

Pb²⁺- Induced Self Assembly of a Thiophene Derived Chalcone With Enhanced Fluorescence in Aqueous Media

Research Article

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Abstract

A new pyrene based chalcone receptor **1** has been synthesized and utilized as a sensor for Pb²⁺ ions in aqueous media. Receptor **1** showed a highly selective and sensitive recognition of Pb²⁺ over other heavy and transition metal ions. The turn "off-on" fluorescent detection of Pb²⁺ by **1** is free from the interference of other metal ions. A possible PET mechanism is proposed for the "off-on" fluorescent enhancement.

Keywords: Chemosensor; Pyrene; Thiophene; Lead; Fluorescence; PET

Introduction

Lead poisoning is the most environmentally caused diseases [1,2]. Pb²⁺ is a kind of dangerous heavy metal ions, which pollute the environment and create a wide variety of health problems, such as irritability, anaemia, muscle paralysis, neurological damage, nerve disorders, mental confusion, memory loss, reduced IQ and particularly to children even at low doses [3-7]. Therefore, the detection of Pb²⁺ has a vital significance in monitoring the environmental pollution and medical diagnostics in environmental and biological samples. Nowadays, the development of fluorescent chemosensors for the detection of metal cations has attracted extensive interest due to their high sensitivity and selectivity [8-11]. Fluorescent techniques have become the potential tool for trace analytes due to operational simplicity, low cost, real time monitoring and high selectivity [12-14]. Though, there are many fluorescence sensors for the selective detection of Pb²⁺ reported earlier, most of them show fluorescence quenching. However sensors with 'turn-on' responses are relatively rare [15-22]. In addition, the probes for Pb²⁺ have several drawbacks:

(i) sensors are interfered with by other metal ions such as Hg²⁺, Zn²⁺ and Cd²⁺; (ii) only a few could work in aqueous solution [23-35]. Thus, developing fluorescent chemosensors that could overcome the disadvantages mentioned above is highly desirable.

Herein, we report a thiophene derived chalcone bearing a pyrene scaffold **1** as a receptor for selective detection of Pb²⁺ in aqueous solution. Pyrene fluorophores have been extensively used for the synthesis of fluorescent chemosensors because of their unique monomer and excimer emissions depending upon the relative proximity between pyrene fluorophores [36]. The synthesis, characterization and the nature of binding of the receptor **1** with Pb²⁺-ion are explained by using fluorescence techniques.

Experimental methods

Materials and measurements

All the reagents were purchased from sigma Aldrich & Merck in analytical grade. ¹H NMR and ¹³C NMR spectra were measured on a Bruker 300 MHz spectrometer, with chemical shifts reported in ppm

(in CDCl_3 , TMS as internal standard). LC-MS were determined on a LC-MSD-Trap-XCT Plus based on infusion methods. Absorption spectra were made on a Shimadzu UV-240 spectrophotometer. Fluorescence measurements were performed on a Jasco FP-8200 spectrofluorimeter equipped with quartz cuvettes of 1 cm path length. The excitation and emission slit widths were 5.0 nm. All emission spectra were recorded at $24 \pm 1^\circ\text{C}$. Stock solutions for analysis were prepared ($2 \times 10^{-3}\text{M}$ for compound **1** in $\text{DMSO-H}_2\text{O}$, 1:1 v/v, HEPES=50 mM, pH=7.4) immediately before the experiments. The solutions of metal ions were prepared from nitrate salts of Na^+ , K^+ , Al^{3+} , Cu^{2+} , Cd^{2+} , Hg^{2+} , La^{3+} , Pb^{2+} , Zn^{2+} , Co^{2+} , Ni^{2+} , Ca^{2+} , Mn^{2+} , Cr^{3+} , Ba^{2+} , Ce^{3+} , Mg^{2+} , Fe^{2+} , Fe^{3+} and Ag^+ .

Synthesis of receptor **1** ((E)-1-(8,10-dihydropyren-1-yl)-(thiophen-2-yl)prop-2-en-1-one)

Aqueous sodium hydroxide (4 ml, 10%) was added to a mixture of 2-thiophenecarboxaldehyde 0.2 g (1.78 mmol), 1-acetylpyrene 0.48 g (1.96 mmol) and 25 ml of ethanol. The reaction mixture was stirred at room temperature for 24 h. The resulting precipitate was collected by filtration, washed with more ice cold ethanol. The precipitate was then recrystallized from Chloroform. Yield 85 %. M.p. $180\text{--}181^\circ\text{C}$, ^1H NMR (300 MHz, CDCl_3): δ : 8.58–8.61 (d, 1H, $J=9$ Hz), 8.12–8.23 (6H, m), 8.00–8.08 (m, 2H), 7.73–7.78 (d, 1H, $J=15$ Hz), 7.41–7.43 (d, 1H, $J=6$ Hz), 7.27–7.28 (d, 1H, $J=3$ Hz), 7.23–7.24 (d, 1H, $J=3$ Hz), 7.04–7.07 (t, 1H) ppm. ^{13}C NMR (100 MHz, CDCl_3): δ = 194.28, 139.10, 137.25, 132.69, 132.18, 131.02, 130.08, 129.62, 128.29, 128.26, 128.13, 128.05, 127.36, 126.13, 125.34, 125.22, 125.05, 124.90, 123.84, 123.66, 123.36, 123.04 ppm. LC-MS: m/z = 339.4852 [$\text{M}^+ - \text{H}$] $^+$.

