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Abiotic Fluorescent Receptors for Detection of Pb²⁺ in Aqueous Media as well as Living Cells: Mechanistic and Sensing Aspects

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Abstract

Substantial utility of lead in numerous industries (storage, batteries, gasoline, cable manufacture, paint and ammunition), has resulted in recurring of environmental contamination. Lead being one of the highly abundant elements in nature, ranks third among the list of toxic substances. Owing to its ever-increasing demand and allied toxicity (memory loss, irritability, anemia, muscle paralysis, and mental retardation), development of novel synthetic receptors suitable to track Pb²⁺ *in vitro* as well as *in vivo* has been an exciting area of research in the past few decades. In this review, emphasis has been diverted to summarize and briefly discuss major contributions made towards development of fluorescent abiotic receptors capable of sensing ionic lead (Pb²⁺) under aqueous environments as well as in living cells.

Keywords: Abiotic fluorescent receptors; Spectrometry; Stoichiometry

Introduction

Lead (Pb) presents one of the most abundant among heavy metals, widely distributed and mobilized in the environment due to its extensive usage in industries like batteries, gasoline, cable, pigments and in our daily lives [1-3]. Low-level environmental exposure to lead is associated with numerous sources (industrial processes, water pipes, petrol, paint, solder in canned foods) and pathways (water, food, air, household dust, street dirt, soil, etc.) [4-6]. Owing to the vulnerability of Pb²⁺ at low concentrations, occupational and environmental exposures remain a serious problem [7-9]. Due to public health campaigns, a decline in its commercial usage has resulted, and hence lead poisoning has become rare. In spite of this, chronic exposure to low levels of lead is still a public health issue, especially among some minorities and socioeconomically disadvantaged groups.

Various instrumentation based methods, such as X-ray absorption spectroscopy (XAS), inductively coupled plasma-optical emission spectroscopy (ICP-OES), anodic stripping voltammetry (ASV), chromatography, atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), cold vapor AAS or flame AAS-ETA (electrothermal atomization) and photometry are used for Pb²⁺ determination at ppm and ppb levels in various matrices [10-29]. Although these conventional methodologies are accurate, but are allied with tedious sample pre-treatment, sophistication, time consumption, expertise, and usually lack convenience for routine analysis of bulk of samples. In addition, such instrumental basis totally lacks potential to imagine Pb²⁺ in live cells or bio systems. Another most crucial drawback of these methods is that it assesses the total lead content in a given sample, and not the ionic form. To monitor lead in ionic form (Pb²⁺) at nano gram (ng) or sub-ng level in a wide variety of systems (*in vitro* and/or *in vivo*), synthetic molecular recognition based approaches (receptors) are highly expedient [30-31]. This can be assigned to the tendency of such abiotic molecular systems to perform *in situ* monitoring in real time, amid high selectivity and sensitivity. Most importantly, abiotic receptors are usually reusable, easy to operate and realize high sample through output. Among wide variety of chemical receptors, fluorescent ones are highly sensitive, lower in cost, easy to access, versatile, offer sub-ng spatial resolution with submicron visualization and sub millisecond temporal resolution [32-34]. Most importantly; wide number of parameters can be tuned with fluorescent based methods in order to optimize the convenient readout. In addition to this, complex analytical setbacks could be removed by controlling the excitation and emission wavelengths, time window of signal collection, and polarization of the excitation beam or of the emitted light [35-37].

Till date, substantial progress has been made in the development of fluorescent receptors for Pb²⁺. Existing molecular systems (organic/inorganic molecules) exploit synthetic strategies as well as use of surfactants, multi-peptide self-assembled biomolecular scaffolds, polymers, nano-material (functional nanoparticles), DNazymes, etc. However in this

review, our focus will be solely on the single molecule based abiotic fluorescent receptors displaying sensing of Pb^{2+} both under aqueous as well living systems.

