Priyanka Munjal
Assistant Professor
LNJN National Institute of Criminology and Forensic Science
Ministry of Home Affairs

Email- priya.chem2008@gmail.com
Phone- +91-9582214189

Brief Academic Profile:

Year	Degree	University/Board	Percentage
2019	**PG Certification in Forensic Medicine and Toxicology (12 months)**	IFS, Pune	75.5 %
2016	PhD in Organic chemistry	Indian institute of Technology, New Delhi	CGPA 8.5
2009	Integrated MSc. (Chemistry Honors)	Guru Nanak Dev University, Amritsar	67.4
2007	BSc. (Chemistry Honors)	Guru Nanak Dev University, Amritsar	66
2004	Higher Secondary	CBSE board	64.4
2002	Secondary	CBSE board	71

Experience

Organization	Designation	From	TO
LNJN National Institute of Crimnology and Forensic Science	Assistant Professor	03-Oct-2019	**Till Date**
LNJN National Institute of Crimnology and Forensic Science	Assistant Professor	02-May-2019	30-Sept-2019
LNJN National Institute of Crimnology and Forensic Science	Assistant Professor	10-Oct-2018	30-Apr-2019
LNJN National Institute of Crimnology and Forensic Science	Assistant Professor	04-Sep-2017	03-Sep-2018
Sharda University Greater Noida	Assistant Professor	05-Aug-2016	30th May 2017
Jubilant Chemsys	Research Trainee	03-Aug-2009	5 Feb 2010

Research Experience:

PhD work: My doctoral research is focused on molecular designing and synthesis of calix[4]arene derivative. We synthesized calix[4]arene derivatives inspired by biological molecules such as plastocyanin and rusticyanin. These proteins contain copper centre for electron transfer during photosynthesis where copper co-ordinate with two nitrogen's from l-histidine and two sulphur from methionine and cysteine amino acids respectively. The hydrophobic environment is provided by different amino acid chains in these proteins. Similarly we appended o-aminothiophenol moiety on calix[4]arene cavity where o-aminothiophenol unit provides two sulphur and two nitrogen and calix[4]arene provides hydrophobic environment. I have also synthesized and evaluate reference compounds to determine the role of calix[4]arene cavity toward recognition property. Apart from ionic recognition, I have also synthesized receptors which have shown bio-molecular recognition characteristics. We made in-situ supramolecular complex of metal ions for recognition of amino acids and further investigation are in progress.

Industrial Research: Worked as a Research Associate (RA) at R&D centre in Jubilant Chemsys, Noida, UP, India. The major responsibilities, I handled there as follows:

- Collaborative and independent handling of the assigned medical chemistry projects, chemistry exploration, route optimization and trouble shooting.
- To design and implement synthetic strategies for target molecules.
- To exercise multi-step synthesis, purification and scale up.
- Interpretation of spectral data like NMR, MASS, LCMS, IR etc.

Publications:
1. H. M. Chawla and **P. Munjal**, P. Goel "A new metalio-supramolecular sensor for recognition of sulfide ions" **Tetrahedron Lett. (ISSN No. , 2015**, 56, 5, .682-685.
2. H. M. Chawla and **P. Munjal**, P. Goel "Synthesis and evaluation of a new colorimetric and ratiometric fluorescence probe for copper ions" **Journal of Luminescence, 2015**, 164, 138-145.
3. H. M. Chawla and P. Munjal " Evaluation of calix[4]arene tethered Schiff bases for anion recognition" **Journal of Luminescence, 2016,** 179, 114-121
4. P. Munjal and H. M. Chawla "Metallo-supramolecular complex of 1,3-di{bis(2-hydroxynaphthyl)}-2-aminothiophenylcalix[4]arene for the detection of L-histidine using secondary interactions" **Journal of Luminescence, 2018**, 203, 364-370.

Poster Presentation/Conference Attended:
1. H. M. Chawla and **P. Munjal**, at **"Advances in Applied Chemical Sciences and Innovative Materials"** August 10th – 12th, **2011**. Indian Institute of Technology , Delhi, India.

2. H. M. Chawla and **P. Munjal**, **Emerging Trends in Development of Drugs and Devices**" January 21st-23rd **2013**, University of Delhi, Delhi, India.
3. **P.Munjal** and H.M. Chawla, **"18th CRSI National Symposium in Chemistry",** 5-7th February, **2016**, Institute of Nano Science and Technology and Punjab University, India.
4. **P. Munjal** and H. M. Chawla, at " **4th International Conference on Asian network for Natural and Unnatural Materials (Annum 4)"** 8-10th June 2016, **National University of Singapore, Singapore**
5. P.Munjal and Z. Chanu, at "**Conference of Magnetic Resonance in Medicine and 25th National Magnetic Resonance Society Meeting**", 13-16th Feb, 2019, All India Institute of Medical Science, Delhi, India.
6. Attented Conference of American Chemical Society On "**ACS on Campus event"** 30th Sept-1 Oct 2015, at Indian Institute of Technology, New Delhi, India.
7. Attended Conference on **"Environmental Health and Lab Safety Unit"** 28th-29th March 2014, at Indian Institute of Technology, New Delhi, India
8. Bio-inspired o-aminothiophenylcalix[4]arene derivative at **"Asian Network for Natural and Un-natural Material (Annum -4 Prelude)"** May 20 2015, Indian Institute of Technology, Delhi, India.

Accolades:
- UGC Junior Research Fellowship held on Dec. 2010 (**All India Rank - 334**).
- GATE (Graduate Aptitude Test in Engineering) 2011 PERCENTILE- 97.2; (**All India Rank - 287**)
- Award of Senior Research Fellowship (SRF), University Grant Commission, 2013
- Got Appreciation Letter from **Sh Javeed Ahmad, IPS**, Director, LNJN National Institute of Criminology and Forensic Science on 30th Oct. 2019.

In Service Trainning:
1. 4-day Training programme on **"Laboratory Quality Management System and Internal Audit"** as **per IS/ISO/IEC 17025:2017",** 5-8th March 2019 at LNJN NICFS conducted by **National Institute of Trainning for standardization, Bureau of Indian Standards.**

Other Administrative Tasks:

- Worked as an Placement Coordinator for Forensic Science Students
- Involved in the work of NSQF (National Skill Qualification File) Allignmnet of the PG Diploma Courses with Skill Ministry
- Involved in the work of NABL Accreditiation and Calibration of Instruments for the Forensic Laboratories
- Organised an event on Waste Material management for Post Graduate Students

- Member for purchasing of UV-Visible Spectrophotometry through GEM in Chemistry and Toxicology Division of LNJN NICFS.
- Involved in other activities such as Assistant Academic Cordinator, Assistant Course Cordinator for training of In service officers, Timemtable Incharge etc.

Scientific skills:
- Operational expertise on various characterization instruments such as NMR, UV-Vis spectrophotometer, Fluorescent spectrophotometer, FT-IR spectrophotometer, High Resolution Mass Spectrophotometer, Flash chromatography, LC-MS/MS, GC-MS.

Technical Skills:
- Chem. draw, chem. craft and chem. 3D
- ACD Labs
- Origin
- Sigma plot
- Sci-finder / Bilestein

Identification and analysis of organic explosives from post-blast debris by nuclear magnetic resonance

Priyanka Munjal [a,*], Bhumika Sharma [a], J.R. Sethi [a], Anu Dalal [b], Shivajirao L. Gholap [b]

[a] Chemistry & Toxicology Division, LNJN National Institute of Criminology and Forensic Science, Ministry of Home Affairs, Sec-3, Rohini, Delhi 110085, India
[b] Chemistry Department, Indian Institute of Technology Delhi, New Delhi 110016, India

ARTICLE INFO

ABSTRACT

The growing threat of terrorism has triggered an urgent need to find effective ways to improve the analysis of explosives. This will aid forensic scientists in analysing the post-blast debris, which in turn helps the law enforcement agencies to frame suitable regulations. Analysis of post-blast debris is challenging as it hosts a massive amount of complexity. There are various techniques reported till date such as mass spectrometry, gas chromatography, high-performance liquid chromatography, Fourier transform infrared spectroscopy, and Raman spectroscopy for the analysis of post-blast residues. However, none of them has been able to identify the structural composition of the explosives. The current research study focuses on identifying the structural composition of the explosives from the post-blast debris using the nuclear magnetic resonance (NMR) technology. The post-blast analytes were extracted from soil samples, cleaned by the solid phase extraction (SPE) method and were rapidly analysed by the nuclear magnetic resonance spectrometer. This paper reports the identification of nitro organic explosives such as pentaerythritol tetranitrate (PETN), trinitrotoluene (TNT) and 2,4,6-trinitrophenylmethylnitramine (tetryl) in post-blast debris by 400 MHz nuclear magnetic resonance spectrometer.

1. Introduction

Explosives undergo rapid chemical change with the release of a large amount of energy as well as toxic gases which results in casualties and severe injuries due to its blast pressure, fragmentation, and thermal effects. This makes explosives the best option for illegal activities such as terrorism. Since global terrorism is on the rise, proper analytical methods are needed for the identification and analysis of post-blast debris. Analysis of explosives and their post-blast debris is a crucial step that not only helps forensic scientists to narrow down their search and uncovering terrorist activities but also in assisting the investigating officers in law enforcement agencies (Hewitt et al., 2005; Marple and LaCourse, 2005; Caygill et al., 2012; Zhang et al., 2018).

Based on the chemical structure, explosives are classified as organic nitro explosives, organic peroxides, inorganic salts and mixtures of oxidising and reducing agents. Some of the organic nitro explosives include nitroaromatic – 2,4,6-trinitrotoluene (TNT), picric acid, 2,4-dinitrotoluene (DNT); Nitrate esters – nitro glycerol (NG), nitrocellulose (NC), pentaerythritol tetranitrate (PETN), ethylene glycol dinitrate (EGDN); Nitramines – [1,3,5-trinitro-1,3,5-triazacyclohexane] (RDX), 1,3,5,7-tetranitro-1,3,5,7-tetrazacyclooctane, 2,4,6-trinitrophenylmethylnitramine (tetryl). Examples of organic peroxides include triacetonetriperoxide (TATP), hexamethylene triperoxide diamine (HMTD), whereas examples of inorganic salts are ammonium nitrate fuel oil (ANFO). Moreover, the examples of mixtures of oxidising and reducing reagents are black powder, potassium chlorate and sugar. The chemical structures of a few nitro organic explosives are shown in Fig. 1.