Results and Discussion

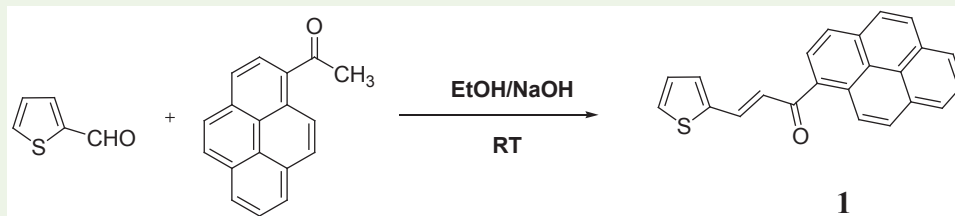
Synthesis and Characterization of Receptor **1**

The fluorescent receptor **1** was concisely synthesized from 2-thiophenecarboxaldehyde and 1-acetylpyrene in a single step condensation reaction (Scheme 1). The final receptor **1** was confirmed by NMR (^1H and ^{13}C) and mass spectrometry. (Supp. Info. S1-S3). The chemosensor is designed in such way that, it has a receptor-linker-fluorophore unit. The pyrene scaffold acts as a fluorophore, which is covalently attached to a thiophene moiety (receptor) through a C=C spacer unit. The hetero atoms, oxygen and sulphur act as binding sites for the metal ion detection.

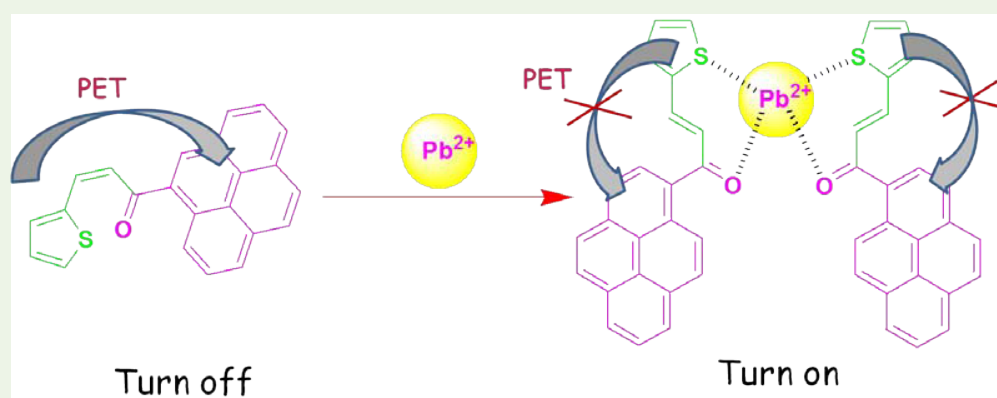
Selectivity studies of receptor **1**

The fluorescence response of the chemosensor **1** towards various metal ions were performed at a physiological pH in $\text{DMSO-H}_2\text{O}$ (1:1 v/v, HEPES=50 mM, pH=7.4) solution. Upon addition of 100 equiv. of Pb^{2+} to a solution of **1**, significant enhancement of fluorescence with an emission maximum at 418 nm was observed compared to the sensor alone under the same experimental conditions. However, under the identical concentration of other metal ions (Na^+ , K^+ , Al^{3+} , Cu^{2+} , Cd^{2+} , Hg^{2+} , La^{2+} , Zn^{2+} , Co^{2+} , Ni^{2+} , Ca^{2+} , Mn^{2+} , Cr^{3+} , Ba^{2+} , Ce^{3+} , Mg^{2+} , Fe^{2+} , Fe^{3+} and Ag^+) sensor **1**, produced no considerable changes in the fluorescence spectra (Figure 1). Therefore, **1** act as a highly selective fluorescence “off-on” probe for Pb^{2+} in $\text{DMSO-H}_2\text{O}$ (1:1 v/v, HEPES=50 mM, pH=7.4) solution.

For practical applications, the possible interferences by other metal ions were carried out through competitive experiments. The fluorescence changes of **1** was measured by the treatment of 100 equiv. Pb^{2+} ions in the presence of same equiv. other interfering metal ions including Na^+ , K^+ , Al^{3+} , Cu^{2+} , Cd^{2+} , Hg^{2+} , La^{2+} , Zn^{2+} , Co^{2+} , Ni^{2+} , Ca^{2+} ,



Scheme 1: Synthesis of Receptor **1**.



Scheme 2: Proposed binding mode of **1** with Pb^{2+} .

