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Research paper

# Upper rim modified calix[4]arene towards selective turn-on fluorescence sensor for spectroscopically silent metal ions

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### ABSTRACT

This work describes the synthesis of a novel sensor calix[4]arene thiosemicarbazone (Lig), consisting of a calix [4]arene thiosemicarbazone moiety capable of detecting  $Cu^{2+}$ ,  $Hg^{2+}$ ,  $Zn^{2+}$  colorimetrically and for the selective detection of  $Zn^{2+}$  by "Turn On" fluorescence change in DMSO-H<sub>2</sub>O (9:1, v/v, pH = 7.2) solution amongst various toxic heavy metal ions. The sensor shows about 240-fold fluorescence enhancements at 474 nm (Stokes shift-140 nm) when bound to a  $Zn^{2+}$  ion with a detection limit of about 15  $\mu$ M for the  $Zn^{2+}$  ion. Such fluorescence could be visualized by normal unaided eyes offering a simple, visual detection process. DFT theoretical study has been used to further support the high efficiency of the sensor. The binding ability of the Lig with  $Zn^{2+}$  ions has been used to evaluate the biofunctional properties of the synthesized ligand. The synthesized Lig is found to possess the cytotoxic activity and a better penetrating ability towards the A549 human lung cancer cell line.

### 1. Introduction

Metal toxicity is caused by both essential and non-essential metals when exceeding their limits in the human environment and the accumulation of excessive amounts of these metals within the body [1,2]. Among the essential metals, zinc cation is the second most abundant that plays crucial functional roles in the human body such as structural [3], catalytic [4], Lewis acid [5] regulation of transcription and translation via its DNA and RNA binding [6], brain function [7] and immune function [8]. Compared to other toxic metals, zinc is considered to be relatively harmless to humans [9]. Normally, the human body contains about 2 g of zinc found in reproductive organs, muscle, liver, and brain. Despite zinc is considered to be a harmless element, exposure to high concentrations results in serious health disorders such as interference of copper uptake for the metabolic process [10]. Alzheimer's disease is a chronic neurodegenerative disease that affects most of the human population worldwide, which may be related to an imbalance in copper, iron, and zinc in human physiology [11]. Deficiency of zinc causes multiple systemic effects such as growth retardation, weight loss, infertility, mental disorders, impaired immune function, skin lesions, and hair loss [12]. Cell growth and cell proliferation metabolic processes are well coordinated with zinc uptake by the human body. Hence, monitoring the level of zinc in the human body is an indirect way of monitoring the uncontrolled cell proliferation processes such as tumor growth [13–15]. Copper is an essential redox-active metal, which regulates many biological functions by its bivalent oxidation states ( $Cu^{2+}$ , Cu<sup>+</sup>) [16,17]. The usage of copper extensively in chemical industries, particularly in electronics, leads to the contamination of the environment. Imbalance in copper levels in the human body disrupts normal biological functions that lead to health disorders such as oxidative stress, hepatocellular carcinoma, neurodegeneration, respiratory deficiency, cardiomyopathy, and pigmentation defects [18]. United States Environment Protection Agency (USEPA) quoted that the permissible concentration of Cu<sup>2+</sup> in water is 1.30 mg/L [19]. Mercury is one of the potentially harmful elements to living organisms when continuously exposed. Mercury vapor easily accumulates in the lungs and it can penetrate the blood-brain barrier (BBB) through vain and gets accumulated in the brain [20]. Once mercury is accumulated in the brain, it reduces the neurotransmitter production, disturbs the nerve cellular process, and decreases the secretion of important hormones, thyroid, and testosterone [21].

According to the United States Environment Protection Agency

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(USEPA) and the World Health Organisation (WHO), the concentration of mercury in drinking water should not exceed 30 nM  $L^{-1}$  [22]. So monitoring the concentration level of zinc, copper, and mercury in the human body as well as in the environment is necessary. Spectroscopically, some heavy metals do not show a unique signature in UV-visible and IR regions because they either have completely filled or empty dorbitals [23]. So the detection of such "spectroscopically silent" heavy metals is quite difficult and need sophisticated instruments like synchrotron [24], X-ray absorption spectroscopy (XANES) [25], flame atomic absorption spectrometry (FAAS) [26], graphite furnace atomic absorption spectrometry (GFAAS) [27], inductively coupled plasmaoptical emission spectrometry (ICP-OES) [28] and inductively coupled plasma-mass spectrometry (ICP-MS) [29]. UV-visible and fluorescence spectroscopies are quite common than the above mentioned sophisticated techniques. There is an endless research effort to develop a new colorimetric and fluorescence sensor for spectroscopically silent heavy metals [30-37].

Thiosemicarbazones are sulfur analogs of semicarbazones derived from the condensation of aldehyde or ketone with thiosemicarbazide, which have been the focus of chemists because of their therapeutic applications [38-41]. Recently, chemists use thiosemicarbazones extensively in various fields that include catalysis [42], sensors [43–45], and corrosion [46]. Thiosemicarbazones exhibit sensing property against both cations and anions owing to their chemical skeletal formulae. The lone pair electrons in azomethine nitrogen and thiocarbonyl moiety are responsible for the cation recognition and the hydrogen present in the nitrogen moiety is responsible for the anion recognition [47]. The applicability of thiosemicarbazone and their metal complexes are extended in the field of biology particularly in cytotoxicity [48-51]. Cytotoxicity means toxic for cells via irreversible lethal cellular damage and alters normal metabolic functions of cells which cause cell death called necrosis (induced) or apoptosis (programmed) [52]. Generally, cytotoxic drugs impair the cellular reproductive integrity by inhibiting the enzymes responsible for cell reproduction or by the interference of the molecular assembly of a protein responsible for cellular reproduction via non-covalent interaction [53]. When thiosemicarbazone enters the biological system, it can coordinate with bio-available metal ions. These complexes are responsible for the release of reactive oxygen species (ROS) which will impair various biomolecules and causes cell death [54]. Thiosemicarbazone and its metal complexes are found to cause cellular death via apoptosis mechanism for cancer cells [55].

The field of supramolecular chemistry starts from the discovery of crown ethers by Charles Pedersen [56]. Supramolecular chemistry has grown massively in various branches of chemistry especially in sensing [57,58]. Calix[4] arenes are oligomers largely used to sense various harmful elements [59-63]. Calix[4] arene mainly acts as a great platform in the sensor field because of its rigid structure, modifications possible with a lower and upper rim, host-guest properties, and self-assembly behaviour [64,65]. With the aid of calix[4]arene platform, numerous strategies are used to sense cations and anions. Familiar strategies such as photoinduced electron transfer (PET), excimer formation, photoinduced charge transfer (PCT) and fluorescence resonance energy transfer (FRET) are broadly studied with calix[4]arene for sensing [66]. So the synthetic receptor having calix[4] arene and thiosemicarbazone moieties may act as a great sensor towards cations and anions. In this work, we synthesized upper rim modified p-tert-butyl calix[4]arene thiosemicarbazone-based sensor (Lig) which was used to sense spectroscopically silent metal ions in aqueous solutions, and its cytotoxic activity against A549 lung cancer cell line was evaluated.