The post-blast debris is usually a soil sample that may contain craters, shrapnel, detonators, etc. left after the explosion. Soil can be classified based on particle size such as sand (0.05–2 mm), silt (0.002–0.05 mm) and clay (< 0.002 mm). More the particle size fraction affects the retention capacity for explosives and chemicals. Larger the particle size, more significant is the retention capacity for explosives and chemicals. Loam soil is a mixture of sand, silt and clay. It contains a high amount of organic matter. In the present study, extraction of explosives was done from loam soil. Nitroaromatic explosives such as TNT and tetryl bind strongly to the exchangeable cations present at the surface of the soil particles, whereas non-aromatic explosives such as PETN exhibit lower adsorption in soil particles. Other physical and chemical properties such as pH, microbial content and percentage of organic matter affect the sorption/desorption

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Full Length Article

Evaluation of calix[4]arene tethered Schiff bases for anion recognition

H.M. Chawla *, Priyanka Munjal

Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, India

ABSTRACT

Two calix[4]arene tethered Schiff base derivatives (**L1** and **L2**) have been synthesized and their ion recognition capability has been evaluated through NMR, UV–vis and fluorescence spectroscopy. **L1** interacts with cyanide ions very selectively to usher a significant change in color and fluorescence intensity. On the other hand **L2** does not show selectivity for anion sensing despite having the same functional groups as those present in **L1**. The differential observations may be attributed to plausible stereo control of anion recognition and tautomerization in the synthesized Schiff base derivatives.

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1. Introduction

There has been intense research activity for development of efficient systems for sensing anionic analytes [1] due to involved academic challenges and realization of the importance of anions in numerous biological, environmental and industrial processes [2]. Unlike cations, anions vary widely in shape, size and charge parameters and they are also very sensitive towards pH and interact with solvents. It is often difficult to match the geometric parameters of anions with those of the neutral molecular receptors to achieve required host-guest fits [3]. Common inorganic anions like fluoride, nitrate, phosphate, chromate, arsenate, sulfate, cyanide and chloride, frequently become critical for analysis of quality of water bodies [4,5]. Cyanide anion, though widely used in electroplating, plastics, metallurgy, gold and silver ion extraction and tanning industries, is extremely toxic and needs to be regularly monitored [6–8]. Likewise, fluoride ion is ubiquitous and essential in small amounts, but it is toxic to living systems when present in excess [9].

The molecular receptors developed for cyanide and fluoride ions fundamentally involve (i) hydrogen bonding (ii) basic character of anionic species (iii) nucleophilicity and (iv) metallo-supramolecular interactions with metal ion displacement protocols [12–18]. All these processes require a linear hydrogen bond formation (D…H…A) with fluoride, and cyanide ions to usher changes in fluorescence, NMR or other measurable parameters [19–24]. Though these interactions with anions may induce electronic or stereo electronic differentials for estimations, they have been scantly investigated in the

past. We have attempted to understand and utilize these week interactions in this paper [25–27].

As we had experience in the area of investigations on calixarenes [10], we envisaged that Schiff bases when tethered to a rigid calixarene base, would provide opportunities to understand stereo control in the recognition processes. Accordingly, two tethered calixarenes **L1** and **L2** were designed to examine anion recognition as both **L1** and **L2** have similar functional groups for interaction with cyanide ion through hydrogen bonding. The present studies reveal that naphthalene analog (**L1**) is a better sensor material for cyanide than the corresponding phenolic Schiff base, **L2** as detailed later.

2. Synthesis

L1 and **L2** both are Schiff bases which should be easily obtainable by the acid and base catalyzed condensation of calixarene amine and aldehyde components. However, when the reactions were attempted with **2** and salicylaldehyde or 2-hydroxynaphthaldehyde by using reported procedures, we faced difficulties in getting calixarene tethered Schiff base structures. After several failed attempts, the designed molecules (**L1** and **L2**) were eventually obtained by using zinc perchlorate as the catalyst as shown in Scheme 1. Accordingly p-*t*-butylcalix[4]arene synthesized by the reaction protocol reported by Gutche and coworkers [28], was refluxed with dibromopropane in the presence of potassium carbonate to yield 26, 28-bis(propoyloxybromo)calix[4]arene (**1**) in good yield [29]. Nucleophilic substitution of **1** with o-aminothiophenol in the presence of tetrabutyl ammonium sulfate as the phase transfer catalyst gave **2** which was condensed with 2-hydroxynaphthaldehyde by using zinc perchlorate as the catalyst [30]. It was noticed that the reaction of

* Corresponding author. Tel.: +91 11 26591517; fax: +91 11 26581 102.
E-mail address: hmchawla@chemistry.iitd.ac.in (H.M. Chawla).
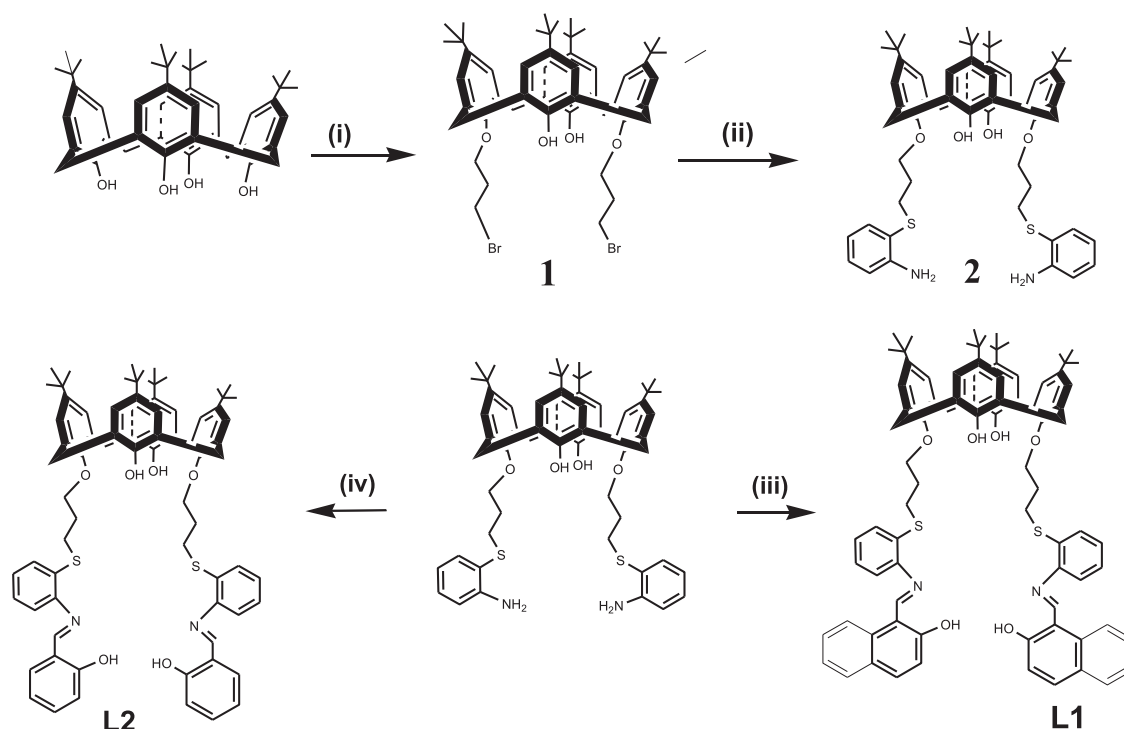
Scheme 1. *Reagents and conditions*: (i) 1,3 dibromopropane, CH$_3$CN, K$_2$CO$_3$, reflux; (ii) NaH, DMF, TBAHSO$_4^-$, o-aminothiophenol, stirring; (iii) 2-hydroxynaphthaldehyde, Zn (ClO$_4$)$_2$, CH$_3$CN:CHCl$_3$ (9:1), stirring; and (iv) salicylaldehyde, Zn(ClO$_4$)$_2$, CH$_3$CN:CHCl$_3$ (9:1), stirring.

amine and 2-hydroxynaphthaldehyde in the presence of zinc per-chlorate in acetonitrile–chloroform mixture (9:1) immediately gave a yellow colored solution which on work-up gave about 87% yield of **L1** with m.p.140–145 °C. It exhibited strong IR absorptions at υ_{max} 3432 (−OH str.), 1623 (C=N str.). HRMS gave *m/z* at 1287.6368 which confirmed the formation of **L1**. In its ^{1}H NMR (CDCl$_3$) spectrum, **L1** exhibited peaks at 15.30 ppm and 9.21 ppm attributable to hydroxyl and imine protons of naphthyl group as well as characteristic signals at 4.3 and 3.2 ppm for ArCH$_2$Ar protons revealing the presence of cone conformation of calix[4]arene. In its ^{13}C NMR spectrum the signals at 33.6 ppm further established the conformation of **L1** as cone. Similarly molecular receptor **L2** was synthesized in 82% yield by nucleophilic substitution of **1** with o-aminothiophenol in the presence of phase transfer catalyst followed by condensation with salicylaldehyde in acetonitrile/chloroform mixture. The molecular ion peak at *m/z* as 1187.6022 in its HRMS confirmed the di-substitution pattern of calix[4]arene. In its ^{1}H NMR spectrum of **L2** exhibited peaks at 13.07 and 8.87 corresponding to hydroxyl and imine protons of salicylaldehyde based derivative of calixarene.

3. Results and discussion

3.1. Colorimetric studies

When tetrabutyl ammonium salts of different anions such as F$^-$, Cl$^-$, Br$^-$, I$^-$, AcO$^-$, CN$^-$, H$_2$PO$_4^-$, HSO$_4^-$ in DMSO were added to a solution of **L1** (0.2 mM), a profound color change was observed only with CN$^-$ and F$^-$ ions. The color change from yellow to orange was observed in the case of CN$^-$ while fluoride indicated a color change from yellow to dark yellow (Fig. 1a). The naked eye colorimetric change was further studied under uv light as shown in ESI Fig. 10.

Likewise, when receptor **L2** was allowed to interact with tet-rabutyl ammonium salts of different anions, no definite color
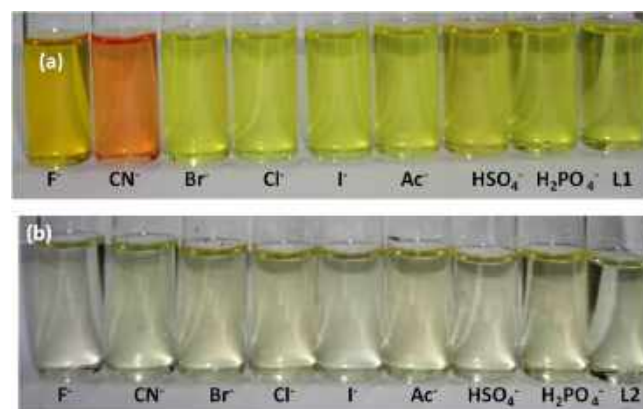

Fig. 1. (a) Colorimetric response of receptor **L1** (0.2 mM) with various anions, and (b) colorimetric response of receptor **L2** (0.2 mM) with various anions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

change was observed (Fig. 1b). Since both **L1** and **L2** had the same functionalities in their constitution, their differential behavior suggested us to explore their interactions further.