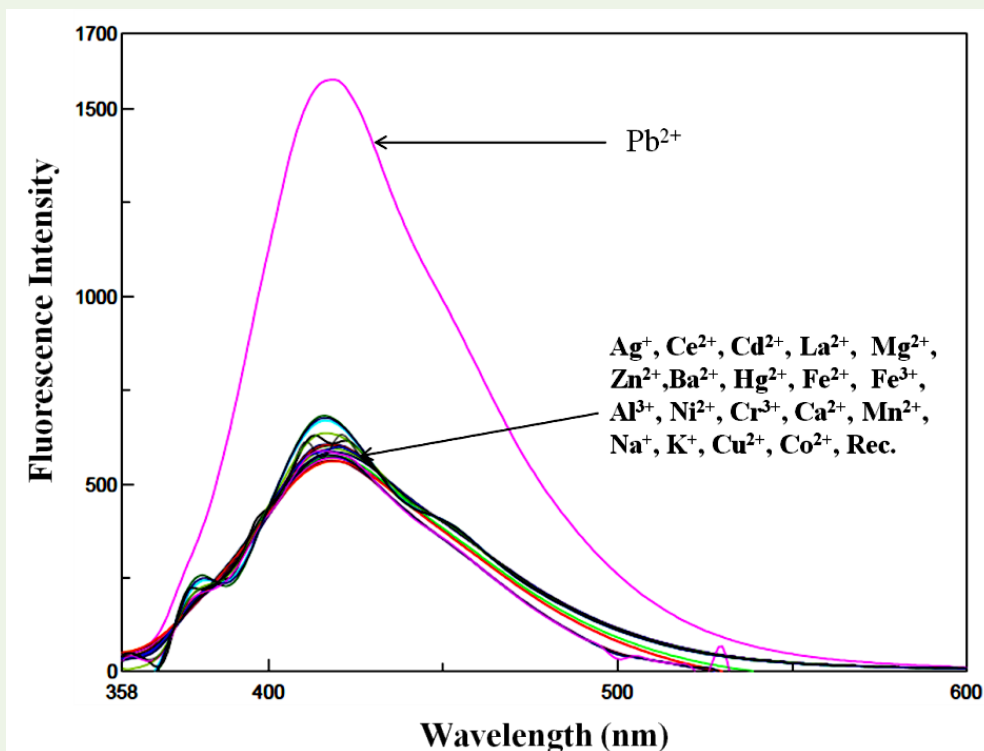


Figure 1: Fluorescence changes of **1** (4×10^{-6} M) DMSO-H₂O solution (1:1 v/v, HEPES=50 mM, pH=7.4) in the presence of various metal ions (100 equiv. of each, excited at 344 nm).

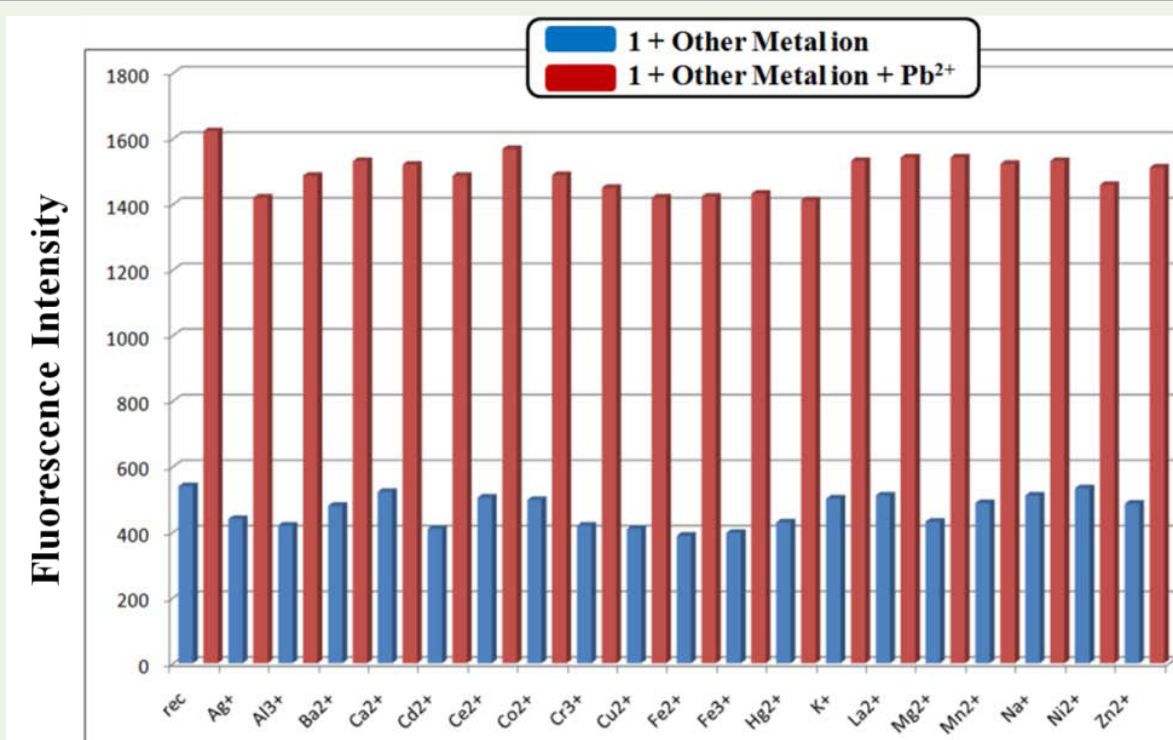


Figure 2: Fluorescence response of **1** (4×10^{-6} M) in DMSO/H₂O (1:1 v/v, HEPES = 50 mM, pH = 7.4) solution. The blue bars represent the fluorescence emission of **1** and 100 equiv. of other metal ions. The red bars represent the fluorescence changes that occur upon addition of 100 equiv. of other metal ions to the solution containing **1** and Pb²⁺ (100 equiv.).