Abiotic fluorescent receptors

Chang et al. synthesized and developed a new fluorescent receptor (**Figure 1**), and studied it for probing Pb^{2+} in living cells [38]. Highly selective fluorescent "turn on" response due to photo induced electron transfer (PET) process was achieved in presence of competing ions under simulated physiological conditions (HEPES, pH 7) [39]. The stoichiometry of the complex was determined to be 1:1. Receptor 1 could detect Pb^{2+} in drinking water up to 15 ppb. Thus possessing sensitivity to Environmental Protection Agency (EPA) levels of lead poisoning [40]. The binding constant (K_d) between Pb^{2+} and 1 was obtained as $23 \pm 4 \mu M$. The developed receptor was successfully probed to track Pb^{2+} in live cell imaging. The designed receptor could also respond to the changes in the intracellular Pb^{2+} levels within mammalian cells.

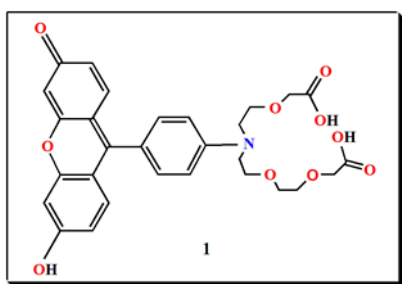


Figure 1: Structure of receptor 1.

Ghosh et al. synthesized and used 2-amino-4-phenyl-4H-benzo[h]chromene-3-carbonitrile based molecular switch 2 (**Figure 2**), as 'turn on' fluorescence receptor for the selective detection of Pb^{2+} [41]. Molecule 2 showed significant increase in the fluorescence intensity as a result of inhibition of PET in methanol solvent. The signal readout was established to occur via 1:1 complex formation. The detection limit for Pb^{2+} was calculated to be $4.14 \times 10^{-4} M^{-1}$. Most importantly proposed receptor design could penetrate living membrane without harming cells, and was further utilized for detection of Pb^{2+} during live cell imaging.

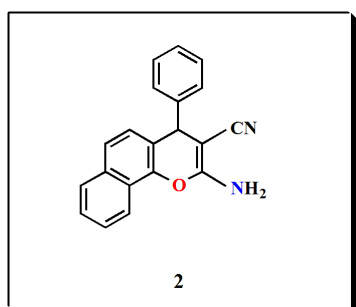


Figure 2: Structure of receptor 2.

Halaweish et al. designed and synthesized a new fluorescent receptor 3 by linking dicarboxylate pseudocrown ether to the BODIPY fluorophore (**Figure 3**) [42,43]. Receptor 3 offered selective recognition of Pb^{2+} under physiological conditions (20 mM HEPES buffer). The fluorescent signaling was achieved through inhibition of PET process. Receptor displayed good photostability and sensitivity toward EPA limits of lead poisoning in drinking water (15 ppb). Besides, compound 3 is applicable to probe intracellular lead contents in live cells. The dissociation constant of complex between probe and Pb^{2+} was found to be $10 \mu M$.

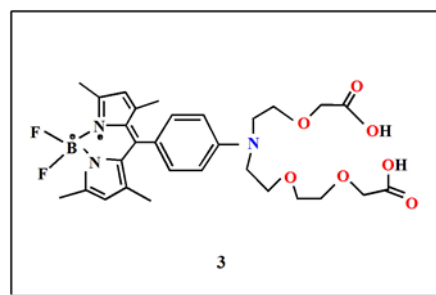


Figure 3: Structure of receptor 3.

Xu et al. successfully developed a naphthalimide based fluorescent receptor 4 in (**Figure 4**) for Pb^{2+} detection in aqueous solution [44]. Receptor 4 exhibited a highly selective fluorescence 'turn-on' response toward Pb^{2+} under aqueous conditions (CH_3CN/H_2O , v/v, 1:1, 10 mM Tris-HCl, pH 7.4) via inhibition of PET process. Besides, sensing mechanism and binding mode of 4 for Pb^{2+} was elucidated through a series of model compounds which were rationally designed and synthesized. The complex formation was determined to be of 1:1 stoichiometry. The limit of detection of probe towards Pb^{2+} was calculated to be $1.63 \times 10^{-7} M^{-1}$. The living cell imaging experiments with 4 demonstrate its potential applications for the detection of Pb^{2+} in biological systems.