3.2. UV–vis spectral observations

The absorption spectrum of **L1** in DMSO (25 μM) exhibited two maxima at 408 nm and 319 nm respectively with a small hump at 469 nm. When tetrabutyl ammonium salts of different anions (CN$^-$, F$^-$, Cl$^-$, Br$^-$, I$^-$, AcO$^-$, H$_2$PO$_4^-$ and HSO$_4^-$) were added to a solution of **L1** in DMSO, both CN$^-$ and F$^-$ were found to cause a bathochromic shift in the absorption spectrum of **L1** (Fig. 2a). It was observed that gradual addition of CN$^-$(up to 8.8 equivalents) led to attenuation of band at 408 nm where a new band appeared at 469 nm (Fig. 2b) which was accompanied by a color change from yellow to orange. A clear isosbestic point
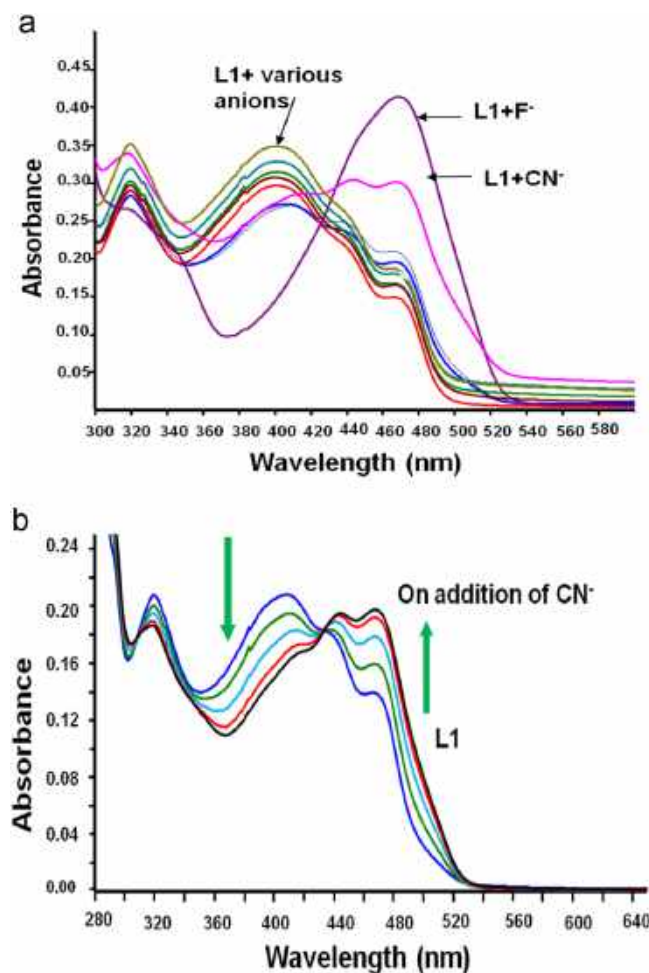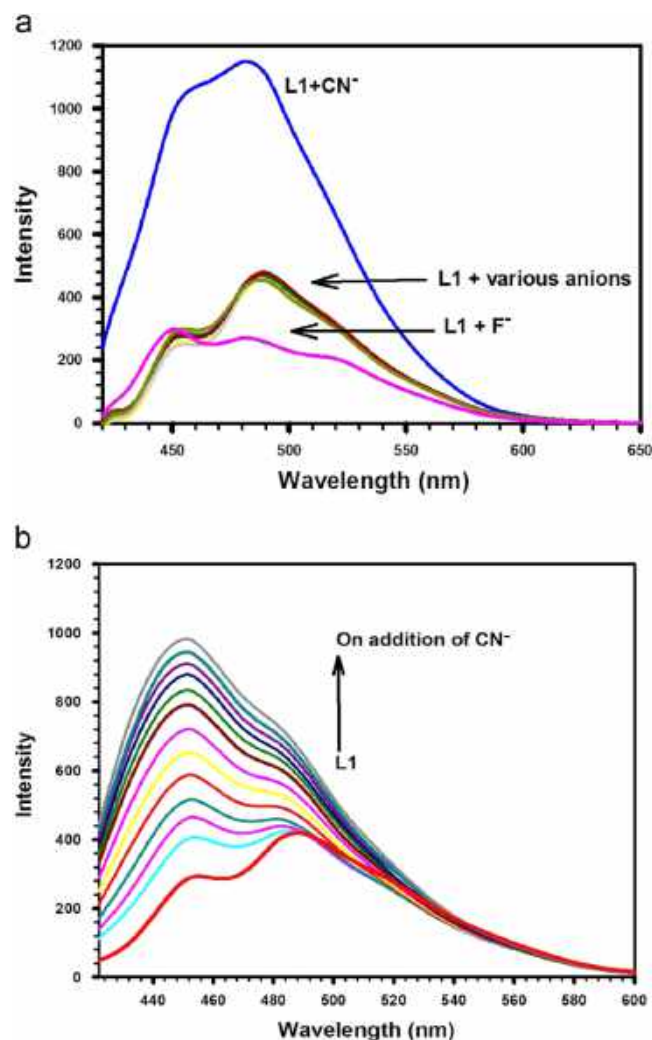
Fig. 2. (a) UV–vis absorption spectrum of L1 (25 μM) on addition of various anions in DMSO and (b) UV–vis spectra of L1 (25 μM) on the titration with cyanide ion in DMSO. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)
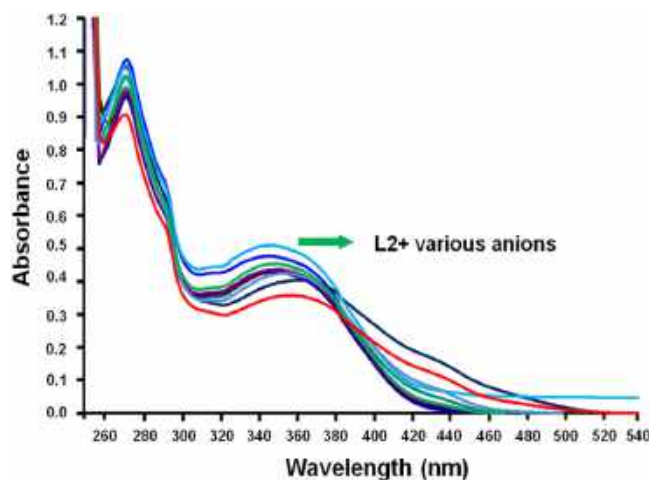
Fig. 3. UV–vis absorption spectrum of L2 (25 μM) on addition of various anions in DMSO.

Fig. 4. (a) Fluorescence spectrum of L1 (25 μM) upon addition of various anions in DMSO (λ_{exc}=402 nm), and (b) Fluorescence spectra of L1 (25 μM) on the titration with cyanide ion in DMSO (λ_{exc}=402 nm).

was observed at 439 nm indicates the formation of a well defined L1-CN$^-$ complex. Job plot analysis of binding of L1 and CN$^-$ revealed a 1:1 binding stoichiometry (ESI Fig. 13).

The absorption spectrum of receptor L2 in DMSO exhibited two maxima at 349 nm and 271 nm. When L2 was examined for its

response towards tetra-butyl ammonium salts of different anions, no discernible spectral changes were observed (Fig. 3).

3.3. Fluorescence spectral changes

When excited at 402 nm, L1 gave strong emission at 492 nm in DMSO with a hump at 452 nm. On addition of 18 equivalents of various anions (CN$^-$, F$^-$, Cl$^-$, Br$^-$, I$^-$, AcO$^-$, H$_2$PO$_4^-$ and HSO$_4^-$) to a 25 μM solution of receptor L1, there was no enhancement in fluorescence intensity in the emission spectrum except when it was treated with CN$^-$. It was observed that on addition of F$^-$ ions, it was the marginal quenching effect on the emission spectrum of L1 (Fig. 4a). Since the fluorescence probe could discriminate F$^-$ and CN$^-$ in DMSO, the effect was further investigated.

On gradual addition of CN$^-$ (up to 18 equivalents), the emission intensity at 492 nm and 452 nm got enhanced by 2.5 folds (Fig. 4b). The binding constant of receptor L1 with CN$^-$ was calculated by application of Benesi–Hildebrand equation as 1.2×10^3 M^{-1} (ESI Fig. 14) [10]. The detection limit was calculated to be 1.9×10^{-5} M as shown in ESI Fig. 15 [13].

Similarly, on gradual addition of fluoride ions, the emission intensity at 492 nm got slightly quenched with a small increase at
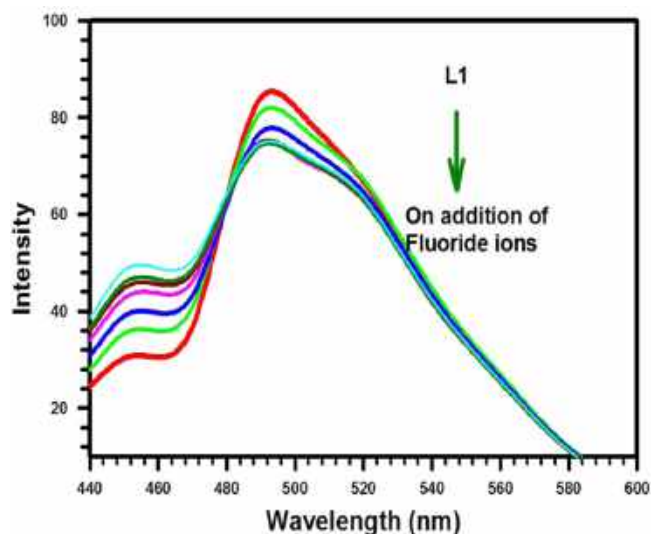
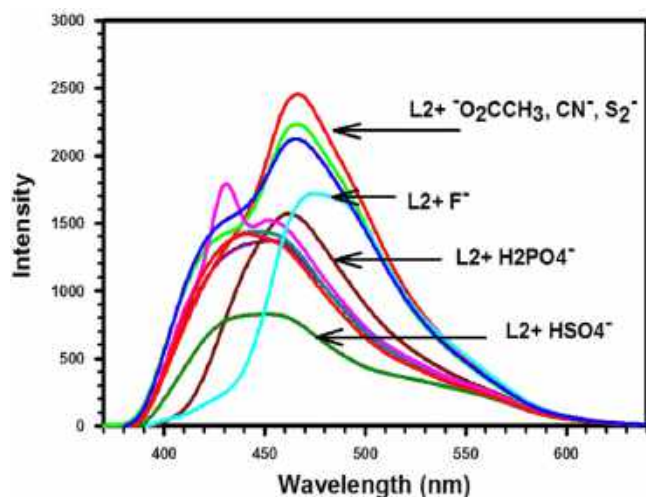**Fig. 5.** Emission spectra of L1 (10 μM) upon titration with of fluoride ions when excited at 402 nm in DMSO.