Mn^{2+} , Cr^{3+} , Ba^{2+} , Ce^{3+} , Mg^{2+} , Fe^{2+} , Fe^{3+} and Ag^+ . The tested interfering metal ions showed no observable interference with the detection of Pb^{2+} ion (Figure 2). These results suggested that receptor **1** could be used for the selective detection of Pb^{2+} -ion in environmental and biological sample analysis.

Fluorescence responses and stoichiometric studies

Furthermore, the fluorescence response of **1** to various concentrations of Pb^{2+} was investigated as titration experiments.

As shown in Figure 3, upon addition of Pb^{2+} (0-60 equiv.), the fluorescence of **1** gradually increased and remained steady when 100 equiv. of Pb^{2+} was added, implying that **1** interacts with Pb^{2+} in 2:1 stoichiometry. The Job's plot method (Figure 4) is used to find the complexation mode between receptor **1** and Pb^{2+} (Host-Guest) complex [37]. The complex showed a maximum mole fraction of **1** is 0.3 at 418 nm established a 2:1 (**1**: Pb^{2+}) binding stoichiometry. The detection limit of the sensor **1** is found to be $0.11 \times 10^{-6} M^{-1}$, which is calculated using $3\delta/S$ [38].

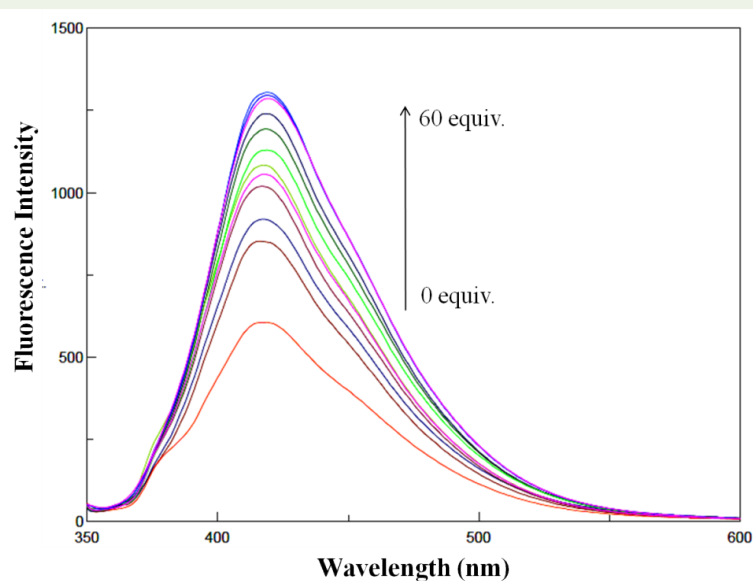


Figure 3: Fluorescence titration spectrum of **1** ($4 \times 10^{-6} M$) in DMSO- H_2O solution (1:1 v/v, HEPES=50 mM, pH=7.4) upon addition of different amount of Pb^{2+} (0-60 equiv. of each, excited at 344 nm) emission = 418 nm.

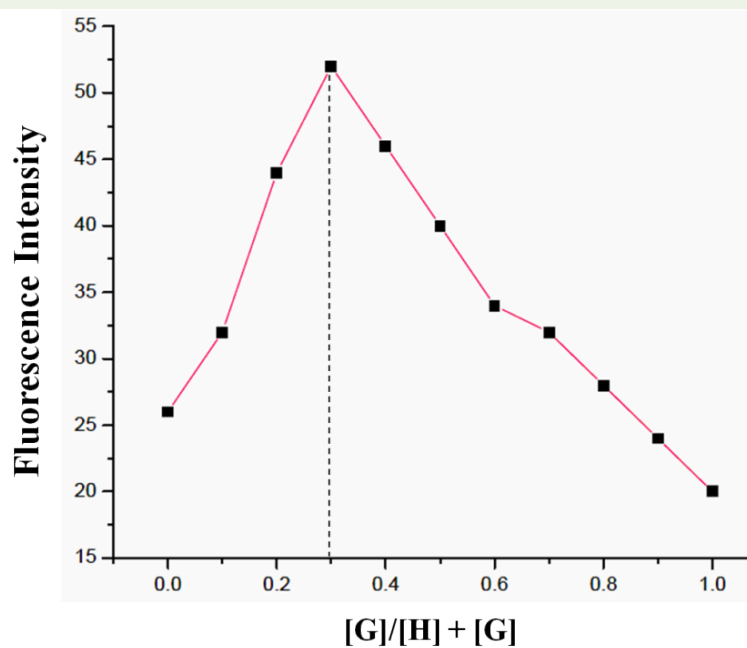


Figure 4: Job plot of **1** and Pb^{2+} in DMSO- H_2O solution (1:1 v/v, HEPES=50 mM, pH=7.4).