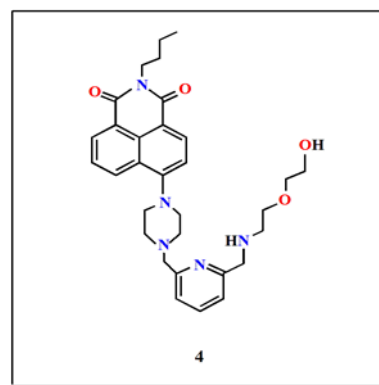


Figure 4: Structure of receptor 4.

Jung et al. reported an easy to synthesize fluorogenic receptor based on BODIPY-functionalized $Fe_3O_4@SiO_2$ core/shell nanoparticles (**Figure 5**), for imaging Pb^{2+} ion in living cells [45]. Receptor 5 exhibit strong affinity for Pb^{2+} over other

competing metal ions in aqueous solvent system (HEPES=20 mM, pH 7.4). Under such conditions, receptor could successfully detect Pb^{2+} in cultured cells. The fluorescence signaling from receptor 5 for Pb^{2+} was attributed to a large Chelation enhanced Fluorescence (CHEF) [46]. The detection limit of 5 for Pb^{2+} was found to be 1.5 ppb (sensitivity below US-EPA levels of lead poisoning in drinking water, 15 ppb). The Jobs studies revealed 1:1 binding mode between 5 and Pb^{2+} . The association constant, K_a for coordination of Pb^{2+} with receptor was found to be $6.3 \times 10^4 M^{-1}$.

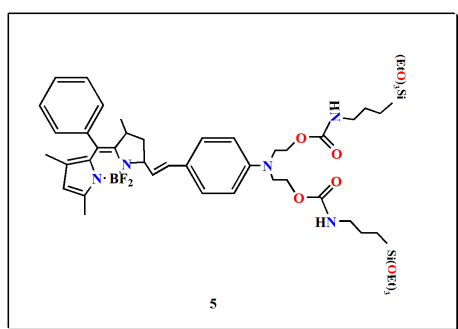


Figure 5: Structure of receptor 5.

Yu et al. developed highly sensitive and selective fluorescent receptors, 6-7 for detection of Pb^{2+} in aqueous solution (Figure 6) [47]. The sensor design is based on dianthracene-cyclen-based 'clickates', and receptor could detect Pb^{2+} through fluorescence enhancement signal transduction. A large CHEF effect was responsible for the fluorescence enhancement in presence of Pb^{2+} . The receptor 6 could more selectively detect Pb^{2+} in aqueous solvent system (HEPES=50 mM, pH 7.4). Contrary to 6, other receptor design 7 showed a relatively small Chelation-enhanced Quenching (CHEQ) effect even in presence of Cu^{2+} and Hg^{2+} also [48]. The stoichiometry of the complexation between 6 and Pb^{2+} was revealed to be 1:1. The binding constant ($\log K = 10.7 \pm 0.5$) showed a strong binding affinity of 6 for Pb^{2+} . Importantly, 6-7 were reported to be the first equal-equivalent responding receptors for Pb^{2+} in aqueous solution. In addition, owing to the selectivity and strong binding affinity of receptor 6, it was successfully utilized for the detection of Pb^{2+} in living cells as well as in fetal calf serum.

Weng et al. devised and synthesized a dual functional molecular receptor (Figure 7) based on rhodamine backbone. Receptor displays a fluorescence "turn on" response selectively in presence of Pb^{2+} [49]. Authors introduced an acyclic diethyl iminodiacetate moiety on to it for achieving binding of Pb^{2+} . The designed receptor 8 could sense Pb^{2+} and Cu^{2+} in water through two different approaches in aqueous media, HEPES buffer with only 1% CH_3CN (v/v). The mechanism of Pb^{2+} recognition was proposed to proceed through rhodamine spirolactam ring-opening mechanism. The probe showed high binding affinity only toward Pb^{2+} even in the presence of other biologically relevant species with low detection limits of $2.5 \times 10^{-7} M$. A 1:1 stoichiometry was demonstrated to exist between Pb^{2+} and receptor during

complex formation. The corresponding binding constant was calculated to be $2.43 \times 10^4 M^{-1}$. Importantly, receptor successfully displayed the "turn on" response with Pb^{2+} in living cells.

