfurization reaction proposed between compound **ILA92** and mercuric ions.

To explore the potential of the probe **ILA92** as a tool for live cell imaging of mercuric ions, we used an in vitro model system (SHSY5Y neuroblastoma cells) and investigated the effects of **ILA92** in the absence and presence of mercuric ions. In addition, it was observed under the microscope that SH-SY5Y cells in the presence of **ILA92** (5 μM) did not induce structural modifications during the whole

trial. It demonstrates that, at the concentrations used it does not present cytotoxic effects.

Thus, considering this control experiment (Fig. 3), the SH-SY5Y cells were first treated with **ILA92** (2 µM) for 20 min, and subsequently a solution containing Hg^{2+} (30 µM) was added and incubated for another 30 min. As shown in Figure 6A, the cells incubated with only **ILA92** emitted fluorescence. However, upon the addition of Hg^{2+} , the fluorescence emission showed an increase of the emission. These results are in accordance with the changes of the emission spectra obtained in aqueous solutions, which demonstrates that the probe **ILA92** is capable of detecting Hg^{2+} in both aqueous solutions and biological samples. And when compared to those reported in the literature [34-36], it has good behavior in biological matrices.

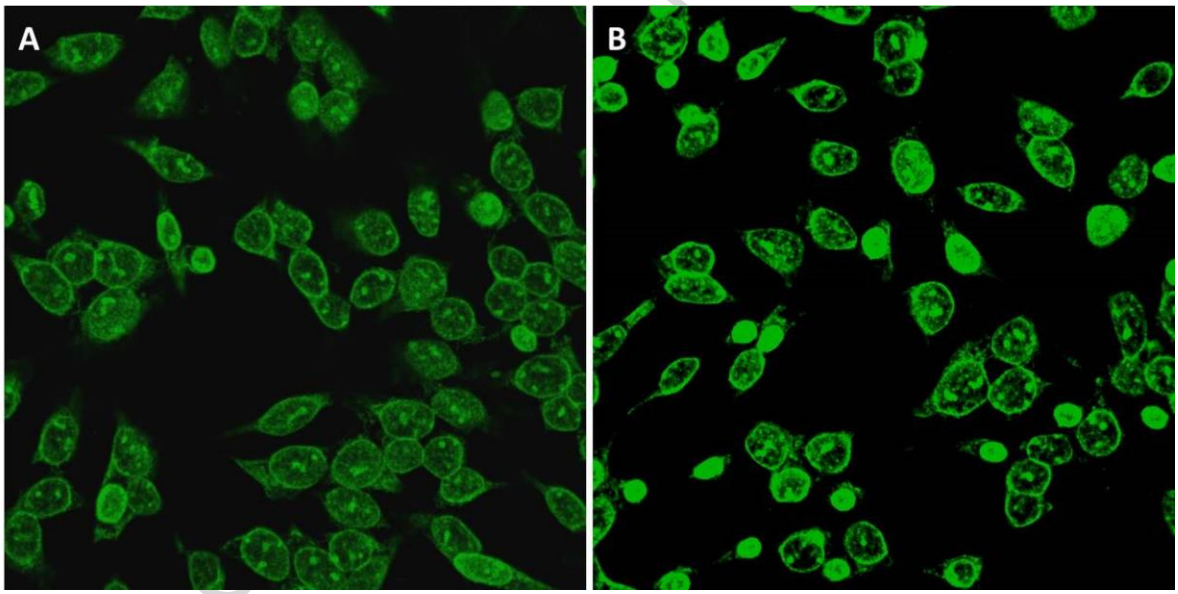


Fig. 6. (A) Basal fluorescence of SH-SY5Y cells lines incubated with the probe **ILA92** (2 µM). (B) Increase in fluorescence after treating the SH-SY5Y cells lines with 30 µM $HgCl_2$ for 30 min.

4. Conclusions

We have reported the synthesis, kinetic study and biological application of a coumarin derivative compound **ILA92** that selectively recognizes Hg^{2+} ions over a

series of other metal ions. The detection limits obtained were (~ 10^{-7} M). The sensing is performed colorimetrically and fluorometrically and it is based on a desulfurization reaction. Fluorescence imaging for Hg^{2+} in living cells shows that sensor **ILA92** could be potentially used for further biological applications.

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Conflicts of interest

There are no conflicts to declare.

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Graphical Abstract

Desulfurization reaction of a thioxothiazolidin coumarin derivative induced by mercuric ions. Exploring its potential for live cell imaging

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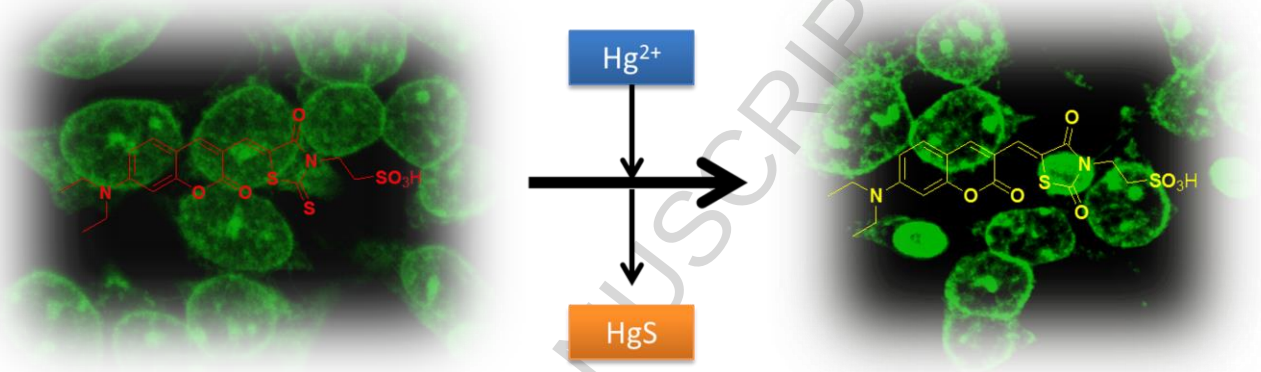

Highligths

- A new thioxothiazolidin coumarin derivative (**ILA92**) is designed for specific sensing of mercury.

- The sensing of mercuric ions is performed colorimetrically and fluorometrically and it is based on the desulfurization reaction of **ILA92**.

- The detection limit of sensing was obtained 2.34×10^{-6} M (Absorbance) and 4.50×10^{-6} M (Fluorescence).

- The **ILA92** can successfully use for sensing Hg^{2+} in aqueous medium and living cells.