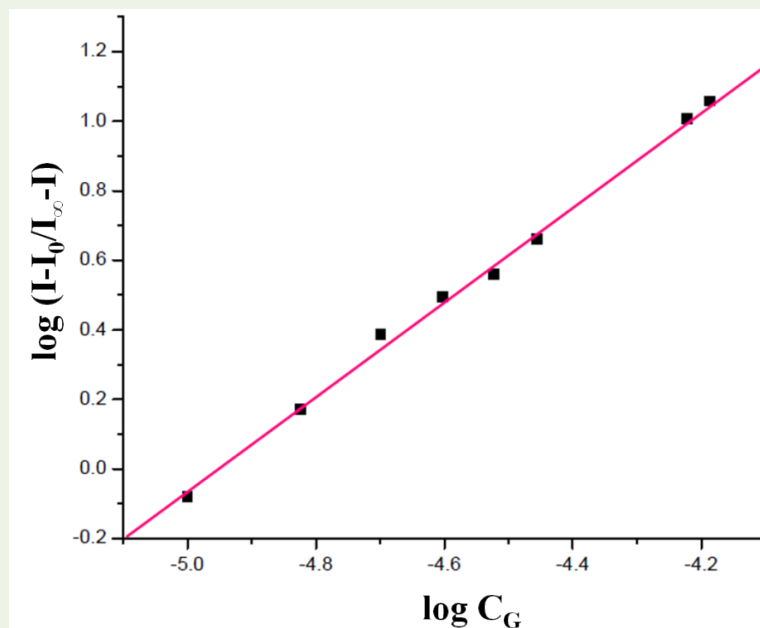


Figure 5: Benesi-Hildebrand plot of 1-Pb²⁺ complex (2:1) binding stoichiometry.

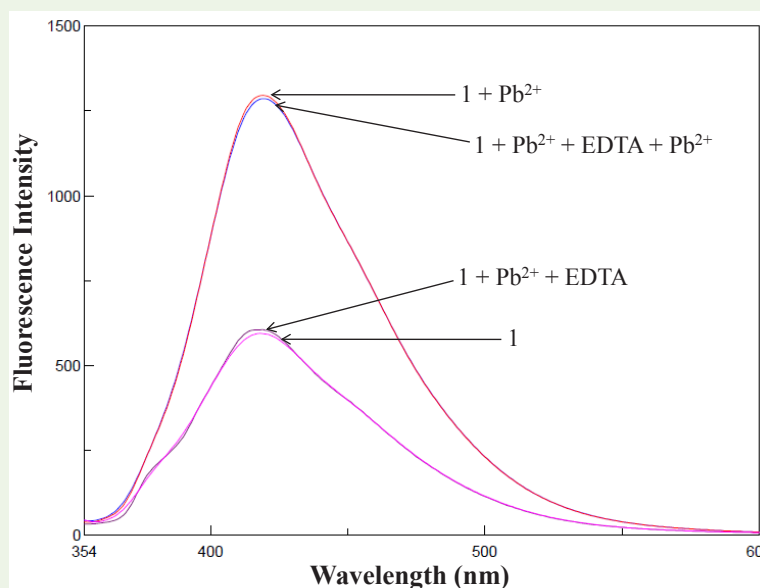


Figure 6: Fluorescence spectra of **1** (4×10^{-6} M) solution (DMSO-H₂O, 1:1 (v/v), HEPES=50 mM, pH=7.4) in the presence of Pb²⁺ & EDTA (100 equiv.)

The binding stoichiometry is further confirmed by the Benesi-Hildebrand plot between **1**-Pb²⁺ (Figure 5) [39]. The association constant was determined to be $K_a = 1.18 \times 10^5 \text{ M}^{-2}$ for the **1**-Pb²⁺ complex.

The recognition reversibility of **1** to Pb²⁺ was verified with the help of EDTA. As already discussed, sensor **1** alone show a weak fluorescence and after addition of Pb²⁺ there is a significant enhancement of the fluorescence intensity as an turn on process. Nevertheless, upon addition of EDTA to the same solution the fluorescence intensity got reduced and peak overlapped with that of

the receptor **1** as an turn on-off process. This study indicates that the Pb²⁺ recognition process is reversible one as shown in Figure 6.

The IR spectrum of chemosensor **1** exhibited characteristic IR band in the region of 1429 and 1668 cm⁻¹ corresponding to C=C and C=O stretching groups. However, as shown in Figure 7, in the IR spectra of **1**+Pb²⁺, C=C stretching band is shifted to 1435 cm⁻¹ and at the same time the C=O group shifted to 1660 cm⁻¹ [40,41]. These differences in the IR band shift further corroborates that the carbonyl group took part in coordination with Pb²⁺ ions.