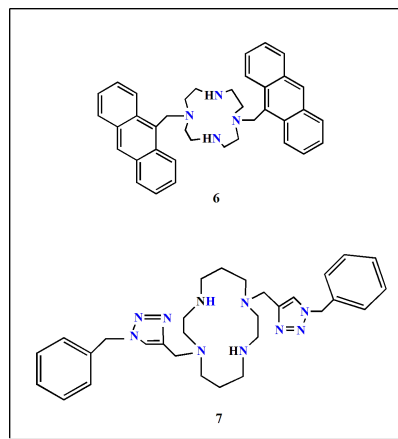


Figure 6: Structure of receptors 6-7.

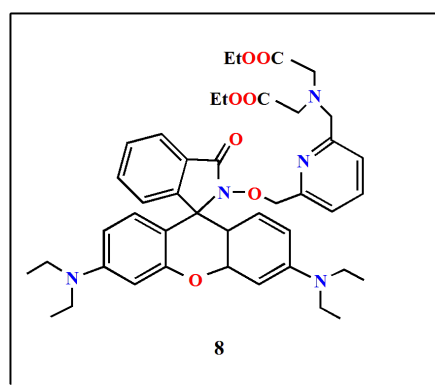


Figure 7: Structure of receptor 8.

Patra et al. proposed an azino bis-Schiff kind of receptor for the detection of Pb^{2+} in aqueous medium 9 in (Figure 8) [50]. Receptor 9 showed remarkable detection ability in a wide pH range between 4-10 in a mixed solvent (MeOH/H₂O, 2:1 v/v, HEPES=100 mM, pH=7.0). The binding of Pb^{2+} with 9 was proposed to increase the rigidity of compound thereby generating efficient CHEF. The complexation was proposed to be of 2:1 stoichiometry between receptor and Pb^{2+} . Receptor 9 was successfully utilized for the determination of Pb^{2+} in aqueous solution of bovine serum albumin protein as well as in different water samples. In addition, response of 9 for Pb^{2+} was successfully reversed by using disodium salt of ethylenediaminetetraacetate (EDTA). The limit of detection was calculated to be 8 nM (lower than the lead toxic level defined by US- EPA).

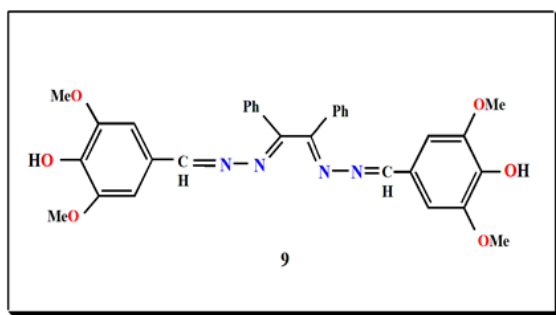


Figure 8: Structure of receptor 9.

Lan et al. synthesized cyclen-functionalized perylene diimide based receptors 10 and 11 (**Figure 9**). The receptor displayed a highly selective Pb^{2+} sensing through fluorescence enhancement response in a neutral HEPES buffer solution. The sensing studies were examined in 10 mM HEPES/DMSO ($v/v=90/10$, $pH=7.2$) [51]. The recognition mechanism involved 1:2 and 1:1 binding stoichiometry between Pb^{2+} and receptors, 10 and 11 respectively. The binding constants for such complexation were calculated to be ($\log K=10.6 \pm 0.5$ and 8.0 ± 0.3) respectively. The presence of aliphatic chain on one side of molecule instead of crown ring resulted in successful penetration of receptor 11 into the living cells. Such characteristic features made the receptor suitable for imaging applications.