Fig. 6. Fluorescence spectrum of **L2** (25 μM) with various anions in DMSO.

452 nm as shown in Fig. 5. The detection limit was found to be 3.1×10^{-4} M (ESI Fig. 16).

Receptor **L2** on excitation at 350 nm in DMSO gave emission maxima at 460 nm. In contrast to **L1**, receptor **L2** showed no selectivity for anions when examined through fluorescence changes (Fig. 6).

3.4. Comparative studies on receptor L1` and L2

The absorption and fluorescence studies revealed that receptor **L2** did not show selectivity towards any of the anions studied, while **L1** exhibits a profound selectivity for CN^- and F^- despite the fact that both these receptors are Schiff bases and have similar functionalities. The differential selectivity was further examined by studying their tautomeric behavior. It is known that Schiff bases exist as an equilibrium mixture of their phenol-imine (O–H….N) and keto-amine (O….H–N) tautomers which usually get stabilized through intra-molecular hydrogen bonding in solution [11]. Consequently, we examined the tautomeric behavior of both L1 and L2 through their phenol-imine and keto-amine forms of calixarene tethered 2-hydroxy Schiff bases derived from hydroxy benzene

and naphthalene aldehydes by ^{1}H NMR and UV–vis spectroscopy. It was observed that the ^{1}H NMR spectrum of naphthyl derivative **L1**, (ESI Fig. 4) shows two doublets at 9.21 and 15.30 ppm due to HC–NH and CH–NH, respectively. These could be ascribed to the keto and amine forms of tethered Schiff bases showing N-H….O hydrogen bonding. On the other hand salicylaldehyde derivative, **L2**, exhibited two singlets at 8.87 and 13.07 ppm that could be ascribed to CH=N and ArOH due to phenol-imine tautomerism as shown in figure given below.

The absorption spectra of receptors **L1** and **L2** revealed low energy bands at 408 nm and 349 nm for **L1** and **L2** respectively. The band at 349 nm in the case of receptor **L2** could be assigned to π–π^* transition of C=N group. The band at 408 nm in the case of receptor **L1** could be attributed to n–π^* transition of C=O group. The observed band at less than 400 nm in **L2** indicated the presence of the penol-imine tautomeric form in concurrence with literature precedents [11]. In case of **L1**, the keto-amine tautomeric form seems to predominate which showed absorption band at greater wavelengths than 400 nm (ESI Fig. 17). These NMR and absorption spectral results clearly indicated that salicylaldehyde tethered calixarene (**L2**) is present as a phenol-imine tautomeric form, while the 2-hydroxy naphthyl analog (**L1**) is present as a keto-amine tautomeric form. The 2-hydroxy naphthyl derivative has extended delocalization of electrons as compared to phenyl ring of the salicylaldehyde derivative. The recognition characteristic of **L1** could thus be traced to the keto-amine form of Schiff base derived from 2-hydroxy naphthyl derivative for cyanide ions recognition (Fig. 7).

3.5. Mass spectrometry

The mass spectrum of receptor **L1** with CN^- was examined by high resolution mass spectroscopy when molecular ion peak of free receptor **L1** appeared at m/z 1287.63 (M^+). Titration of **L1** with CN^- resulted in an ion peak at m/z 1336.6199 which corresponded to $[L1+CN^-]$, suggesting that the receptor **L1** formed a 1:1 complex with CN^- (Fig. 8).

3.6. Competitive measurements

The selectivity of receptor **L1** towards CN^- in the presence of different anions was determined by competitive experiments. When **L1** was titrated with one equivalent of cyanide ion in combination with the same equivalents of other competing anions, only negligible difference was observed in the emission spectra (Fig. 9).

4. Plausible binding mechanism of cyanide ion with receptor L1

The binding mechanism has been determined through analysis of UV–vis, fluorescence and NMR spectroscopy. The red shifted absorption spectrum of receptor **L1** on addition of cyanide ion showed that receptor **L1** binds with the CN^- ions in the ground state. On addition of CN^- to the solution of receptor **L1** led to a 61 nm bathochromic shift due to increase in conjugation of 2-hydroxynaphthyl ring. On excitation at 408 nm, the emission maximum of receptor **L1** at 492 nm got enhanced by 2.5 folds on addition of cyanide ion. To further support the mechanism the ^{1}H NMR titration of receptor **L1** with cyanide ion has been performed in CDCl$_3$. ^{1}H NMR spectrum of free receptor **L1** revealed protons for 2-hydroxynaphthalene derivative at 15.30 ppm (D$_2$O exchangeable) and 9.02 ppm due to CH–NH and CH–NH (H$_a$) protons respectively. On addition of 0.2 equivalents of CN^- ions to receptor **L1**, the characteristic doublet at 15.30 ppm got broadened
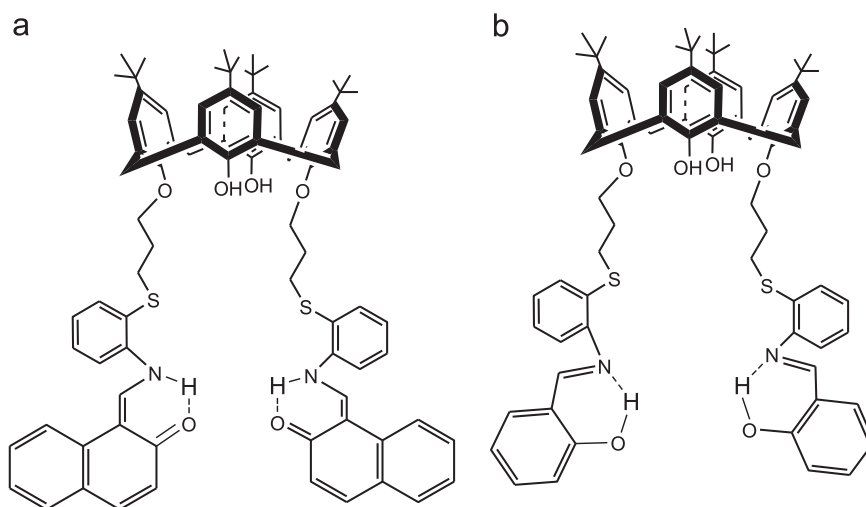
a

b

Fig. 7. (a) Keto-amine tautomeric form of naphthyl derivative **L1** (b) phenol-imine tautomeric form of salicylaldehyde derivative **L2**.
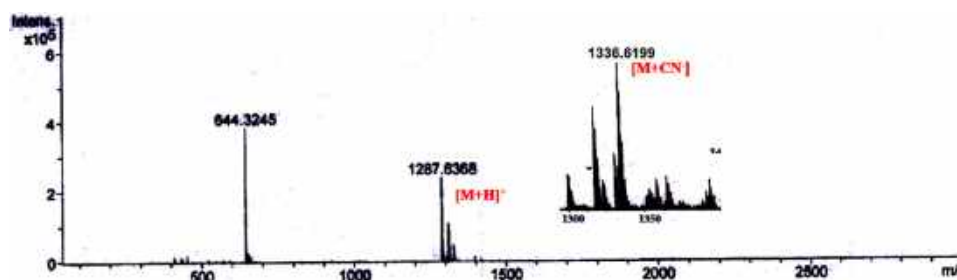

Fig. 8. HRMS spectrum of receptor **L1**. Inset HRMS spectrum obtained in the titration of receptor **L1** with cyanide ions.
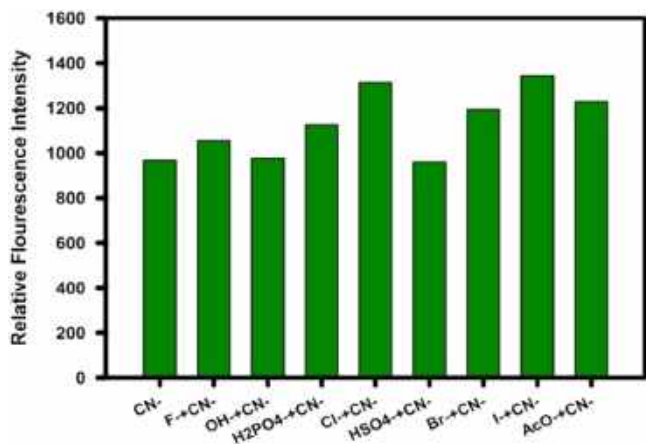

Fig. 9. (a) Fluorescence spectrum of **L1** (25 μM) in the presence of various anions in DMSO in response to cyanide ions.

5. Conclusion

Colorimetric and fluorimetric titrations reveal that **L1** selectively recognizes CN^- and fluoride ions from a plethora of similar anions with a fast color change from light yellow to orange and light yellow to dark yellow respectively. The receptor **L2** did not show expected selectivity towards anions despite the presence of similar functionalities as those present in **L1**. This could be ascribed to the existence of profound keto-enol tautomerisms, and better stability of keto-amine form of 2-hydroxy naphthyl tethered calixarene.

6. Experimental section

All the reagents used in the study were purchased from Sigma Aldrich or Merck and were considered chemically pure. The solvents were dried and distilled prior to use. HPLC grade solvents were used for UV and fluorescence measurements. ^1H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker DPX 300 instrument using tetramethylsilane (TMS) at 0.00 as an internal standard. Mass spectra were recorded on a Bruker Compass Data Analysis 4.0 Mass spectrometer while IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer in KBr disks. A Perkin Elmer Lambda 35 double beam spectrophotometer with variable bandwidth was used for the acquisition of the UV–vis molecular absorbance. All the data were acquired using 1 cm² path length quartz cuvettes. The spectral response from appropriate blanks of reference solutions was subtracted before data analysis. Dilution correction was also carried out wherever it was felt necessary. Melting points were determined on an electrothermal melting point apparatus obtained from Toshcon-Toshniwal (India).
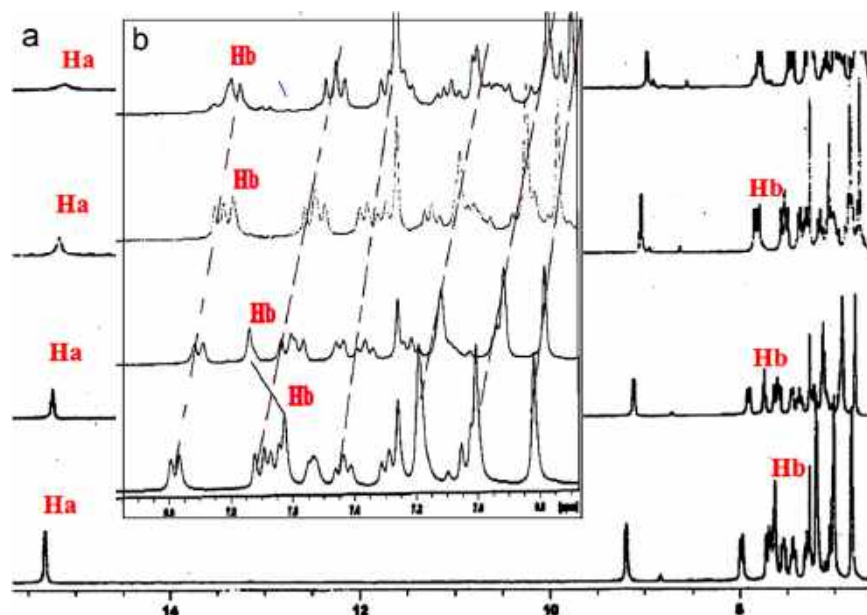
and eventually disappeared. This result indicates that the NH (H_a) protons undergo hydrogen bonding followed by deprotonation which increases the conjugation in the aromatic ring to result in upfield shift of aromatic protons (Fig. 10). The aromatic hydrogen of naphthalene ring at 7.6 ppm (H_b) seems to interact with nitrogen through intramolecular hydrogen bonding. This is supported by the NMR evidence when signal for H_b proton apparently moved downfield (figure given below). The schematic binding mechanism is shown in Fig. 11 [31–33].