Proposed coordination mode of the complex

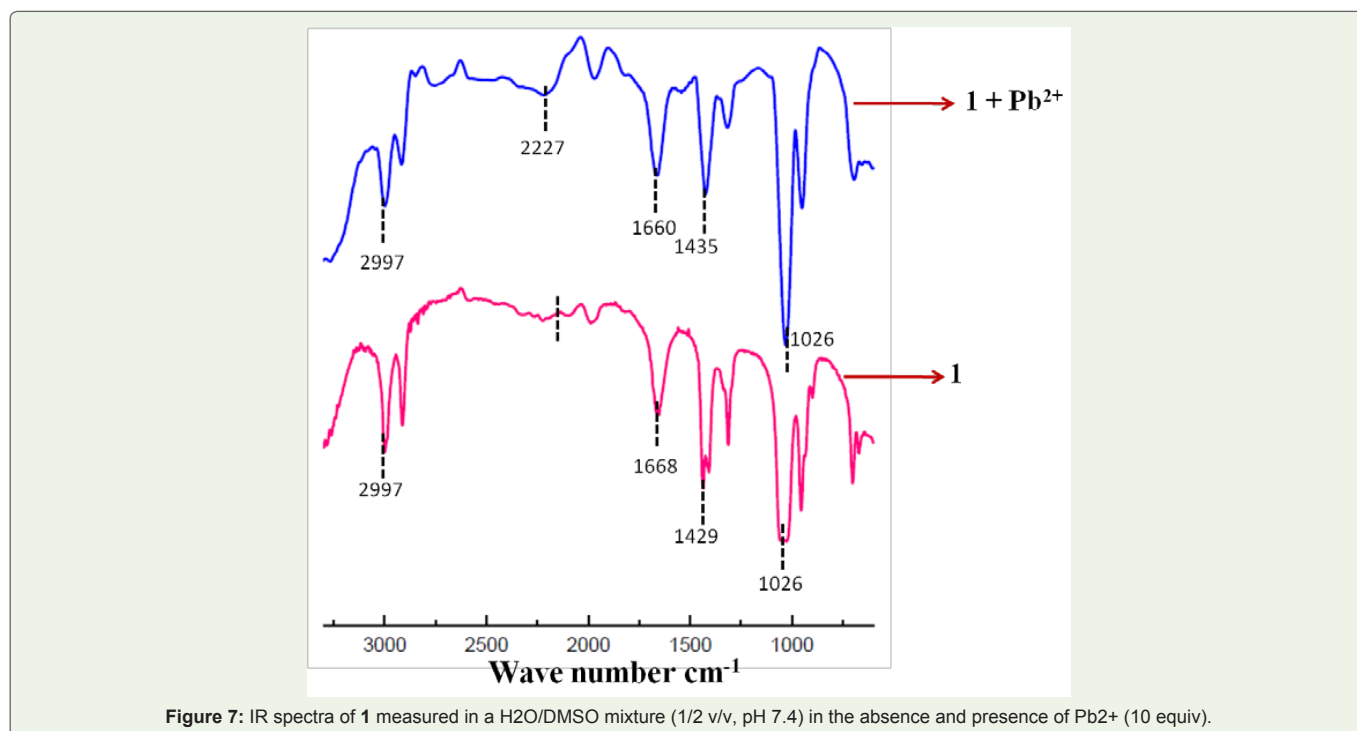


Figure 7: IR spectra of **1** measured in a H₂O/DMSO mixture (1/2 v/v, pH 7.4) in the absence and presence of Pb²⁺ (10 equiv).

The proposed coordination mechanism of **1**-Pb²⁺ was investigated through fluorescence changes as shown in scheme 2. For the sensor **1**, have no accountable fluorescence (turn-off) due to intramolecular photo-induced-electron-transfer (PET) process within the chalcone based pyrene unit [42-44]. Upon the addition of Pb²⁺-ions to **1**, there is a gradual increase in the fluorescence (turn-on) enhancement which eventually inhibits the PET process. By combing all the above results such as, fluorescence titration spectrum, job's plot and non linear curve fitting methods the possible coordination modes of **1**-Pb²⁺-ion complex was found to be 2:1 (Host: Guest) binding stoichiometry.

In addition, a pH titration experiment showed that receptor **1** and **1**+Pb²⁺ had stable fluorescence properties over a wide pH range of 5-9 (Figure S4), which suggests that receptor **1** is suitable for biological application under the physiological conditions. The effective time taken by the receptor **1** for the detection of Pb²⁺ was also studied. The bar diagram (Figure S5) reveals that the receptor **1** can complex with 100 equiv. Pb²⁺ completely in 2 min. However, after that no considerable change in the fluorescence intensity was observed.

Conclusion

In summary, we have designed and synthesized a new chalcone based pyrene receptor **1** as a sensor for Pb²⁺ ions in aqueous solution. Receptor **1** showed highly selective and sensitive recognition of Pb²⁺ over other heavy and transition metal ions. The turn "off-on" fluorescent detection of Pb²⁺ by **1** is free from the interference of other metal ions. This approach could be applied for the real level sensing and detection of Pb²⁺ in the environmental and biological samples.