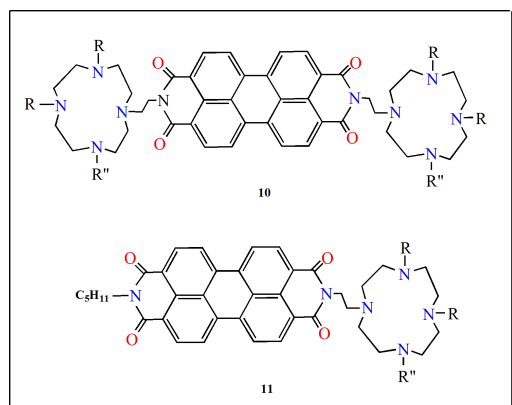


Figure 9: Structure of receptor 10-11.

Conclusions

This review summarizes the foremost progress made in the development of fluorescent receptors for detection of Pb^{2+} in aqueous as well as living cells. Emphasis has been given mainly to reports diverted towards practical applications, with lower detection limits, increased selectivity, and hence reliable in terms of quality assurance. Selected references are being mentioned which will provide the ground work for an individual to know quickly the proposed and developed strategies, and their applications for Pb^{2+} recognition. In this direction, some of the crucial design considerations, such as

choice of fluorophore, selectivity, and mechanism of recognition are momentarily discussed.

Research till date has been conducted nearly in the academic arena, and little attention is paid towards incorporation of long wavelength fluorophores. Thus there exists a plenty of room for further advances, mostly in advancing applications towards sensitivity and selectivity in diverse environmental and biological matrices. It is these facets that require the next stage of innovation.

We hope this review inspires researchers with unique analytical, synthetic, material and physical chemistry tools, to delve into this existing area of research, and to those who have something to do with or interest in the biology or medical sciences.

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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.
- Castelino N, Castelino P, Sannolo N (2008) Inorganic Lead Exposure: Metabolism and Intoxication, *The Agency for Toxic Substances and Disease* 8: 55-64.
- Flegal AR, Smith DR (1992) Current needs for increased accuracy and precision in measurements of low levels of lead in blood. *Environ Res* 58: 125-133.
- Winder C, Carmichael NG, Lewis PD (1982) Neural Models of Plasticity: Experimental and Theoretical Approaches. *Trends Neurosci* 5: 207-214.
- Araki S, Sato H, Yokoyama K, Murata K (2000) subclinical neurophysiologic effects of lead: A review on peripheral, central and autonomic nervous system effects in lead workers. *Am J Ind Med* 37: 193-204.
- Claudio ES, Godwin HA, Magyar JS (2003) Lead: its Effect on Environment and Health. *Prog Inorg Chem* 51: 1-144.
- Quang DT, Kim JS (2010) Fluoro-and Chromogenic Chemosensors for Heavy Metal ion Detection in solution and Biospecimens. *Chem Rev* 110: 6280-6301.
- Chen CT, Huang WP (2002) A Highly Selective Fluorescent Chemosensor for Lead Ions. *J Am Chem Soc* 124: 6246-6247.
- 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.
- Tahan JE, Granadillo VA, Romero RA (1994) Noval colorimetric immunoassay for the detection of aflatoxin B1. *Anal Chim Acta* 295: 187-191.