Fig. 10. (a) The Partial ^{1}H NMR spectrum of receptor **L1** with cyanide ions and (b) expansion of spectrum from 7 to 8 ppm.
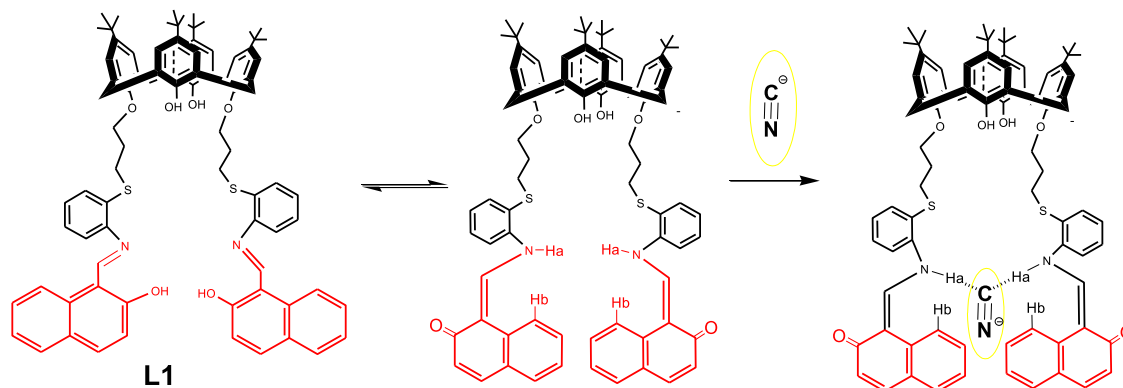

Fig. 11. Plausible binding mechanism of receptor **L1** with cyanide ions.

6.1. Synthesis of compound 1

1 was synthesized by refluxing *p-tert*- butylcalix[4]arene (2 g, 1 mmol) and 1,3-dibromopropane (1.24 g, 2 mmol) in acetonitrile in the presence of K_2CO_3 (4.25 g, 10 equivalents). On completion, (observed on TLC) the reaction mixture was filtered and the solvent was evaporated under reduced pressure. The remaining solid was extracted with chloroform and washed with water twice. The crude product was further re-crystallized from CHCl$_3$/MeOH in 69% yield. MS-FAB gave *m/z* for $C_{50}H_{66}Br_2O_4$ at 889.8 [M$^+$].

^{1}H NMR spectrum (CDCl$_3$, δ_H ppm): 1.00 (s, 18H, –C(CH$_3$)$_3$), 1.25 (s, 18H, –C(CH$_3$)$_3$), 2.50 (m, 4H, –OCH$_2$–CH$_2$–CH$_2$Br), 3.35 (d, 4H, J=12 Hz, ArCH$_2$Ar), 4.0 (t, 4H, J=5.6 Hz, –OCH$_2$–CH$_2$–CH$_2$Br), 4.09 (t, 4H, J=6.5 Hz, –OCH$_2$–CH$_2$CH$_2$Br), 4.26 (d, 4H, J=12 Hz, ArCH$_2$Ar), 6.86 (s, 4H, ArHcalix), 7.03 (s, 4H, ArH$_{calix}$), 7.68 (s, D$_2$O exchangeable, 2H, ArOH). ^{13}C NMR (CDCl$_3$, δ_H ppm): 29.8, 30.5, 31.2, 31.3, 32.9, 33.5, 72.8, 124.6, 125.1, 127, 132.3, 141.8, 148.4, 150.1.

6.2. Synthesis of compound 2

To a suspension of NaH (0.107 g, 4.48 mmol) in DMF (50 mL) was added o-aminothiophenol (0.42 g, 3.36 mmol) at 0 °C. The reaction mixture was stirred for 15 min and tetrakis-(p-tert-butyl) 26, 28-bis(bromopropyloxy)calix[4]arene (1 g, 1.12 mmol) was added to the reaction mixture. On completion of the reaction, the solvent was removed under reduced pressure and the reaction mixture was extracted with CHCl$_3$. After removal of the solvent, the crude product was subjected to column chromatography by using ethyl acetate/hexane (2:1) as the eluent. The separated compound could be re-crystallised from methanol-water to give thin creamish crystals in 65% yield. M.pt. 75–77 °C, UV (λ_{max}, CH$_3$CN): 293 nm. IR absorption band at (υ_{max} cm^{-1}): 3343, 2954, 1607, 1497, 1361, 1310, 780. HRMS (ESI-MS) *m/z*: calculated for $C_{62}H_{79}N_2O_4S_2$ is 979.5480, found 979.5476 [M+H] $^+$. ^{1}H NMR (DMSO-d$_6$, δ_H in ppm): 1.119 (s, 18H, –C(CH$_3$)$_3$), 1.24 (s, 18H, –C(CH$_3$)$_3$), 2.08 (m, 4H, –OCH$_2$CH$_2$CH$_2$), 3.29 (t, 4H, SCH$_2$), 3.43 (d, 4H, ArCH$_2$Ar), 4.02 (t, 4H, J=6.2 Hz, –OCH$_2$), 4.19 (d, 4H, J=12.3 Hz, ArCH$_2$Ar), 5.3 (s, 4H, NH$_2$, D$_2$O exchangeable), 6.5 (t, 2H, J=7.2 Hz, (aminothiophenol)ArH), 6.7 (d, 2H, J=7.8 Hz, (aminothiophenol)ArH), 7.02 (t, 2H, J=7.5 Hz, (aminothiophenol)ArH), 7.28 (s, 8H, ArH), 7.3 (d, 2H, J=7.2 Hz, -(aminothiophenol) ArH), 8.57 (s, 2H, –OH, D$_2$O exchangeable); ^{13}C NMR (CDCl$_3$ δ_H in ppm): 150.2, 149.1, 148, 146.5, 140.9, 135.5, 132.3, 129.1, 127.2, 125, 124.6, 117.9, 114.4, 73.7, 33.5, 33.3, 31.3, 31.2, 30.5, 29.31.

6.3. Synthesis of receptor L1

L1 was synthesized by stirring 2-aminothiophenyl derivative of calix[4]arene (500 mg, 0.50 mmol) and 2-hydroxy naphthalde-hyde (264 mg, 1.5 mmol) in the presence of 3–4 mg of zinc

perchlorate taken as a catalyst in $CH_3CN:CHCl_3$ (9:1). The color of the solution immediately changed to yellow. On completion of the reaction as observed on TLC, the solvent was evaporated and the crude oily product left was triturated with methanol to yield a yellow color fine solid precipitate which was filtrated. The precipitate was re-crystallized from methanol/water to give a compound in 87% yield. M.pt.140–145 °C. It exhibited strong IR absorption at (υ_{max} in cm^{-1}): 3432 (–OH str.), 1623 (C=N str.). HRMS gave m/z as 1287.6368, calculated for $C_{84}H_{90}O_6N_2S_2$ [M+] is 1287.6313. ^{1}H NMR (CDCl$_3$, δ_H in ppm): 0.967 (s, 18H, –C(CH$_3$)$_3$), 1.278 (s, 18H, –C(CH$_3$)$_3$), 2.24 (m, 4H, (CH$_2$CH$_2$CH$_2$), 3.25 (d, 4H, ArCH$_2$Ar, J=12.9 Hz), 3.395 (t, 4H, –SCH$_2$, J=13.8 Hz), 4.066 (t, 4H, –OCH$_2$, J=11.1 Hz); 4.3 (d, 4H, ArCH$_2$Ar, J=12.9 Hz), 6.78 (s, 4H, ArH-calix), 7.005 (s, 4H ArH-calix), 7.04 (d, 2H, ArH-aminothiophenyl, J=7.8 Hz), 7.197 (bm,4H, ArH-naphthalene), 7.29 (t, 2H, ArH-aminothiophenyl, J=7.6 Hz), 7.441 (t, 2H, ArH-aminothiophenyl, J=12.8 Hz), 7.52 (d, 4H, ArH-naphthalene, J=7.4 Hz), 7.63 (d, 2H, ArH-naphthalene, J=8.2 Hz), 7.72 (d, 2H, ArH-naphthalene, J=8.9 Hz), 7.9 (d, 2H, ArH-naphthalene, J=8.7 Hz), 8.849 (s, 2H, –OH-calix, D$_2$O exchangeable), 9.21 (s, 2H, –CH–NH), 15.30 (s, 2H, –CH–NH, D$_2$O exchangeable); ^{13}C NMR (CDCl$_3$, δ_H ppm): 29.46, 30.81, 30.98, 3.64, 33.76, 33.93, 74.40; 76.54, 76.97, 77.39, 109.10, 117.34, 119.07, 121.82, 123.39, 125.04, 125.50, 126.82, 127.32, 127.65, 127.85, 129.21, 129.86, 132,74, 136.31, 141.53, 149.47, 150.52, 154.27.