### 2. Experimental details

### 2.1. Materials and methods

1-Napththyl isothiocyanate and hexamethylenetetramine (HMTA) were purchased from Sigma-Aldrich. p-*tert*-butylcalix[4]arene,

bromoethane, hydrazine monohydrate and trifluoroacetic acid were purchased from Alfa Aesar. Dry solvents and spectroscopic grade solvents were used for the entire synthesis and spectral studies, respectively. Metal salts used were nitrate or chloride salts. Double distilled (DD) water was used in all experiments. T90 + UV/Visible spectrometer was used for UV–visible spectral studies and Shimadzu (RF-5301 PC) spectrofluorometer was used for fluorescence experiments. Thermo Scientific Nicolet iS5 FT-IR spectrometer was used for FT-IR spectral measurements. <sup>1</sup>H and <sup>13</sup>C NMR analysis were recorded by Bruker Advance DPX 500 MHz spectrometer. pH adjustment was carried out by Eutech digital pH meter. Time-resolved fluorescence lifetime experiments were carried out using Horiba Jobin Yvon nanosecond pulse diode laser-based time-correlated single-photon counting (TCSPC) spectrometer and data was processed by IBH DAS 6.2 data analyzer.

### 2.2. Synthesis of N(naphthalen-1-yl)hydrazinecarbothioamide (1)

The compound 1 was synthesized using a modified procedure available in the literature [67]. In a 100 ml RB flask, 1-Napthylisothiocyanate (740.96 mg, 4 mmol) was weighed and 30 ml of dry ethanol was added. Hydrazine monohydrate (250 ŵl slightly excess than 4 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 24 h and the resultant precipitate was filtered off from the reaction mixture. The precipitate was washed with ethanol and then recrystallized in methanol. The colorless product yield was 87%. FT-IR (KBr, cm<sup>-1</sup>) (Fig. S1) data showed 3345, 3299, 3248, 3188, 3047, 1595, 1492, 1213, 1073, 892, 805, 768, 507. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) (Fig. S2)  $\delta$ : 9.45 (s, 1H), 8.04 (s, 1H), 7.90 (m, 3H), 7.84 (t, 3H), 4.08 (s, 2H). <sup>13</sup>C NMR (500 MHz, DMSO-*d*<sub>6</sub>) (Fig. S3)  $\delta$ : 181.7, 135.7, 134.1, 130.2, 128.5, 126.4, 125.8, 125.3, 123.1.

# 2.3. Synthesis of 1, 3, 5, 7-tetra-tert-butyl-3, 7-diethoxy-calix[4]arene(2)

The compound (2) was synthesized as per a method available in the literature [68]. In a 100 ml RB flask, p-tert-butylcalix[4]arene (1.2979 g, 2 mmol) and potassium carbonate (580.48 mg, 4.2 mmol) were weighed. Then 70 ml of dry acetonitrile was added. This mixture was refluxed for 2 h to dissolve p-tert-butylcalix[4]arene. After 2 h, this mixture was cooled to room temperature and a slight excess of bromoethane (373 µl) was added to the reaction mixture. This reaction mixture was further refluxed for 48 h. After the completion of the reaction, acetonitrile was distilled out from the mixture and the precipitate obtained was redissolved in dichloromethane. The dichloromethane solution was washed with water and brine solution. The organic layer was collected and dichloromethane was distilled off by a rotary evaporator to obtain a colorless precipitate. This precipitate was purified by column chromatography using hexane:ethylacetate (80:20) as eluent. Yield: 83%. FT-IR (KBr, cm<sup>-1</sup>) (Fig. S4): 3455, 2961, 1635, 1485, 1194, 1032, 869. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) (Fig. S5) δ: 7.83 (s, 2H), 7.03 (s, 4H), 6.85 (s, 4H), 4.32 (d, 4H), 4.10 (q, 4H), 3.33 (d, 4H), 1.64 (t, 6H), 1.27 (s, 18H), 1.01 (s, 18H). <sup>13</sup>C NMR (500 MHz, CDCl<sub>3</sub>) (Fig. S6)  $\delta$ : 150.6, 150.1, 146.7, 141.5, 133.1, 128.1, 125.5, 122.1, 71.9, 34.0, 33.8, 32.0, 31.7, 31.1, 15.4.

# 2.4. Synthesis of 1, 5-diformyl –3, 7-di-tert-butyl-3, 7-diethoxy-calix[4] arene (3)

The compound (3) was prepared following a procedure reported in the literature [68]. In a 100 RB flask, 1, 3, 5, 7-tetra *tert*-butyl-3, 7-diethoxy-calix[4]arene (705.03 mg, 1 mmol) and Hexamethylenetetramine (HMTA) (5.7659 g, 41.13 mmol) were weighed. 80 ml of trifluoroacetic acid was added. This mixture was refluxed for 48 h. After the completion of the reaction, the mixture was poured into 500 ml of ice water. A yellow color precipitate was formed. This precipitate was redissolved in chloroform and washed with water and brine solution. The chloroform



Conditions:(a) 2 Eq  $K_2CO_{3}$ ,2 Eq Bromoethane, ACN-48 Hrs Reflux. (b) HMTA,TFA-48 Hrs Reflux. (c) 2 Eq Comd 1, Dry-Ethanol, RT 24 Hrs Stirr.

Scheme 1. Synthesis of calix[4]arene thiosemicarbazone (Lig).

was removed by a rotary evaporator to obtain a yellow precipitate. This precipitate is purified by column chromatography using hexane:ethylacetate (90:10) as eluent. Yield: 80%. FT-IR (KBr, cm<sup>-1</sup>) (Fig. S7): 3349, 2963, 2798, 2719, 1685, 1597, 1479, 1133, 1027. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) (Fig. S8):  $\delta$ : 9.77 (s, 2H), 9.17 (s, 2H), 7.62 (s, 4H), 6.94 (s, 4H), 4.32 (d, 4H), 4.13 (d, 4H), 3.50 (q, 4H), 1.70 (t, 6H), 1.05 (s, 18H). <sup>13</sup>C NMR (500 MHz, CDCl<sub>3</sub>) (Fig. S9)  $\delta$ : 191.0, 159.5, 149.8, 148.1, 132.0, 129.1, 128.7, 126.0, 72.3, 54.5, 34.2, 31.6, 31.2, 15.3.

### 2.5. Synthesis of ligand Calix[4]arene thiosemicarbazone (Lig)

In a 100 ml RB flask, compound 1 (304.2 mg, 1.4 mmol) was weighed and 30 ml of dry ethanol was added. This solution was stirred at room temperature for 2 min. Compound 3 (390 mg, 0.6 mmol) was dissolved in a minimum amount of dry ethanol and added dropwise to the above solution. This mixture stirred at room temperature for 24 h. A yellow color precipitate was formed. This precipitate was filtered off and washed with ethanol. Yield: 84%. m.pt: 188 °C, FT-IR (KBr, cm<sup>-1</sup>) (Fig. S10): 3326, 3183, 2962, 1599, 1517, 1475, 1274, 1196, 1102, 1028, 771, 515. <sup>1</sup>H NMR (500 MHz, DMSO- $d_6$ ) (Fig. S11)  $\delta$ : 11.75 (s, 2H), 10.15 (s, 2H), 9.29 (s, 2H), 8.02 (d, 2H), 8.00 (s, 1H), 7.94 (d, 2H), 7.88 (d, 2H), 7.77 (s, 4H), 7.60 (m, 4H), 7.55 (m, 4H), 7.26 (s, 4H), 4.23 (d, 4H), 4.09 (q, 4H) 3.51 (d, 4H), 1.65 (t, 6H), 1.10 (s, 18H). <sup>13</sup>C NMR (500 MHz, DMSO-d<sub>6</sub>) (Fig. S12) δ: 177.9, 154.8, 150.0, 148.0, 143.3, 136.2, 134.2, 133.6, 131.2, 129.9, 128.4, 127.4, 127.1, 126.5, 126.5, 126.3, 126.22, 125.9, 123.7, 72.2, 34.6, 31.5, 31.2, 15.4. ESI Mass (Fig. S13) Calculated: 1047.39, Found: 1047.61.