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References

- Erel Y, Axelrod T, Veron A, Mahrer Y, Katsafados P, et al. (2002) Transboundary atmospheric lead pollution. *Environ Sci Technol* 36: 3230-3233.
- Campbell LM, Dixon DG, Hecky RE (2003) A Review Of Mercury In Lake Victoria, East Africa: Implications For Human And Ecosystem Health *J Toxicol Environ Health, Part B*, 6: 325-356.
- Araki S, Sato H, Yokoyama K, Murata K (2000) Subclinical neurophysiological effects of lead: A review on peripheral, central, and autonomic nervous system effects in lead workers. *Am J Ind Med* 37: 193-204.
- Rifai N, Cohen G, Wolf M, Cohen L, Faser C, et al. (1993) Incidence of lead poisoning in young children from inner-city, suburban, and rural communities. *Ther Drug Monit* 15: 71-74.
- Cory-Slechta DA (1984) The Behavioral Toxicity of Lead: Problems and Perspectives. *Adv Behav Pharmacol* 4: 211-255.
- Winder C, Carmichael NG, Lewis PD (1982) Effects of chronic low-level lead exposure on brain development and function. *Trends Neurosci* 5: 207-209.
- Lee KM, Chen X, Fang W, Kim JM, Yoon J (2011) A dual colorimetric and fluorometric sensor for lead ion based on conjugated polydiacetylenes. *Macromol Rapid Commun* 32: 497-500.
- Chen CT, Huang WP (2002) A highly selective fluorescent chemosensor for lead ions. *J Am Chem Soc* 124: 6246-6247.
- Prabhu J, Velmurugan K, Nandhakumar R (2014) A highly selective and sensitive naphthalene-based chemodosimeter for Hg²⁺ ions. *Journal of Luminescence* 145: 733-736.
- Kim HN, Ren WX, Kim JS, Yoon J (2012) Fluorescent and colorimetric sensors for detection of lead, cadmium, and mercury ions. *Chem Soc Rev* 41: 3210-3244.