6.4. Synthesis of receptor L2

L2 was synthesized by using the same reaction protocols as adopted for **L1** except that salicylaldehyde (0.250 g, 0.25 mMol) was taken in place of 2-hydroxynaphthaldehyde. % yield was 82. M.pt.175–178 °C. IR absorption band at (υ_{max} cm^{-1}) 3371, 2955, 1615, 1477. HRMS gave m/z as 1187.6022, calculated for $C_{76}H_{87}O_6N_2S_2$ [M+] is 1187.6000. ^{1}H NMR (DMSO-d$_6$, δ_H in ppm): 1.097 (s, 18H, –C(CH$_3$)$_3$), 1.159 (s, 18H, –C(CH$_3$)$_3$), 2.20 (m, 4H, –CH$_2$CH$_2$CH$_2$), 3.24 (d, 4H, ArCH$_2$Ar, J=12.9 Hz), 3.21 (t, 4H, –SCH$_2$, J=13.7 Hz), 4.01 (t, 4H, –OCH$_2$, J=11.3 Hz), 4.16 (d, 4H, ArCH$_2$Ar, J=12 Hz), 7.11 (d, 8H, ArH-calix, J=7.8 Hz), 6.92–7.59 (m, 8H, Ar–H), 8.52 (s, 2H, –OH-calix, D$_2$O exchangeable), 8.87 (s, 2H, N=CH–), 13.07 (s, 2H, OH–naphthalene, D$_2$O exchangeable); ^{13}C NMR (CDCl$_3$, δ_H in ppm): 28.63, 29.25, 31.04, 31.73, 33.82, 33.96, 117.39, 117.54, 118.90, 119.32, 125.089, 125.54, 125.86, 127.508, 127.646, 127.725, 132.317, 12.647, 133.167, 141.413, 146.175, 146.947, 149.532, 150.718, 161.213.

6.5. UV–vis and fluorescence experiments

The spectroscopic grade of DMSO was used as the solvent for titration experiments which were carried out in 10 mm quartz cuvettes at 25 °C. The molecular receptors **L1** and **L2** were dissolved in DMSO to afford a concentration of 25 µM stock solution. Anions were used as their tetra butyl ammonium salts (10 mM) in DMSO while 25 µM host solution was used for the titration experiments.

6.6. ^{1}H NMR titration experiments

The ^{1}H NMR titrations were performed at 298 K by using the solution of receptor **L1** (0.02 M) in DMSO-d6 (400 µL) which was titrated by adding 25 µL solution of tetrabutylammoinum salt of cyanide ions (0.1 M). The chemical shift changes were monitored by taking tetramethyl silane as the standard.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jlumin.2016.06.045.

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Synthesis and evaluation of a new colorimetric and ratiometric fluorescence probe for copper ions

Har Mohindra Chawla *, Priyanka Munjal, Preeti Goel

Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, India

ABSTRACT

Synthesis and spectroscopic evaluation of compounds **3a**, **3b** and **4** reveal that cone conformer of 25,27-bis(o-aminothiophenyl propyloxy) -tetra-*p*-*tert*-butylcalix[4]arene **3a** can function as a highly selective ratiometric and colorimetric fluorescence probe for copper ions.

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1. Introduction

Copper ions play a significant role in both chemical and biological reactions especially in controlling the kinetics and transport of ions within the cell [1]. Major alteration in cellular level concentration of copper ions ushers serious neurodegenerative diseases which may include Alzheimer's and Parkinson diseases [2]. Copper is the third most abundant transition metal ion in the human body and is known to act as a co-factor for metalloenzymes as redox centre [3]. It is extensively used in chemical, electrical, textile and organic chemical industries and can be a cause for serious environmental concern warranting new approaches for its analysis and mitigation [4].

Calixarenes are macrocyclic metacyclophanes which have been extensively examined as molecular receptors. This is primarily due to their easy functionalization, hydrophobic cavity and manipulable conformational characteristics to confer selectivity for metal ion binding [5]. Calixarene scaffolds can be linked to fluorescence functions to offer changes in color and fluorescence characteristics on interaction with low concentrations of metal ions. Research on calixarene based systems has provided significant knowledge on their use, sensitivity and capacity to provide visual detection of analyte with fast response times [6]. General mechanisms involved in calixarene based systems for metal ion detection are mainly photo-induced electron transfer (PET) [7], fluorescence resonance energy transfer (FRET) [8], photon induced charge transfer (PCT) [9] or intramolecular charge transfer (ICT) protocols [10] for which considerable information is available for distinguishing different mechanisms involved in the recognition process.

Though a number of fluorescence based methods have been reported for copper ion recognition, most of them are based on fluorescence quenching to reveal so called 'on–off' methodology [11,12]. A relatively less number of methods have been investigated which depend upon enhancement of fluorescence intensity on interaction with metal ions [13]. Sadly most of these methods are markedly affected by system perturbations and intermolecular interactions. Recently our group has also reported selective interaction of Schiff base appended calixarenes with copper ions [14]. All these advances in the literature suggest the need for continuation of research on the development of copper selective molecular probes that can function through a ratiometric change (which can show fluorescence changes at two different wavelengths) for better scrutiny and reliability as well as a colorimetric response with anticipated rapidity [15].

In this communication, we report the synthesis of o-aminothiophenyl appended calix[4]arene derivative which selectively and sensitively interacts with copper ions to direct more research into the approach suggested. The selection of calixarene scaffold with useful functionalities based upon a study of active sites of copper containing proteins (e.g., plastocyanin) has been envisaged for ensuring measurement of analyte concentration with built in corrections for environmental and equipment flaws [16]. The designed molecular probe is different from recently investigated fluorophores

* Corresponding author. Tel.: +91 11 265 91517; fax: +91 11 265 81102.
  E-mail addresses: hmchawla@chemistry.iitd.ernet.in,
hmchawla@chemistry.iitd.ac.in (H.M. Chawla).
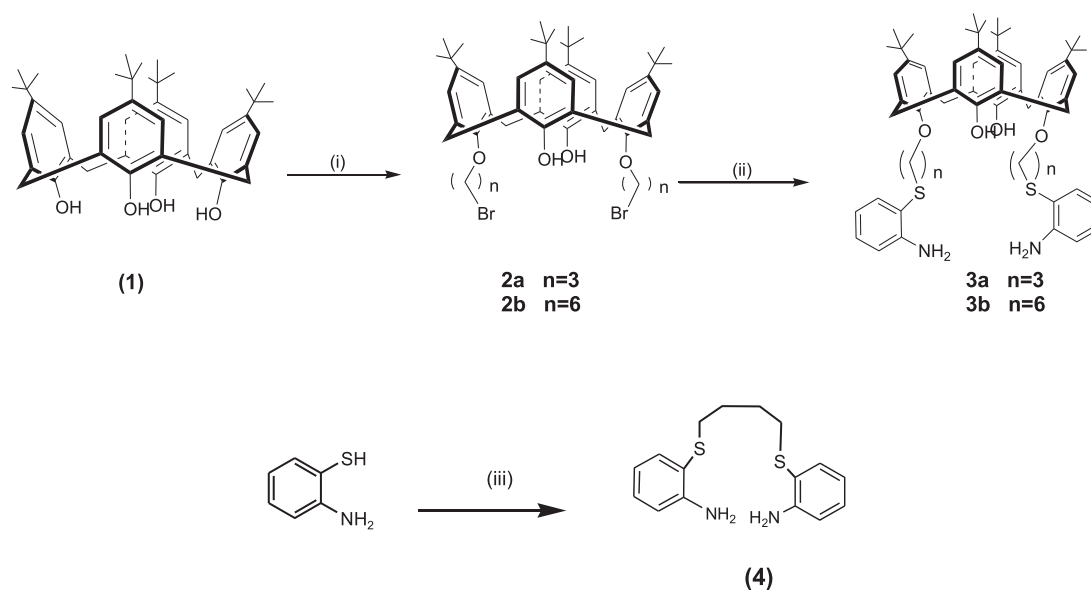
Scheme 1. Synthesis of novel receptor **3a**. Reagents and conditions: (i) 1,3-dibromopropane, CH_3CN, K_2CO_3, reflux; (ii) NaH, DMF, $\overline{TBAHSO_4}$, o-aminothiophenol, stirring and (iii) 1,4-dibromobutane, NaH, DMF, $\overline{TBAHSO_4}$, o-aminothiophenol, stirring.

like coumarin, 1,8-naphthalimide, quinoline and anthraquinone derivatives [17] and takes into cognizance of active site of plastocyanin which is known to play a prominent function in the initiation of biological processes. Since many copper containing proteins and enzymes deploy sulfur and nitrogen containing amino acids separated by a significant hydrophobic pocket in their constitution [18], we envisaged that if we could have nitrogen and sulfur binding sites appended to a hydrophobic cone or partial cone conformer of calix[4]arene (to take care of stereochemical and hydrophobic factors and a propyloxy chain to ensure adequate rigidity/flexibility ratio), the resulting molecular receptor would be able to interact with copper ions in a specific manner. This hypothesis was contemplated since calixarenes have been known to provide useful molecular receptors and sensor materials for transition metal ions.

The synthesis of the designed molecular receptor **3a** has been accomplished by minor modification of chemical reactions (Scheme 1) while its evaluation has been done by spectroscopic investigations for ionic recognition [19]. A reference compound **4** with similar binding sites and signaling units as those present in **3a** was also synthesized for determining the role of hydrophobic calixarene cavity. The design of **4** was adjusted in such a way that it contained the functionalites available in **3a** for interaction with copper ions. The size of calix[4]arene cavity and spacer length was also varied by synthesizing another calixarene derivative (**3b**) with appended hexyl chain in place of propyl chain. The synthetic compounds were investigated by UV–vis, NMR and fluorescence spectroscopy to determine the role of calixarene, spacer unit and conformation. The results obtained on evaluation of these molecular receptors indicate that 25,27-bis(o-aminothiophenyl propyloxy) tetra *p-tert*-butylcalix[4]arene (**3a**) can act as an efficient molecular probe for copper ions and cavity dimensions are important for copper interactions, thereby paving the way to design more efficient plastocyanin based copper ion receptors.

2. Results and discussion

2.1. Synthesis of the molecular receptors 3a and 3b and their characterization

The synthesis of the calixarene based molecular receptor **3a** was achieved through selective alkylation of *p-tert*-butylcalix[4]arene with 1,3-dibromopropane in the presence of potassium carbonate followed by a nucleophilic substitution reaction with o-aminothiophenol (Scheme 1). The reaction mixture on work up and column chromatography using ethyl acetate/hexane (2:1) as the eluent, gave creamish white solid (65% yield), with a m.p. 75–78 °C. It exhibited strong IR absorptions at υ_{max} 3343 (NH_2, str.), 1607 (NH, bend), 1361 (C–N, str.). In its ^1H NMR spectrum in DMSO-d_6, compound **3a** revealed the appearance of protons at δ 3.27 (–SCH_2; 4H) and D_2O exchangeable singlets at δ 5.30 and δ 8.57 for NH_2 (aminothiophenol) and hydroxyl protons of calix[4]arene respectively. The disappearance of a triplet at δ 3.97 ppm for –$BrCH_2$ present in the precursor **2a** confirmed the introduction of di-substituted aminothiophenol at the lower rim of calix[4]arene (Fig. 1) which was further confirmed by the appearance of a molecular ion peak at m/z 979.548 $[M+H]^+$ in its high resolution mass spectrum.