Fig. 1. Absorption change of Lig (10  $\mu M)$  when addition (10  $\mu L)$  of three pH buffer solutions.

### 3. Results and discussion

The synthetic pathway of Lig is shown in scheme 1. The characterization details are provided in the supporting information (S1-S13). The first step was the synthesis of naphthalene thiosemicarbazide in ethanol at normal room temperature. The peak at 1213 cm<sup>-1</sup> (Fig. S1) in FT-IR spectrum confirms the formation of thiosemicarbazide. The product of the first step was also confirmed by <sup>13</sup>C NMR spectroscopy. The peak at 181.7 ppm (Fig. S3) indicates the formation of thiosemicarbazide. The second step was the lower rim modification of p-tert-butyl calix[4]arene with ethyl bromide at an elevated temperature in the presence of potassium carbonate. This modification was confirmed by <sup>1</sup>H NMR spectrum of compound 2. The two singlets appeared at 1.01 and 1.27 ppm (Fig. S5) indicate that only two phenolic moieties are functionalized as ether and the conformation is maintained as a cone structure. It is possible to tune the upper rim selectivity of calix[4]arene by its lower rim functionalization [68]. The lower rim bi-functionalization can help for the selective bis-formylation of the upper rim of calix[4]arene. The third step was the bis-formylation of calix[4]arene on its upper rim via ipso substitution of a formyl group with hexamethylenetetramine and trifluoroacetic acid at an elevated temperature. There is a strong absorption observed at 1685 cm<sup>-1</sup> and weak doublet absorption observed at 2798 and 2719  $\rm cm^{-1}$  (Fig. S7) due to the stretching vibration of the carbonyl group and Fermi resonance of aldehyde protons, respectively. The disappearance of strong singlet around 1.27 ppm, the appearance of a strong singlet at 9.77 ppm in <sup>1</sup>H NMR spectrum (Fig. S8), and the appearance of a strong peak at 191.07 ppm in <sup>13</sup>C NMR spectrum (Fig. S9) confirm the ipso substitution of formyl group in the upper rim of calix[4] arene moiety. After ipso formylation, the conformation of the molecule is maintained in cone structure and is confirmed by the strong singlet that appeared at 1.05 ppm in the proton spectrum of compound 3 (Fig. S8). The final step is the synthesis of thiosemicarbazone from bisformylated calix[4]arene and naphthalene thiosemicarbazide by stirring at room temperature in an ethanol medium. The peak at 1196  $\rm cm^{-1}$ (Fig. S10) corresponds to the thiocarbonyl stretching frequency confirming the formation of Lig. The presence of a singlet at 1.18 ppm is responsible for the cone conformation of the entire molecule (Fig. S11). The peaks at 11.75 and 10.15 ppm (Fig. S11) correspond to N-H protons of thiourea moiety which show singlet confirming the cone conformation. The presence of a singlet peak at 8.02 in <sup>1</sup>H NMR spectrum (Fig. S11) corresponds to imine protons and peaks at 177.9 &143.3 ppm in <sup>13</sup>C NMR spectrum (Fig.S12) correspond to thiocarbonyl carbon and imine carbon, respectively, also confirming the formation of desired

Absorbance



Fig. 2. Absorption spectral changes of Lig (10  $\mu M)$  with various metal ions (1 eq.)



Fig. 3. Incremental addition of  $Zn^{2+}$ (up to 1 eq.) with Lig (10  $\mu$ M). Inset fig. absorption variation in 380 nm for  $[Zn^{2+}]$  (1 eq.).

p-tert-butylcalix[4]arene thiosemicarbazone Lig.

### 3.1. pH studies

The synthesized Lig is enriched with acidic hydrogen in thiosemicarbazone moiety. So the pH of the medium plays a major role while sensing an analyte. To investigate the photophysical properties of synthesized Lig with respect to pH, UV–visible spectra were recorded at three different pH conditions. The absorbance difference at 400 nm of the Lig with and without pH buffer was measured and plotted (Fig. 1). The absorption difference is large at pH 9.2 (basic medium), and ligand shows a considerable color change from colorless to a yellow color as a consequence of tautomerization from thione to thiol [69]. These results indicate that the synthesized Lig is best suitable for sensing at acidic and neutral pH conditions and not suitable for metal ions in a basic medium. In this work, all sensing studies were carried out in aqueous neutral pH conditions.

### 3.2. UV-Visible absorption studies

The absorption studies of the synthesized Lig were investigated in



**Wavelength (nm) Fig. 4.** Incremental addition of Cu<sup>2+</sup>(upto 1 eq.) with Lig (10 mg/L). Inset fig. absorption variation in 380 nm with respect to [Cu<sup>2+</sup>] (1 eq.).

400

450

300

250

350



**Fig. 5.** Incremental addition of  $Hg^{2+}$  (upto 1 eq) with Lig (10 mg/L). Inset fig. absorption variation in 380 nm with respect to  $[Hg^{2+}]$  (1 eq.).

DMSO:H<sub>2</sub>O (9:1, v/v, pH = 7.2) solvent. The Lig shows a sharp absorption band in 255 nm ( $\varepsilon_{255} = 4,870 \text{ L mol}^{-1}\text{cm}^{-1}$ ) and broad absorption band at 334 nm ( $\varepsilon_{334} = 5,840 \text{ L mol}^{-1}\text{cm}^{-1}$ ). The absorption bands at 255 nm, 334 nm correspond to  $\pi$ - $\pi$ \* and n- $\pi$ \*, respectively. The sensing ability of the synthesized Lig was investigated with various metal ions that included  $Ag^+$ ,  $Al^{3+}$ ,  $Cd^{2+}$ ,  $Cu^{2+}$ ,  $Fe^{3+}$ ,  $Hg^{2+}$ ,  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Na^+$ ,  $Ni^{2+}$ ,  $Pb^{2+}$ , and  $Zn^{2+}$ . Among the metal ions, the Lig showed selectively towards  $Zn^{2+}$ ,  $Cu^{2+}$ , and  $Hg^{2+}$  (Fig. 2) (Fig. S14). To study the UV-Visible spectral changes and sensing capability of Lig with these three metal ions, absorption titration was carried out individually. While titrating with  $Zn^{2+}$ , the absorption intensity at 255 nm, 334 nm decreased gradually and absorption intensity increased at 380 nm (Fig. 3). The isosbestic point noticed at 360 nm indicates that there is coordination between Lig and Zn<sup>2+</sup> metal ion. While titrating with Zn<sup>2+</sup> ion the absorption intensity at 380 nm gradually increases (6 fold) with increasing concentration of zinc ion (Fig. S15). The linearity curve is not straight: shows the first bend at first half equivalence and a second bend at the second half equivalence of zinc metal ion concentration (inset Fig. 3). The stoichiometry ratio between Lig and  $Zn^{2+}$  is 1:1 found by the job's plot method (Fig. S16). The binding constant and limit of detection for the  $Zn^{2+}$  metal ion is 1.99X10<sup>4</sup> M, 6.05 (±3) mg/L, respectively (Fig. S17) [67]. When Lig was titrated against  $Cu^{2+}$  ions peaks at 280

20 µM

30 ...M

40 uM

500

70 uM

80 uM

90 μM 100 μl

600

550



Fig. 6. Fluorescence spectrum of Lig (10 mg/L) with various metal ions (1 eq.) ( $\lambda_{Ex}$  = 334 nm,  $\lambda_{Em}$  = 474).