11. Bowins RJ, McNutt RH (1994) Determination of ultra-trace amounts of cobalt in ocean water by laser-excited atomic fluorescence. *J Anal At Spectrom* 9: 1233.
12. Feldman BJ, Osterloh JD, Hata BH, Dalessandro A (1994) Determination of lead in blood by square wave anodic stripping voltammetry at a carbon disk ultra microelectrode. *Anal Chem* 66: 1983-1987.
13. Cave MR, Butler O, Cook JM, Cresser MS, Miles DL (2000) Environmental analysis. *J Anal At Spectrom* 15: 181-235.
14. Cave MR, Butler O, Chenery RN, Cook JM, Cresser MS, Miles DL (2001) Atomic Spectrometry Update. Environmental analysis. *J Anal At Spectrom* 16: 194-235.
15. Willis JB (1962) Determination of Lead and Other Heavy Metals in Urine by Atomic Absorption Spectroscopy. *Anal Chem* 34: 614-617.
16. Scaccia S, Zappa G, Basili N (2001) Development and application of a high resolution liquid chromatographic method for the analysis of complex pigment distribution. *J Chromatogr A* 915: 167-177.
17. Li YF, Chen CY, Li B, Sun J, Wang JX, et al. (2006) Elimination efficiency of different reagents for the memory effect of mercury using ICP-MS. *J Anal At Spectrom* 21: 94-96.
18. Becker JS, Zoriy MV, Pickhardt C, Palomero-Gallagher N, Zilles K (2005) Imaging of copper, zinc, and other elements in thin section of human brain samples (hippocampus) by laser ablation inductively coupled plasma mass spectrometry. *Anal Chem* 77: 3208-3216.
19. Iyengar V, Wolttlez J (1988) Trace elements in human clinical specimens: evaluation of literature data to identify reference values *Clin Chem* 34: 474-481.
20. Townsend AT, Miller KA, Mc S, Aldous S (1998) The determination of copper, zinc, cadmium and lead in urine by high resolution ICP-MS. *J Anal At Spectrom* 13: 1213-1219.
21. Cave MR, Butler O, Chenery RN, Cook JM, Cresser MS, et al. (2001) Investigation of the role of hollow cathode (vaporization) temperature on the performance of particle beam-hollow cathode atomic emission spectrometry (PB-HC-AES). *J Anal At Spectrom* 16: 194-235.
22. Willis JB (1962) Determination of Lead and Other Heavy Metals in Urine by Atomic Absorption Spectroscopy. *Anal Chem* 34: 614-617.
23. Scaccia S, Zappa G, Basili N (2001) Development and application of a high resolution liquid chromatographic method for the analysis of complex pigment distributions. *J Chromatogr A* 915: 167-177.
24. Li YF, Chen CY, Li B, Sun J, Wang JX, et al. (2006) Elimination efficiency of different reagents for the memory effect of mercury using ICP-MS. *J Anal At Spectrom* 21: 94-96.
25. Dragoe D, Spataru N, Kawasaki R., Manivannan A, Spataru T, et al. (2006) Detection of trace levels of Pb²⁺ in tap water at boron-doped diamond electrodes with anodic stripping voltammetry. *Electrochimica Acta* 51: 2437-2441.
26. Yanaz Z, Filik H, Apak R (2010) Development of an optical fibre reflectance sensor for lead detection based on immobilised arsenazo (3) *Sens Actuators B* 147: 15-22.
27. Li J, Lu F, Umemura T, Tsunoda K (2000) Determination Of Lead By Hydride Generation Inductively Coupled Plasma Mass Spectrometry. *Anal Chim Acta* 419: 65-72.
28. Fang G, Meng S, Zhang G, Pan J (2001) Spectrophotometric determination of lead in foods with dibromo-p-methyl-bromosulfonazo. *Talanta* 54: 585-589.
29. Arancibia V, Nagles E, Cornejo S (2009) Determination of lead in the presence of morin-5'-sulfonic acid and sodium dodecyl sulfate by adsorptive stripping voltammetry. *Talanta* 80: 184-188.
30. Oehme I, Wolfbeis OS (1997) Optical Sensors for Determination of Heavy Metal Ions. *Microchim Acta* 126: 177-192.
31. Gellman SH (1997) Introduction: Molecular Recognition. *Chem Rev* 97: 1231-1232.
32. Silva AP, Gunaratne QN, Gunnlaugsson TH, Huxley AJM, et al. (1997) Signaling Recognition Events with Fluorescent Sensors and Switches. *Chem Rev* 97: 1515-1566.
33. Amendola V, Fabbrizzi L, Licchelli M, Mangano C, Pallavicini P, et al. (2000) A Highly Selective Fluorescent Chemosensor for Lead ion. *Coord Chem Rev* 192: 649-669.
34. De Silva AP, Fox DB, Huxley AJM, Moody TS (2000) Combining fluorescence, coordination chemistry and electron transfer for signalling purposes. *Coord Chem Rev* 205: 41-57.
35. Carter KP, Young AM, Palmer AE (2014) Fluorescent sensors for measuring metal ions in living systems. *Chem Rev* 114: 4564-4601
36. Du J, Hu M, Fan J, Peng X (2012) Colorimetric detection of heavy metal ions using label-free gold nanoparticles and alkanethiols. *Chem Soc Rev* 41: 4511-4535.
37. Kim JS, Quang DT (2007) Fluoro- and chromogenic chemodosimeter for heavy metal ion detection in solution and biospecimens. *Chem Rev* 107: 3780-3799.
38. 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.
39. Qian J, Qian X, Xu Y, Zhang S (2008) Multiple molecular logic functions and molecular calculations facilitated by surfactant's versatility. *Chem Commun* 4141-4143.
40. Kim HN, Ren WX, Kim JS, Yoon Y (2012) Fluorescent and colorimetric sensors for detection of lead, cadmium and mercury ions. *Chem Soc Rev* 41: 3210-3244.
41. Sinha S, Koner RR, Kumar S, Mathew J, Roy A, et al. (2013) Structurally turned benzo[h] chromen derivative as Pb²⁺ selective 'turn-on' fluorescence sensor for living cell imaging. *J Lumin* 143: 355-360.
42. Baruah M, Huntimer ED, Ahmed MS, Hoppe AD, Halaweish FT (2012) Selective BODIPY® based fluorescent chemosensor for imaging Pb ion in living cells. *Tetrahedron Lett* 53: 4273-4275.
43. Coskun A, Akkaya EU (2008) A Multianalyte Chemosensor on a Single Molecule: Promising Structure for an Integrated Logic Gate. *J Am Chem Soc* 73: 5008-5014.
44. Un HL, Huang CB, Huang J, Huang C, Jia T, et al. (2014) A Naphthalimide-Based Fluorescence "Turn-On" Probe for the Detection of Pb²⁺ in Aqueous Solution and Living Cells. *Chem Asian J* 9: 3397-3402.
45. Son H, Lee HY, Lim J, Kang MD, Han WS, et al. (2010) A Highly Sensitive and Selective Turn-On Fluorogenic and Chromogenic Sensor Based on BODIPY-Functionalized Magnetic Nanoparticles for Detecting Lead in Living Cells. *Chem Euro J* 16: 11549-11553.