Compound **3b** was synthesized by using a similar method as adopted for **3a** except that tetrakis-(p-tert-butyl) 26,28-bis(hexyloxybromo)calix[4]arene (**2b**) was taken instead of tetrakis-(p-tert-butyl) 26,28-bis(propyloxybromo)calix[4]arene (**2a**). In its ^1H NMR spectrum in CDCl$_3$, compound **3b** revealed the appearance of protons at δ 3.01 (–SCH_2; 4H) and disappearance of a triplet at δ 3.53 for –$BrCH_2$ present in the precursor **2b** which confirmed the formation of the compound **3b**. The conservation of the original fixed cone conformation of both the derivatized calix[4]arenes was confirmed by the presence of two sharp doublets of methylene bridges at δ 4.4 and δ 3.3 in the ^1H NMR spectrum and a signal at δ 33 in the ^{13}C NMR spectrum.

2.2. Synthesis of the reference compound 4 and its characterization

Compound **4** was synthesized by using the same method as adopted for the synthesis of **3** when 1,4-dibromobutane (0.250 g, 0.25 mmol) was taken instead of tetrakis-(p-tert-butyl) 26,28-bis (propyloxybromo)calix[4]arene **2**. In its ^1H NMR spectrum, compound **4** revealed the appearance of protons at δ 2.62 (–SCH_2, 4H) and D_2O exchangeable singlet at δ 4.0 for NH_2 (aminothiophenol). The introduction of di-substituted aminothiophenol at 1,4-dibromobutane was confirmed by the appearance of a molecular ion peak at 305.1179 in its high resolution mass spectrum.
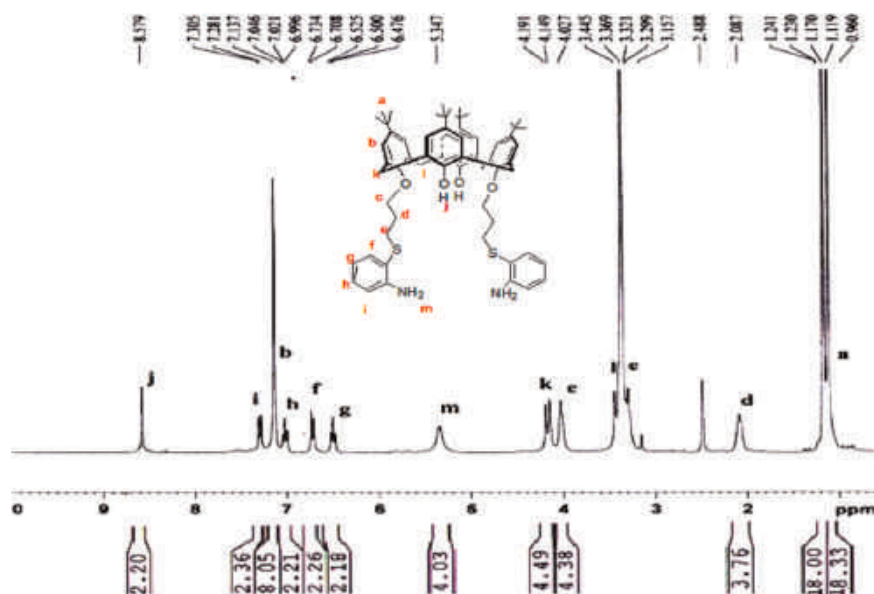
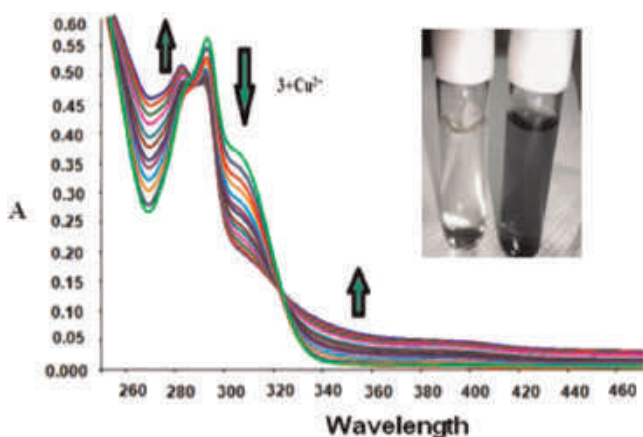**Fig. 1.** ^{1}H NMR spectra of the synthesized compound **3a** in DMSO-d$_6$.

Fig. 2. UV–vis spectra of o-aminothiophenyl substituted propyloxy tetra *p-tert*-butylcalix[4]arene (40 μM) in CH$_3$CN obtained by adding 1 eq of Cu^{2+} solution. Inset shows color change from colorless to blue on addition of 10 eq of Cu^{2+} ion to the sensor solution. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.3. Colorimetric studies

3a (4.0×10^{-5} M) was evaluated by observing its colorimetric response on interaction with diverse variety of metal ions. The solution of the metal ions (Na$^+$, Li$^+$, Cs$^+$, Ag$^+$, Mn^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+} in the form of their perchlorate salts) in dry CH$_3$CN was first added individually to a solution of synthesized molecular receptor (4.0×10^{-5} M) in dry CH$_3$CN. A distinct color change from colorless to greyish blue was observed only on addition of 10 eq of Cu^{2+} ions (Fig. 2 inset). No change in color was noticed when Cu^{2+} ions were added to the solution of **4**. This selective color change in **3a** on addition of copper ions with other interfering ions only prompted us to further investigate the synthesized calixarene derivative **3a**.

2.4. UV–vis spectroscopic measurements

Following preliminary colorimetric analysis, binding and sensing properties of the designed receptor molecule were studied by UV–vis spectroscopic measurements in dry CH$_3$CN. In the UV–vis absorption

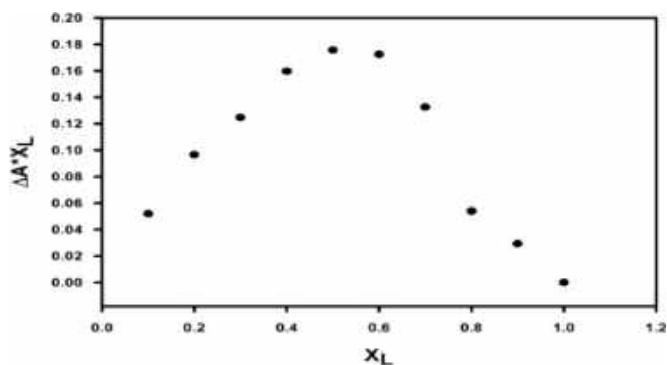**Fig. 3.** Job's plot of Cu^{2+} complex formation. X_L is the mole fraction of ligand **3a**.

spectrum, receptor **3a** (4.0×10^{-5} M) was found to exhibit a strong absorption band at 293 nm with a hump at 309 nm. It was observed that only Cu^{2+} amongst various tested metal ions (Na$^+$, Li$^+$, Cs$^+$, Ag$^+$, Mn^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+} in the form of their perchlorate salts) displayed noticeable changes in the absorption spectrum of receptor **3a** (ESI Fig. S3). Upon gradual addition of Cu^{2+} to 4×10^{-5} M solution of **3a**, the intensity of absorption band at 293 nm decreased with the appearance of another absorption band at 282 nm. It was determined that the addition of 1 eq of Cu^{2+} resulted in a blue-shift of 11 nm in the absorption spectra with a clear isosbestic point at 288 nm as shown in Fig. 2. The color change observed could be ascribed to the absorption spectrum between 320–500 nm which was broadened on addition of Cu^{2+} ions to suggest that **3a** can be used for its visual recognition by the naked eye.

2.5. Stoichiometry

The stoichiometry of **3a-Cu^{2+}** was determined from continuous variation Job's plot which showed the maximum at 0.5 mol% indicating the formation of a 1:1 molecular complex (Fig. 3). The 1:1 stoichiometry was also confirmed by HRMS data when main mass peak was observed at m/z 1041.4536 due to $[CuC_{62}H_{78}S_2N_2O_4]^+$ (Fig. 4).
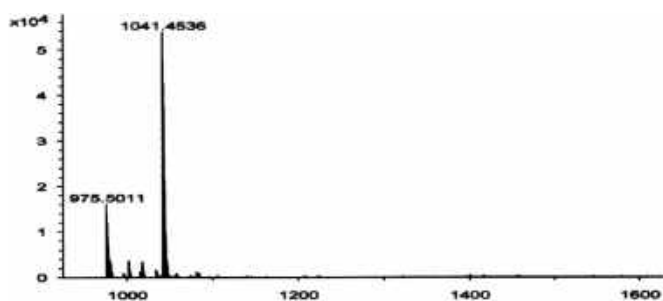
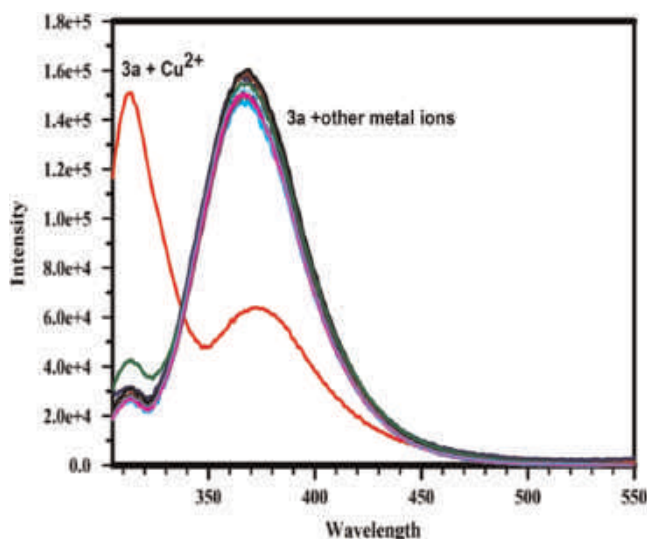Fig. 4. Mass spectrum of complex $[CuC_{62}H_{78}S_2N_2O_4]^+$.

Fig. 5. Fluorescence emission spectra of compound **3a** with other metal ions. (λ_{ex} 293 nm).

2.6. Fluorescence spectroscopic measurements

Changes observed on interaction of different metal ions with receptor **3a** and the reference are discussed below:

The emission spectrum of **3a** (4×10^{-5} M) exhibited a maximum at 367 nm with a small band at 313 nm, when excited at 293 nm. The recognition characteristics of **3a** in dry CH_3CN were determined by recording the changes in its fluorescence spectrum on addition of various tested metal ions e.g., Na^+, Li^+, Cs^+, Ag^+, Mn^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+} in the form of their perchlorates. The fluorescence spectrum remained unaffected by the presence of all tested metal ions except when copper ions were added (Fig. 5) thereby indicating a high selectivity for Cu^{2+} ions over other heavy transition metal ions. The change in the fluorescence spectrum resulted in a blue-shift of 50 nm with an increase in intensity of 313 nm emission band at the expense of 367 nm in its emission spectrum.