Fig. 7. Incremental addition of  $Zn^{2+}$  with Lig (up to 1 eq.) Inset fig. Intensity variation in 474 nm with respect to concentration of  $Zn^{2+}$  ( $\lambda_{Ex}=334$  nm,  $\lambda_{Em}=474$ ).

nm, 380 nm gradually increased and the peaks at 255 nm and 334 nm gradually decreased (Figs. 4 & S18). The presence of isosbestic point at 360 nm clearly shows the coordination between Lig and  $Cu^{2+}$  ion. The absorbance at 380 nm increases gradually with increasing concentration of  $Cu^{2+}$  and stable after 0.6 eq of  $Cu^{2+}$  ion concentration (Inset Fig. 4). The calculated binding constant and limit of detection for the Cu<sup>2+</sup> metal ion is  $8.79 \times 10^3$  M and  $15.03 (\pm 3)$  mg/L, respectively (Fig. S19) [67]. When the Lig was titrated against  $Hg^{2+}$  ions, the peaks at 255 nm and 334 nm gradually decreased with increasing concentration of Hg<sup>2+</sup> ions (Figs. 5 & S20). The 334 nm peak shows a bathochromic shift towards higher wavelength along with hypochromic shift and the presence of an isosbestic point at 360 nm infer the coordination between Lig and Hg<sup>2+</sup> ion. The absorption intensity at 380 nm with respect to the concentration of Hg<sup>2+</sup> ion shows the first bend at 0.5 eq and increase further addition (Inset Fig. 5). The calculated binding constant and limit of detection for  $Hg^{2+}$  ion are 5.69X10<sup>4</sup> M and 8.21 (±3) mg/L, respectively (Fig. S21) [70].

### 3.3. Fluorescence studies

The fluorescence property of synthesized Lig was investigated by titrating with various metal ions such as  $Ag^+$ ,  $Al^{3+}$ ,  $Cd^{2+}$ ,  $Cu^{2+}$ ,  $Fe^{3+}$ ,  $Hg^{2+}$ ,  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Na^+$ ,  $Ni^{2+}$ ,  $Pb^{2+}$ , and  $Zn^{2+}$ . When the synthesized Lig was excited at 334 nm, it did not show any emission at 474 nm in free form. The reason behind the non-fluorescent nature of Lig at 474 nm is due to the absence of isomerization of the imine C=N double bond in the excited state [67]. After the addition of 1 eq. metal ions, only Zn<sup>2+</sup> show considerable fluorescence change at 474 nm indicating the isomerization of imine C=N double bond in the excited state (Fig. 6). In the case of Cu<sup>2+</sup> and Hg<sup>2+</sup> ions, Lig did not show any considerable fluorescence enhancement, due to the quenching behavior of these ions. The quenching ability of  $\mathrm{Cu}^{2+}$  and  $\mathrm{Hg}^{2+}$  is a consequence of the paramagnetic nature of copper and spin-orbit coupling of mercury ions. Upon the addition of  $Zn^{2+}$  ions, the emission peak at 474 nm shows a 242 fold hyperchromic shift along with a 24 nm bathochromic shift (Fig. 7). The selectivity of Lig towards  $Zn^{2+}$  ions is shown in the fluorescence bar diagram (Fig. S22). The stoichiometric ratio of Lig with  $Zn^{2+}$  calculated by fluorescence 15.25 (±3) mg/L, 140 nm respectively (Figs. S24 & S25) [69]. The detection limit of synthesized Lig is compared with earlier reported journals (Table S1). Competitive titration was carried out to study the fluorescence interference ability of other cations towards Zn<sup>2+</sup> ion-induced fluorescence of Lig. By the addition of 2 equivalents of other cations, we did not observe any fluorescence quenching of the Lig-Zn<sup>2+</sup> complex. The fluorescence intensity at 474 nm was increased during the addition of other interfering cations which is the consequence of combining effect other of metal ions with  $Zn^{2+}$  ion (Fig. S26). From the above results, the synthesized Lig selectively senses Zn<sup>2+</sup> ion over various toxic heavy metals without any interference by fluorescence spectroscopy in aqueous solutions is confirmed.

### 3.4. Naked-eye detection

A complete and efficient sensor should detect heavy metals via visible color change; this will enhance the practical applicability of the sensors. If the color change is detectable to normal unaided eyes, then the sensing process will be simple and efficient. The naked eye detection was carried out. For this analysis, a higher concentration of Lig (1 mM) was used. Upon addition of one equivalent heavy metal ions to Lig solution, only  $Cu^{2+}$ ,  $Hg^{2+}$  and  $Zn^{2+}$  showed a considerable visible color change from colorless to a vellow color (Fig. S27) due to the metal ions induced conjugation extension of imine double bond. When the same solution was kept under UV light, only Zn<sup>2+</sup> showed chartreuse color fluorescence.  $Cu^{2+}$ ,  $Hg^{2+}$  ions quench the blue fluorescence of Lig. The observed quenching may be due to the paramagnetic nature of  $Cu^{2+}$  and spin–orbit coupling of  $Hg^{2+}$  ions. In order to find the lowest detection limit of Zn<sup>2+</sup> ion by normal unaided eye analysis, we titrated the synthe sized Lig with  ${\rm Zn}^{2+}$  ion having various concentrations. We observed the considerable color change from colorless while the addition of 100  $\mu L$  of  $0.05 X 10^{-2}$  mol/L  $Z n^{2+}$  ion to the synthesized Lig solution (Fig. S28). When exceeding more than 1 eq., the turbid solution was observed. This turbidity is due to the metal-Lig complex reaches its maximum level. If we add a suitable solvent to this complex dissolution will occur. This may improve the detection limit of the synthesized Lig. From this result, we confirmed that the synthesized Lig could sense Cu<sup>2+</sup>, Hg<sup>2+</sup>, and Zn<sup>2+</sup> ions via color change having the lowest detection limit of  $\text{Zn}^{2+}$  by normal eye is 500  $\mu M$  concentration range. The synthesized Lig shows better selectivity towards Zn<sup>2+</sup> ion over various metal ions via fluorescence change also confirmed.

### 3.5. Lifetime study

Excited-state lifetime was investigated by the TCSPC fluorescence technique. The synthesized Lig was excited at 455 nm laser and the



Fig. 8. TCSPC lifetime spectrum of Lig (10 mg/L) and Lig +  $Zn^{2+}$  (1 eq. of  $Zn^{2+}$  ion).

emission was recorded at 474 nm. Similar experiments were carried out with the addition of  $Zn^{2+}$  to the Lig solution. The data was processed by IBH DAS 6.2 data analyzer using biexponential fit. From this analysis, the excited state lifetime of synthesized Lig was determined to be 0.40 ns. After the addition of one equivalent of  $Zn^{2+}$  ion, there is a slight change in a lifetime and the value is 0.086 ns (Fig. 8 prompt curve omitted). This slight change in a lifetime is due to the polarity change in the solvent medium. So the  $Zn^{2+}$  ion is not acting as a fluorescence quencher to the synthesized Lig and does not affect the fluorescence property of the molecule (Figs. S29 & S30). From the above results, it is concluded that this Lig is very much useful to detect  $Zn^{2+}$  ion in the aqueous medium.