46. Goswami S, Chakrabarty R (2010) Highly Selective Colorimetric Fluorescent Sensor for Pb²⁺. *Eur J Org Chem* 3791-3795.
47. Xu HR, Li K, Liu Q, Wu TM, Wang MQ, et al. (2013) Dianthracene–cyclen conjugate: the first equal-equivalent responding fluorescent chemosensor for Pb in aqueous solution. *Analyst* 138: 2329-2334.
48. Chae MY, Yoon J, Czarnik AW (1996) Chelation-enhanced fluorescence chemosensing of Pb (II), an inherently quenching metal ion. *J Mol Recog* 9: 297-303.
49. Li M, Jiang XJ, Wu HH, Lu HL, Li HY, et al. (2015) Aminopyridine derivatives controlled the assembly and various properties of Cu-BTC metal-organic frameworks. *Dalton Trans* 44: 14008-14018.
50. Ghorai A, Mondal J, Saha R, Bhattacharya S, Patra GK (2016) A highly sensitive reversible fluorescent-colorimetric azino bis-schiff base sensor for rapid detection of Pb in aqueous media. *Anal Methods* 8: 2032-2040.
51. Zhou R, Li B, Wu N, Gao G, You J, et al. (2011) Cyclen-functionalized perylenbisimides as sensitive and selective fluorescent sensors for Pb²⁺ in aqueous solution. *Chem Commun* 47: 6668-6670.