11. Zhang B, Lu L, Hu Q, Huang F, Lin Z (2014) ZnO nanoflower-based photoelectrochemical DNAzyme sensor for the detection of Pb²⁺. *Biosens Bioelectron* 56: 243-249.
12. de Silva AP, Fox DB, Huxley AJM, Moody TS (2000) Combining luminescence, coordination and electron transfer for signalling purposes *Coord. Chem Rev* 205: 41-57.
13. Hu ZQ, Lin CS, Wang XM, Ding L, Cui CL, et al. (2010) Highly sensitive and selective turn-on fluorescent chemosensor for Pb²⁺ and Hg²⁺ based on a rhodamine-phenylurea conjugate. *Chem Commun* 46: 3765-3767.
14. Thakur A, Mandal D, Deb P, Mondal B, Ghosh S (2014) Synthesis of triazole linked fluorescent amino acid and carbohydrate bio-conjugates: a highly sensitive and skeleton selective multi-responsive chemosensor for Cu(II) and Pb(II)/Hg(II) ions *RSC Advances* 4: 1918-1928.
15. Kwon JY, Jang YJ, Lee YJ, Kim KM, Seo MS, et al. (2005) A highly selective fluorescent chemosensor for Pb²⁺. *J Am Chem Soc* 127: 10107-10111.
16. Baruah M, Huntimer ED, Ahmed MS, Hoppe AD, Halaweish FT (2012) Selective BODIPY[®]-based fluorescent chemosensor for imaging Pb²⁺ ion in living cells. *Tetrahedron Letters* 53: 4273-4275.
17. Lee KM, Chen X, Fang W, Kim J, Yoon J (2011) A dual colorimetric and fluorometric sensor for lead ion based on conjugated polydiacetylenes. *Macromol Rapid Commun* 32: 497-500.
18. Lee HY, Bae DR, Park JC, Song H, Han WS, et al. (2009) A selective fluoroionophore based on BODIPY-functionalized magnetic silica nanoparticles: removal of Pb²⁺ from human blood. *Angew Chem Int Ed Engl* 48: 1239-1243.
19. Sinha S, Koner R, Kumar S, Mathew J, Roy A, et al. (2013) Structurally tuned benzo[h]chromene derivative as Pb²⁺ selective 'turn-on' fluorescence sensor for living cell imaging. *Journal of Luminescence* 143: 355-360.
20. Li CY, Zhou Y, Li YF, Kong XF, Zou CX, et al. (2013) Colorimetric and fluorescent chemosensor for citrate based on a rhodamine and Pb²⁺ complex in aqueous solution. *Analytica Chimica Acta* 774: 79-84.
21. Xu HR, Li K, Liu Q, Wu TM, Wang MQ, et al. (2013) Dianthracene-cyclen conjugate: the first equal-equivalent responding fluorescent chemosensor for Pb²⁺ in aqueous solution. *Analyst* 138: 2329-2334.
22. Liu J, Wu K, Li S, Song T, Han Y, et al. (2013) A highly sensitive and selective fluorescent chemosensor for Pb²⁺ ions in an aqueous solution. *Dalton Transactions* 42: 3854-3859.
23. Yang JE, Zhou CJ, Liu C, Li Y, Liu HB, et al. (2012) A dual sensor of fluorescent and colorimetric for the rapid detection of lead. *Analyst* 137: 1446-1450.
24. Ju H, Lee MH, Kim J, Kim JS, Kim J (2011) Rhodamine-based chemosensing monolayers on glass as a facile fluorescent "turn-on" sensing film for selective detection of Pb²⁺. *Talanta* 83: 1359-1363.
25. Wang ZD, Lee JH, Lu Y (2008) Label-Free Colorimetric Detection of Lead Ions with a Nanomolar Detection Limit and Tunable Dynamic Range by using Gold Nanoparticles and DNAzyme. *Adv Mater* 20: 3263-3267.
26. Yin BC, Zuo P, Huo H, Zhong XH, Ye BC (2010) DNAzyme self-assembled gold nanoparticles for determination of metal ions using fluorescence anisotropy assay. *Anal Biochem* 401: 47-52.
27. Sun M, Shangguan DH, Ma HM, Nie LH, Li XH, et al. (2003) Simple PbII fluorescent probe based on PbII-catalyzed hydrolysis of phosphodiester. *Biopolymers* 72: 413-420.
28. Chen JH, Liu J, Fang ZY, Zeng LW (2012) Random dsDNA-templated formation of copper nanoparticles as novel fluorescence probes for label-free lead ions detection. *Chem Commun* 48: 1057-1059.
29. Yang XR, Xu J, Tang X, Liu H, Titan D (2010) A novel electrochemical DNAzyme sensor for the amplified detection of Pb²⁺ ions. *Chem Commun* 46: 3107-3109.
30. Lan T, Furuyab K, Lu Y (2010) A highly selective lead sensor based on a classic lead DNAzyme. *Chem Commun* 46: 3896-3898.
31. Deo S, Godwin HA (2000) A Selective, Ratiometric Fluorescent Sensor for Pb²⁺. *J Am Chem Soc* 122: 174-175.
32. Li J, Lu Y (2000) A Highly Sensitive and Selective Catalytic DNA Biosensor for Lead Ions. *J Am Chem Soc* 122: 10466-10467.
33. He Q, Miller EW, Wong AP, Chang CJ (2006) A selective fluorescent sensor for detecting lead in living cells. *J Am Chem Soc* 128: 9316-9317.
34. Kim IB, Dunkhorst A, Gilbert J, Bunz UHF (2005) Sensing of Lead Ions by a Carboxylate-Substituted PPE: Multivalency Effects. *Macromolecules* 38: 4560-4562.
35. Wu FY, Bae SW, Hong JI (2006) A selective fluorescent sensor for Pb(II) in water. *Tetrahedron Lett* 47: 8851-8854.
36. Wang F, Nandhakumar R, Moon JH, Kim KM, Lee JY, et al. (2011) Ratiometric fluorescent chemosensor for silver ion at physiological pH. *Inorg Chem* 50: 2240-2245.
37. Tang L, Caia M, Huanga Z, Zhonga K, Houa S, et al. (2013) Rapid and highly selective relay recognition of Cu(II) and sulfide ions by a simple benzimidazole-based fluorescent sensor in water. *Sens and Actu B* 185: 188-194.
38. Irving HMNH, Freiser H, West TS (1981) IUPAC compendium of analytical nomenclature, definitive rules, Pergamon, Oxford.
39. Tang L, Wang N, Zhang Q, Guo J, Nandhakumar R (2013) A new benzimidazole-based quinazoline derivative for highly selective sequential recognition of Cu²⁺ and CN⁻. *Tetrahed Lett* 54: 536-540.
40. Sung YM, Wu SP (2014) Highly selective and sensitive colorimetric detection of Ag(I) using N-1-(2-mercaptoethyl)adenine functionalized gold Nanoparticles. *Sensors and Actuators B* 197: 172-176.
41. Silverstein RM, Bassler GC, Morrill TC (1981) *Spectrometric Identification of Organic Compounds*, 4th edition. New York, John Wiley and Sons.
42. Zheng Y, Huo Q, Kele P, Andreopoulos FM, Pham SM, et al. (2001) A new fluorescent chemosensor for copper ions based on tripeptide glycyl-histidyllysine (GHK). *Org Lett* 3: 3277-3280.
43. Zhou X, Li P, Shi Z, Tang X, Chen C, et al. (2012) A highly selective fluorescent sensor for distinguishing cadmium from zinc ions based on a quinoline platform. *Inorg Chem* 51: 9226-9231.
44. Wang F, Nandhakumar R, Hu Y, Kim D, Kim KM, et al. (2013) BINOL-based chiral receptors as fluorescent and colorimetric chemosensors for amino acids. *J Org Chem* 78: 11571-11576.