### 3.6. Computational study and mode of binding

Based on DFT theoretical study, it is inferred that the electron clouds are concentrated on the thiosemicarbazone moiety of the Lig in the ground state (HOMO). Whereas in the excited state (LUMO), the

electron cloud found concentrated on the naphthalene moiety. Such an electron cloud movement may be due to due to isomerization of imine C=N double bond in the excited state. This will facilitate some sort of charge-transfer complex which results in the electron injection between the Lig moiety and the metal ions. The HOMO-LUMO energy difference of metal-free Lig frontier molecular orbitals is 1.07 eV and metal bound Lig frontier molecular orbital is 0.15 eV. Such decrement of the energy gap from metal-free form to metal bounded form is the consequence of stabilization by  $Zn^{2+}$  both HOMO and LUMO of Lig orbitals (Fig. 9). The binding mode of synthesized Lig was confirmed by FT-IR, <sup>1</sup>H NMR, and Mass spectrometry. After the addition of one equivalent of  $Zn^{2+}$  to Lig the disappearance of the peak at  $3326 \text{ cm}^{-1}$  and shift as well as intensity decrement of 1517 cm<sup>-1</sup>, 1196 cm<sup>-1</sup> peaks noticed which confirms the binding of the  $Zn^{2+}$  ion to Lig (Fig. S31). The disappearance of NH protons at 11.75, 10.15 ppm, and downfield shift of imine proton also confirm the binding of synthesized Lig with  $Zn^{2+}$  (Fig. S32). The singlet appeared around 1 ppm is remain singlet after the addition of metal ions. This observation confirms that there is no conformation changes occurred. Besides, found Lig +  $Zn^{2+}$  mass adduct peak at 1168.8 M/Z in the HR-LCMS spectrum also confirms the binding ability of the synthesized Lig towards  $Zn^{2+}$  ion (Fig. S33). The singlet appeared around 1 ppm is remain singlet after the addition of metal ions. This observation confirms that there is no conformation changes occurred. Based on the above results, how a binding mode occurs between Lig with  $Zn^{2+}$  ion is described in Fig. 10. Further, the formation of azine may be responsible for the color change of Lig after metal binding.

### 3.7. Cytotoxicity study

Normally apoptosis occurs during aging to maintain the cell population in tissues. Apoptosis leads to cell death through cell shrinkage whereas necrosis leads to cell death through cell swelling. Drugs having cytotoxic activity are used to trigger the apoptosis mechanism in cancer chemotherapy treatment. The cytotoxicity activity of the synthesized Lig was investigated against A549 human lung cancer cell lines by MTT assay. This study revealed that the half-maximal inhibitory concentration (IC<sub>50</sub>) value of the synthesized Lig is  $58 \pm 0.5 \,\mu$ g/ml against cancer cell lines after 24 h *in vitro* treatment (Fig. S34). This study shows that the synthesized Lig possesses cytotoxic activity against cancer cells. To investigate the mechanism for killing cancer cells, AO/EB (Acridine



**Fig. 9.** DFT optimized structure of Lig and Lig  $+ Zn^{2+}$  complex.



Fig. 10. Proposed binding mode of Lig with  $Zn^{2+}$  ion.



Fig. 11. AO/EB staining images of A549 lung cancer cell lines control sample (a), cancer cell lines + Lig treated cell (up to 24 h) (b), Hoechst staining images of A549 lung cancer cell lines control sample (c), cancer cell lines + Lig treated cell (up to 24 h) (d).

Orange/ Ethidium Bromide) and Hoechst staining studies were carried out. The AO/EB study can show which type of morphology (apoptosis, necrosis) is observed during the cell death process. These features are not observed in Lig untreated control (normal) cancer cell lines (Fig. 11 (a)). During 24 h *in vitro* treatment, the Lig treated cells show early apoptosis features such as cell shrinkage, condensation, and fragmentation (Fig. 11 (b)). A very few necrosis types of cell morphology were also observed with Lig treated cancer cells. In the Hoechst study control sample (Fig. 11(c)) the apoptosis type of morphology is observed during the cell death process after 24 h *in vitro* treatment of cancer cells with the synthesized Lig (Fig. 11 (d)). This again confirms the observations of AO/EB Staining study. From these observations, it may be concluded that the synthesized Lig shows anticancer activity against A549 human lung cancer cell lines and the mode of cell death majorly followed in apoptosis (programmed cell death) pathway compared to necrosis (Induced cell death) (Figs. S35 & S36).

# Control-A549 Cancer cell line A549 Cancer cell line+ A549 Cancer cell line+ Lig+Zn<sup>2+</sup>(6H Incubated) Lig+Zn<sup>2+</sup>(12H Incubated) (a) (b) (c) (b) (c) (c) (c) (c) (c)



### 3.8. Cellular uptake study

The relative extent of cellular uptake was observed in terms of intracellular fluorescence intensity exhibited by the treatment of synthesized calix[4]arene thiosemicarbazone Lig in the presence of  $Zn^{2+}$  ions. A549 cells were incubated with the Lig for 6 and 12 h followed by exposure to  $Zn^{2+}$  ions to identify their localization. (Control sample Fig. 12. (a)). The Lig mainly be present in the cytoplasm as well as in the nucleus of the cells. Green fluorescence was observed in the treated cells. It can be seen that only a small amount of the Lig penetrates through the cell wall after 6 h (Fig. 12(b)) incubation, whereas after 12 h (Fig. 12(c)), the Lig penetrates deeply into the cell line. Further studies are required to confirm the interaction of cellular organelles with the synthesized Lig.

### 4. Conclusion

In summary, calix[4]arene based thiosemicarbazone Lig was synthesized and characterized by various analytical techniques such as FT-IR, NMR, etc. The sensing of  $Zn^{2+}$ ,  $Cu^{2+}$ ,  $Hg^{2+}$  metal ions by the synthesized Lig has been demonstrated. The Lig shows selectivity towards  $Zn^{2+}$  ions as demonstrated by fluorescence studies. The detection of metal ions with normal unaided eyes is also studied. The synthesized calix[4]arene thiosemicarbazone based Lig shows better cytotoxicity against A549 human lung cancer cell line and the mode of cell death is mainly by apoptosis pathway as confirmed by AO/EB and Hoechst staining methods. The penetrating ability of the synthesized Lig into cells was confirmed by cellular uptake studies.

### CRediT authorship contribution statement

Ambigapathi Anandababu: Conceptualization, Formal analysis, Writing - original draft. Sambandam Anandan: Conceptualization, Investigation, Resources, Writing - original draft, Supervision, Funding acquisition. Asad Syed: Investigation. Najat Marraiki: Investigation, Writing - review & editing. Muthupandian Ashokkumar: Formal analysis, Writing - review & editing.

### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

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### References

- L.R. Goodwin, D. Francom, F.P. Dieken, J.D. Taylor, M.W. Warenycia, R. J. Reiffenstein, G. Dowling, Determination of sulfide in brain tissue by gas dialysis/ ion chromatography: postmortem studies and two case reports, J. Anal. Toxicol. 13 (1989) 105–109, https://doi.org/10.1093/jat/13.2.105.
- [2] G.F. Nordberg, B.A. Fowler, M. Nordberg, N.T. Friberg, Handbook on the Toxicology of Metals. Elsevier, 3rd edn,, 2007.
- [3] K.A. McCall, C.C. Huang, C.A. Fierke, Function and mechanism of zinc metalloenzymes, J. Nutr. 130 (2000) 1437–1446, https://doi.org/10.1093/jn/ 130.5.1437S.
- [4] M. Ugarte, N.N. Osborne, Recent advances in the understanding of the role of zinc in ocular tissues, Metallomics 6 (2014) 189–200, https://doi.org/10.1039/ c3mt00291b
- [5] M. Reiter, S. Vagin, A. Kronast, C. Jandl, B.A. Rieger, Lewis acid β-diiminato zinccomplex as all-rounder for co- and terpolymerization of various epoxides with carbon dioxide, Chem. Sci. 8 (2017) 1876–1882, https://doi.org/10.1039/ C6SC04477H.
- [6] W.H. Hudson, E.A. Ortlund, The structure, function and evolution of proteins that bind DNA and RNA, Nat. Rev. Mol. Cell Biol. 15 (2014) 749–760, https://doi.org/ 10.1038/nrm3884.
- [7] A. Takeda, M. Nakamura, H. Fujii, H. Tamano, Synaptic Zn<sup>2+</sup> homeostasis and its significance, Metallomics. 5 (2013) 417–423, https://doi.org/10.1039/ C3MT20269K.
- [8] H. Haase, L. Rink, Multiple impacts of zinc on immune function, Metallomics 6 (2014) 1175–1180. https://doi.org/10.1039/C3MT00353A.
- [9] L.M. Plum, L. Rink, H. Haase, The essential toxin: impact of zinc on human health, Int. J. Environ. Res. Public Health. 7 (2010) 1342–1365, https://doi.org/10.3390/ ijerph7041342.
- [10] D.J. Lanska, B. Remler, Myelopathy among zinc-smelter workers in Upper Silesia during the late 19<sup>th</sup> century, Neurology 82 (2014) 1175–1179, https://doi.org/ 10.1212/WNL.0000000000270.
- [11] N.T. Watt I.J. Whitehouse N.M. Hooper Int, Article ID 971021 J Alzheimers Dis. 2011 (2010) 10.4061/2011/971021971021.
- [12] A.S. Prasad, Discovery of human zinc deficiency: its impact on human health and disease, Adv. Nutr. 4 (2013) 176–190, https://doi.org/10.3945/an.112.003210.
  [13] R.S. MacDonald, The role of zinc in growth and cell, Proliferation. J Nutr. 130
- (2000) 1500–1508, https://doi.org/10.1093/jn/130.5.15008. [14] J.F. Zhang, S. Kim, J.H. Han, S.J. Lee, T. Pradhan, O.Y. Cao, S.J. Lee, C. Kang, .
- [14] J.F. Zhang, S. Kim, J.H. Han, S.J. Lee, T. Pradhan, Q.Y. Cao, S.J. Lee, C. Kang, J. S. Kim, Pyrophosphate-selective fluorescent chemosensor based on 1,8-naphthalimide-DPA-Zn(II) complex and its application for cell imaging, Org. Lett. 13 (2011) 5294–5297, https://doi.org/10.1021/ol202159x.
- [15] S. Lee, J.H. Lee, T. Pradhan, C.S. Lim, B.R. Cho, S. Bhuniya, S. Kim, J.S. Kim, Fluorescent turn-on Zn<sup>2+</sup> sensing in aqueous and cellular media, Sens. Actuator B-Chem. 160 (2011) 1489–1493, https://doi.org/10.1016/j.snb.2011.07.065.

### A. Anandababu et al.

- [16] S.J. Lippard, J.M. Berg, Principles of Bioinorganic Chemistry, University Science Books, Mill Valley, 1994.
- [17] K.J. Franz, Copper shares a piece of the π, Nat. Chem. Bio. 4 (2008) 85–86, https:// doi.org/10.1038/nchembio0208-85.
- [18] M.M. Fetherolf, S.D. Boyd, D.D. Winkler, D.R. Winge, Oxygen-dependent activation of Cu, Zn-superoxide dismutase-1, Metallomics 9 (2017) 1047–1059, https://doi. org/10.1039/C6MT00298F.
- [19] U.S. EPA, Aquatic life ambient freshwater quality criteria copper. U. S. Environmental Protection Agency, Washington, D.C, 2007, Report number: EPA-822/R-07-001.
- [20] M. Korbas, J.L. Odonoghue, G.E. Watson, I.J. Pickering, S.P. Singh, G.J. Myers, T. W. Clarkson, G.N. George, The Chemical Nature of Mercury in Human Brain Following Poisoning or Environmental Exposure, ACS Chem. Neurosci. 1 (2010) 810–818, https://doi.org/10.1021/cn1000765.
- [21] S.V.S. Rana, Perspectives in endocrine toxicity of heavy metals-a review, Biol Trace Elem Res. 160 (2014) 1–14, https://doi.org/10.1007/s12011-014-0023-7.
- [22] G.H. Chen, W.Y. Chen, Y.C. Yen, C.W. Wang, H.T. Chang, C.F. Chen, Detection of mercury(II) ions using colorimetric gold nanoparticles on paper-based analytical devices, Anal. Chem. 86 (2014) 6843–6849, https://doi.org/10.1021/ac5008688.
- [23] J.E.P. Hahn, Characterization of "spectroscopically quiet" metals in biology, Coord. Chem. Rev. 249 (2005) 161–177, https://doi.org/10.1016/j.ccr.2004.03.011.
- [24] S. Moreira, M. Ficaris, A.E.S. Vives, V.F.N. Filho, O.L.A.D. Zucchi, R.C. Barroso, E. F.O. de Jesus, Heavy metals in groundwater using synchrotron radiation total reflection X-ray analysis, Instrum Sci. Technol. 34 (2006) 567–585, https://doi.org/10.1080/10739140600811682.
- [25] T.M. Minkina, A.V. Soldatov, D.G. Nevidomskaya, G.V. Motuzova, Y. S. Podkovyrina, S.S. Mandzhiev, New approaches to studying heavy metals in soils by X-ray absorption spectroscopy (XANES) and extractive fractionation, Geochem. Int. 2 (2016) 197–204, https://doi.org/10.1134/S001670291512006X.
- [26] S.Z. Mohammadi, D. Afzali, D. Pourtalebi, Flame atomic absorption spectrometric determination of trace amounts of lead, cadmium and nickel in different matrixes after solid phase extraction on modified multiwalled carbon nanotubes, Cent. Eur. J. Chem. 8 (2010) 662–668, https://doi.org/10.2478/s11532-010-0029-8.
- [27] E.M. Martinis, R.A. Olsina, J.C. Altamirano, R.G. Wuilloud, Sensitive determination of cadmium in water samples by room temperature ionic liquid-based preconcentration and electrothermal atomic absorption spectrometry, Anal. Chim. Acta. 628 (2008) 41–48, https://doi.org/10.1016/j.aca.2008.09.001.
- [28] Q. He, Z. Hu, Y. Jiang, X. Chang, Z. Tu, L. Zhang, Preconcentration of Cu(II), Fe(III) and Pb(II) with 2-((2-aminoethylamino)methyl)phenol-functionalized activated carbon followed by ICP-OES determination, J. Hazard. Mater. 175 (2010) 710–714, https://doi.org/10.1016/j.jhazmat.2009.10.067.
- [29] A.R. Bowie, A.T. Townsend, D. Lannuzel, T.A. Remenyi, P. Merwe, Modern sampling and analytical methods for the determination of trace elements in marine particulate material using magnetic sector inductively coupled plasma-mass spectrometry, Anal. Chim. Acta. 676 (2010) 15–27, https://doi.org/10.1016/j. aca.2010.07.037.
- [30] E. Feng, Y. Tu, C. Fan, G. Liu, S. Pu, A highly selective and sensitive fluorescent chemosensor for Zn<sup>2+</sup> based on a diarylethene derivative, RSC Adv. 7 (2017) 50188–50194, https://doi.org/10.1039/C7RA09966E.
- [31] A. Visscher, S. Bachmann, C. Schnegelsberg, T. Teuteberg, R.A. Mata, D. Stalke, Highly selective and sensitive fluorescence detection of Zn<sup>2+</sup> and Cd<sup>2+</sup> ions by using an acridine sensor, Dalton Trans. 45 (2016) 5689–5699, https://doi.org/ 10.1039/C6DT00557H.
- [32] S. Lohar, S. Pal, M. Mukherjee, A. Maji, N. Demitrib, P. Chattopadhyay, A turn-on green channel Zn<sup>2+</sup> sensor and the resulting zinc(II) complex as a red channel HPO<sub>4</sub><sup>2-</sup> ion sensor: a new approach, RSC Adv. 7 (2017) 25528–25534, https://doi. org/10.1039/C7RA02175E.
- [33] W.J. Qu, J. Guan, T.B. Wei, G.T. Yan, Q. Lin, Y.M. Zhang, A turn-on fluorescent sensor for relay recognition of two ions: from a F selective sensor to highly Zn<sup>2</sup> <sup>+</sup>selective sensor by tuning electronic effects, RSC Adv. 6 (2016) 35804–35808, https://doi.org/10.1039/C6RA05381E.
- [34] S. Pramanik, V. Bhalla, M. Kumar, Hexaphenylbenzene-based fluorescent aggregates for detection of zinc and pyrophosphate ions in aqueous media: tunable self-assembly behaviour and construction of a logic device, New J. Chem. 41 (2017) 4806–4813, https://doi.org/10.1039/C6NJ03953G.
- [35] S. Erdemir, S. Malkondu, Calix[4]arene based a NIR-fluorescent sensor with an enhanced stokes shift for the real-time visualization of Zn(II) in living cells, Sensors Actuators B: Chem. 306 (2020), 127574, https://doi.org/10.1016/j. snb.2019.127574.
- [36] H. Kaur, P. Raj, H. Sharma, M. Verma, N. Singh, N. Kaur, Highly selective and sensitive fluorescence sensing of nanomolar Zn<sup>2+</sup> ions in aqueous medium using Calix[4]arene passivated Carbon quantum dots based on fluorescence enhancement: Real-time monitoring and intracellular investigation, Anal. Chim. Acta. 1009 (2019) 1–11, https://doi.org/10.1016/j.aca.2017.12.048.
- [37] S. Ullmann, R. Schnorr, M. Handke, C. Laube, B. Abel, J. Matysik, M. Findeisen, R. Ruger, T. Heine, B. Kersting, Zn2+-ion sensing by fluorescent Schiff base Calix [4]arene macrocycles, Chem. Euro. J. 23 (2017) 3824–3827, https://doi.org/ 10.1002/chem.201700253.
- [38] B.M. Paterson, P.S. Donnelly, Copper complexes of bis(thiosemicarbazones): from chemotherapeutics to diagnostic and therapeutic radiopharmaceuticals, Chem. Soc. Rev. 40 (2011) 3005–3018, https://doi.org/10.1039/C0CS00215A.
- [39] B. Hu, B. Wang, B. Zhao, Q. Guo, Z.H. Li, X.H. Zhang, G.Y. Liu, Y. Liu, Y. Tang, F. Luo, Y. Du, Y.X. Chen, L.Y. Ma, H.M. Liu, Thiosemicarbazone-based selective proliferation inactivators inhibit gastric cancer cell growth, invasion, and migration, Med. Chem. Commun. 8 (2017) 2173–2180, https://doi.org/10.1039/ C7MD00353F.

- [40] A.E. Stacy, D. Palanimuthu, P.V. Bernhardt, D.S. Kalinowski, P.J. Jansson, D. R. Richardson, Zinc(II)–Thiosemicarbazone Complexes Are Localized to the Lysosomal Compartment Where They Transmetallate with Copper Ions to Induce Cytotoxicity, J. Med. Chem. 59 (2016) 4965–4984, https://doi.org/10.1021/acs.jmedchem.6b00238.
- [41] A. P. King, H. A. Gellineau, J. E. Ahn, S. N. MacMillan, J. J. Wilson, bis (thiosemicarbazone) complexes of cobalt(III). Synthesis, characterization, and anticancer potential. Inorg. Chem. 56 (2017) 6609-6623. https://doi.org/10.1021/ acs.inorgchem.7b00710.
- [42] D. Larsen, L.M. Langhorn, O.M. Akselsen, B.E. Nielsen, M. Pittelkow, Thiosemicarbazone organocatalysis: tetrahydropyranylation and 2-deoxygalactosylation reactions and kinetics-based mechanistic investigation, Chem. Sci. 8 (2017) 7978–7982, https://doi.org/10.1039/C7SC03366D.
- [43] R. Bhowmick, R. Alam, T. Mistri, K.K. Das, A. Katarkar, K. Chaudhuri, M. Ali, Thiosemicarbazone based chemo and fluorogenic sensor for Zn<sup>2+</sup>with CHEF and ESIPT behaviours: computational studies and cell imaging application, RSC Adv. 6 (2016) 11388–11399, https://doi.org/10.1039/C5RA25653D.
- [44] K.N. Farrugia, D. Makuc, A. Podborska, K. Szaciłowski, J. Plavec, D.C. Magri, UVvisible and 1H–15N NMR spectroscopic studies of colorimetric thiosemicarbazide anion sensors, Org. Biomol. Chem. 13 (2015) 1662–1672, https://doi.org/ 10.1039/C4OB02091J.
- [45] G. Buncic, P.S. Donnelly, B.M. Paterson, J.M. White, M. Zimmermann, Z. Xiao, A. G. Wedd, A water-soluble bis(thiosemicarbazone) ligand. A sensitive probe and metal buffer for zinc, Inorg. Chem. 49 (2010) 3071–3073, https://doi.org/10.1021/ic902370a.
- [46] Y. Meng, W. Ning, B. Xu, W. Yang, K. Zhang, Y. Chen, L. Li, X. Liu, J. Zheng, Y. Zhang, Inhibition of mild steel corrosion in hydrochloric acid using two novel pyridine Schiff base derivatives: a comparative study of experimental and theoretical results, RSC Adv. 7 (2017) 43014–43029, https://doi.org/10.1039/ C7RA08170G.
- [47] I.E. Tolpygin, Thiosemicarbazones as Effective Fluorescent Sensors for Cations and Anions, Russ. J. Gen. Chem. 82 (2012) 1533–1536, https://doi.org/10.1134/ S1070363212090137.
- [48] A.Y. Lukmantara, D.S. Kalinowski, N. Kumar, D.R. Richardson, Structure–activity studies of 4-phenyl-substituted 2'-benzoylpyridine thiosemicarbazones with potent and selective anti-tumour activity, Org. Biomol. Chem. 11 (2013) 6414–6425, https://doi.org/10.1039/C3OB41109E.
- [49] F. Bacher, O. Domotor, A. Chugunova, N.V. Nagy, L. Filipovic, S. Radulovic, E. A. Enyedy, V.B. Arion, Strong effect of copper(II) coordination on antiproliferative activity of thiosemicarbazone– piperazine and thiosemicarbazone morpholine hybrids, Dalton Trans. 44 (2015) 9071–9090, https://doi.org/10.1039/ C5DT01076D.
- [50] A.A. Ibrahim, H. Khaledi, P. Hassandarvish, H.M. Ali, H. Karimian, Indole-7carbaldehyde thiosemicarbazone as a flexidentate ligand toward Zn(II), Cd(II), Pd (II) and Pt(II) ions: cytotoxic and apoptosis-inducing properties of the Pt (II) complex, Dalton Trans. 43 (2014) 3850–3860, https://doi.org/10.1039/ C3D153032A.
- [51] M. Sobiesiak, M. Cieslak, K. Krolewska, J.K. Baranska, B. Pasternak, E. Budzisz, The thiosemicarbazone-derived copper(II), cobalt(II) and nickel(II) complexes as potential anticancer agents: nuclease activity, cytotoxicity and apoptosis studies, New J. Chem. 40 (2016) 9761–9767, https://doi.org/10.1039/C6NJ02899C.
- [52] L.H. Mervin, Q. Cao, I.P. Barrett, M.A. Firth, D. Murray, L. McWilliams, M. Haddrick, M. Wigglesworth, O. Engkvist, A. Bender, Understanding cytotoxicity and cytostaticity in a high-throughput screening collection, ACS Chem. Biol. 11 (2016) 3007–3023, https://doi.org/10.1021/acschembio.6b00538.
- [53] M.C. Berenbaum, The mode of action of cytotoxic drugs, Proc R Soc Med. 63 (1970) 1063–1066.
- [54] D.S. Kalinowski, P. Quach, D.R. Richardson, Thiosemicarbazones: the new wave in cancer treatment, Future Med. Chem. 1 (2009) 1143–1151, https://doi.org/ 10.4155/fmc.09.80.
- [55] A. Sirbu, O. Palamarciuc, M.V. Babak, J.M. Lim, K. Ohui, E.A. Enyedy, S. Shova, D. Darvasiova, P. Rapta, W.H. Ang, V.B. Arion, Copper(II) thiosemicarbazone complexes induce marked ROS accumulation and promote nrf2-mediated antioxidant response in highly resistant breast cancer cells, Dalton Trans. 46 (2017) 3833–3847, https://doi.org/10.1039/C7DT00283A.
- [56] C.J. Pedersen, The discovery of crown ethers, J. Incl. Phenom. Mol. Recognit. Chem. 12 (1992) 7–10. Https://doi.org/10.1007/978-94-011-2532-1\_1.
- [57] I.V. Kolesnichenko, E.V. Anslyn, Practical applicatuions of supramolecular chemistry, Chem. Soc. Rev. 46 (2017) 2385–2390, https://doi.org/10.1039/ C7CS00078B.
- [58] D.B. Amabilino, D.K. Smith, J.W. Steed, Supramolecular materials, Chem. Soc. Rev. 46 (2017) 2404–2420, https://doi.org/10.1039/C7CS00163K.
- [59] J.S. Kim, H.J. Kim, H.M. Kim, S.H. Kim, J.W. Lee, S.K. Kim, B.R. Cho, Metal ion sensing novel calix[4]crown fluoroionophore with a two-photon absorption property, J. Org. Chem. 71 (2006) 8016–8022, https://doi.org/10.1021/ jo0610098.
- [60] X.H. Sun, W. Li, P.F. Xia, H.B. Luo, Y. Wei, M.S. Wong, Y.K. Cheng, S. Shuang, Phenyl-calix[4]arene-based fluorescent sensors: cooperative binding for carboxylates, J. Org. Chem. 72 (2007) 2419–2426, https://doi.org/10.1021/ jo062258z.
- [61] S. Memon, A.A. Bhatti, U.U. Ocak, New calix[4]arene based highly selective fluorescent probe for Al<sup>3+</sup> and I<sup>-</sup>, Anal. Methods. 7 (2015) 5114–5121, https://doi. org/10.1039/C5AY00452G.
- [62] A.B. Othman, J.W. Lee, J.S. Wu, J.S. Kim, R. Abidi, P. Thuéry, J.M. Strub, A. V. Dorsselaer, J. Vicens, Calix[4]arene-Based, Hg<sup>2+</sup>-induced intramolecular

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### Inorganica Chimica Acta xxx (xxxx) xxx

fluorescence resonance energy transfer chemosensor, J. Org. Chem. 72 (2007) 7634–7640, https://doi.org/10.1021/jo0712260.

A. Anandababu et al.

- [63] P.G. Sutariya, A. Pandya, A. Lodhab, S.K. Menon, Fluorescence switch on-off-on receptor constructed on quinoline allied calix[4]arene for selective recognition of Cu<sup>2+</sup> from blood serum and F from industrial waste water, Analyst. 138 (2013) 2531–2535, https://doi.org/10.1039/C3AN00209H.
- [64] F. Zhang, Y. Sun, D. Tian, W.S. Shin, J.S. Kim, H. Li, Selective molecular recognition on calixarene-functionalized 3D surfaces, Chem. Commun. 52 (2016) 12685–12693, https://doi.org/10.1039/C6CC05876K.
- [65] P.K. Lo, M.S. Wong, Extended Calix[4]arene-Based Receptors for Molecular Recognition and Sensing, Sensors. 8 (2008) 5313–5335, https://doi.org/10.3390/ s8095313.
- [66] J.S. Kim, D.T. Quang, Calixarene derived fluorescent probes, Chem. Rev. 107 (2007) 3780–3799, https://doi.org/10.1021/cr068046j.
- [67] H.A. Benesi, J.H. Hildebrand, Spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons, J. Am. Chem. Soc. 71 (1949) 2703–2707, https://doi.org/10.1021/ja01176a030.
- [68] H.M. Chawla, N. Pant, B. Srivastava, S. Upreti, Convenient direct synthesis of bisformylated calix[4]arenes via ipso substitution, Org. Lett. 8 (2006) 2237–2240, https://doi.org/10.1021/ol0605124.
- [69] L. Tang, Z. Huang, Z. Zheng, K. Zhong, Y. Bian, A new thiosemicarbazone-based fluorescence "turn-on" sensor for Zn<sup>2+</sup> recognition with a large stokes shift and its application in live cell imaging, J Fluoresc. 26 (2016) 1535–1540, https://doi.org/ 10.1007/s10895-016-1827-y.
- [70] S. Samanta, U. Manna, T. Raya, G. Das, Aggregation-Induced Emission (AIE) active probe for multiple targets: Fluorescent sensor for Zn<sup>2+</sup> and Al<sup>3+</sup> & colorimetric sensor for Cu<sup>2+</sup> and F, Dalton Trans. 44 (2015) 18902–18910, https://doi.org/ 10.1039/C5DT03186A.