Addition of increasing concentration of Cu^{2+} ions to a solution of **3a** (4×10^{-5} M) led to a gradual decrease in the intensity of the 367 nm emission band with a simultaneous increase in its emission band at 313 nm (Fig. 6a). When the intensity ratio of emission band at 313 nm to that of 367 nm (I_{313}/I_{367}) was plotted as a function of Cu^{2+} ion concentration, a good linear relationship was observed (Fig. 6a inset), thereby indicating a useful ratiometric relevance of measurement with **3a**.

Similarly, ion recognition properties of compound **3b** (4×10^{-5} M) in dry CH_3CN were investigated by fluorescence emission spectroscopy. When excited at 293 nm, **3b** exhibited an emission maximum at 313 nm. It was observed that the emission spectrum of compound **3b** did not lead to any change in the intensity on addition of 1 eq of perchlorate salt of Cu^{2+} ions as shown in Fig. 6b. A comparison of

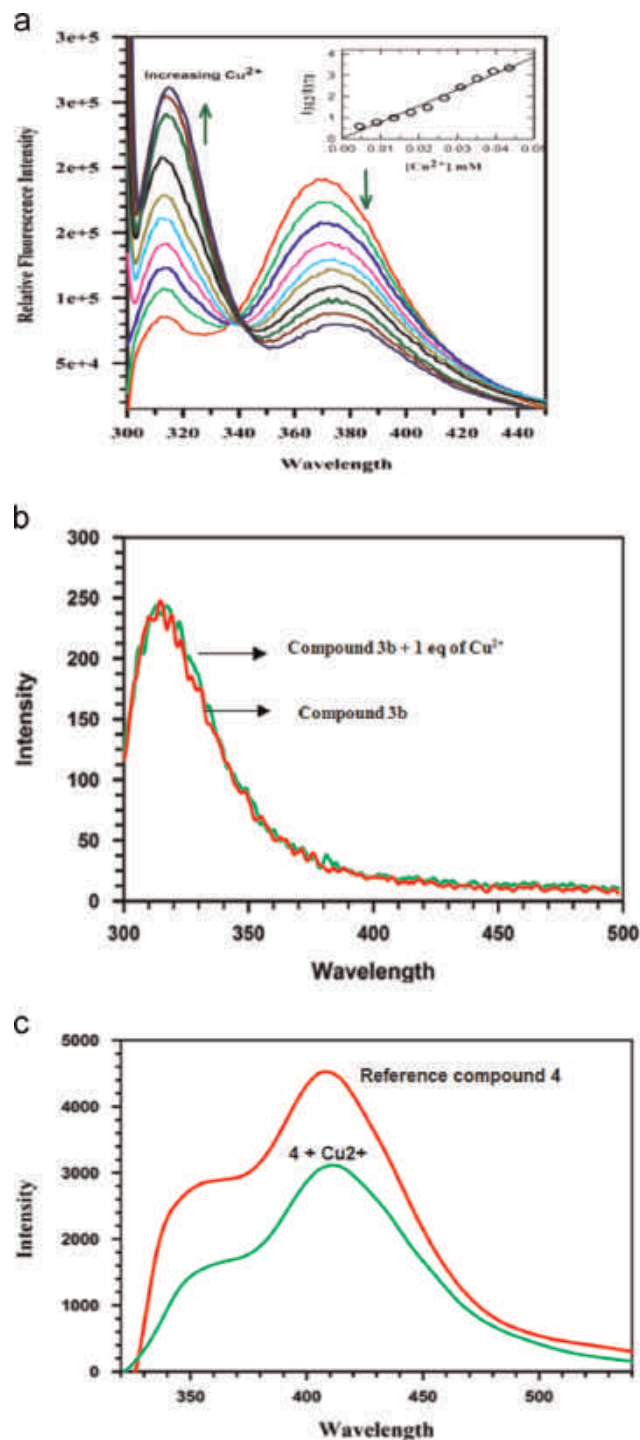Fig. 6. (a) Fluorescence emission spectra of **3a** at different concentrations of Cu^{2+}. Inset: ratiometric calibration curve I_{312}/I_{371} as a function of Cu^{2+}. (b) Fluorescence emission spectra of **3b** (in red) and compound **3b** on addition of 1 eq Cu^{2+} (in green). (c) Fluorescent emission spectra of reference compound **4** on addition of 1 eq Cu^{2+}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

fluorescence titrations of compound **3a** and **3b** indicated that calixarene cavity provides rigidity to the molecular receptor which apparently plays an important role for efficient interaction of **3a** with Cu^{2+} ions. This was supported by the fact that the increase in size of the spacer unit does not provide efficient interaction with copper ions and rigidity conferred by calixarene cavity is an important factor evolving.

Hence compound **3b** happened to be a poorer receptor than compound **3a.**

2.7. With compound 4

Ion recognition properties of another reference compound **4** in dry CH_3CN were also investigated by fluorescence emission spectroscopy. The reference compound **4** when excited at 310 nm, exhibited an emission maximum at 407 nm. It was observed that the emission spectrum of compound **4** led to 25% quenching when 1 eq of copper ions were added to its solution as shown in Fig. 6c. Since **4** is hydrophobic but cavity is absent in its structure, it implies that calixarene cavity is very specific for interaction with copper ions.

2.8. Quantitative studies

The quantitative response of **3a** towards copper ions was studied by repeated fluorescence titrations. The association constant K_a for interaction of **3a** and copper ions was calculated by using Benesi–Hildebrand plot by application of the equation as given below.

$$\frac{1}{F_0 - F} = \frac{a}{a - b} \times \left[\frac{1}{K_a [M]} + 1 \right]$$

where K_a is association constant; F_0 is the observed fluorescence in the absence of cation at 367 nm; F is the observed fluorescence after every addition of 10 μL of Cu^{2+} to 40 μM solution of **3a** at 367 nm and $[M]$ is the concentration of the cation-added.

The plot of $[1/(F-F_0)]$ versus $1/[M]$ was found to exhibit a linear relationship (Fig. 7a). The association constant (K_a=intercept/slope) of **3a** in the presence of Cu^{2+} ion was determined to be $1.19 \times 10^4 \, M^{-1}$. The high magnitude of stability constant for copper indicated the presence of astrong interaction between **3a** and copper ions.

The detection limit of **3a** as a fluorescence sensor for Cu^{2+} ions was also calculated from a plot of $\Delta F/F$ versus $\log [Cu^{2+}]$ by using the method available in the literature [20]. The detection limit was determined to be 2.29 μM which is sufficiently low for the detection of sub milli molar concentration of Cu^{2+} ion found in many chemical systems (Fig. 7b).

2.9. Selectivity and competitive ionic recognition experiments

The selectivity of the receptor **3a** was determined by using competitive experiments with other metal ions. For this 1 eq of Cu^{2+} was used in combination with the same equivalents of other competing metal ions. It was observed that data of all other metal ions (Na^+, Li^+, Cs^+, Ag^+, Mn^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+} in the form of their perchlorates) had marginal effect on the fluorescence emission spectrum for the detection of copper ions by **3a** (Fig. 8).

2.10. NMR titrations

The ^1H NMR spectrum revealed that calixarene hydroxyls were not involved in the complexation process with copper ions. The –NH_2 and –SCH_2 protons of 25,27-bis(o-aminothiophenyl propyloxy) tetra *p-tert*-butylcalix[4]arene (**3a**) were observed at δ 5.3 and δ 3.2 respectively. At low concentration of copper ions (-0.45 equivalents) the peak at δ 5.3 led to its disappearance and the peak at δ 3.2 got broadened. This observation suggested that the –NH_2 and –S of o-aminothiophenyl group were involved in the interaction with Cu^{2+} ions through the cone conformation of calix[4]arene scaffold of receptor **3a** as shown in Fig. 9. It was observed that when concentration of copper ions was increased, it led to
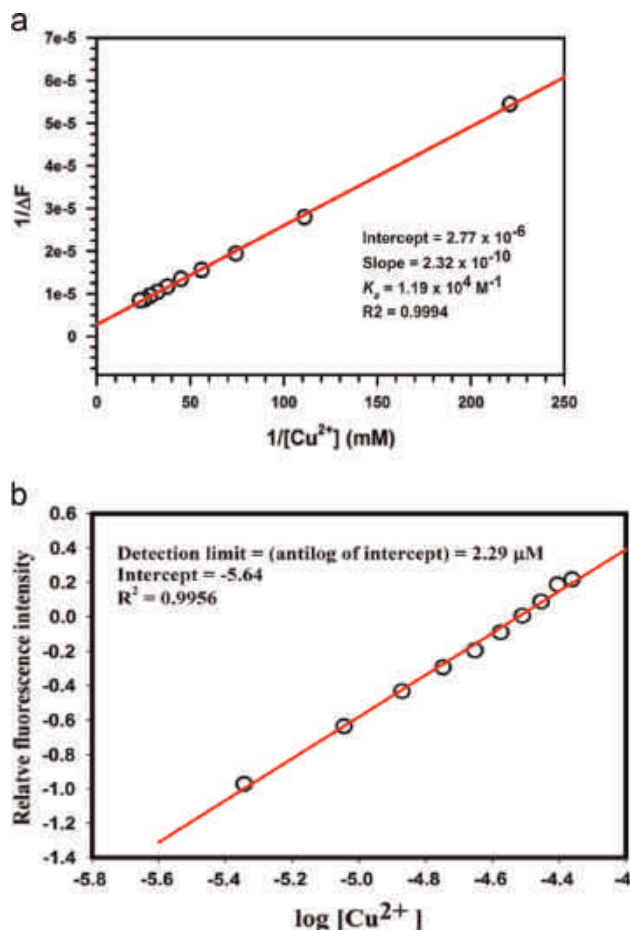

Fig. 7. (a) Benesi–Hildebrand plots of **3a** at different Cu^{2+} concentrations. (b) Detection of limit for compound **3a**.
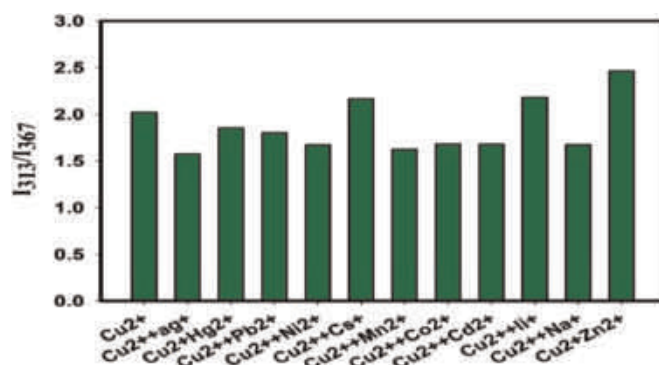

Fig. 8. Ratio responses of **3a** to Cu^{2+} (10 μM) in the presence of various interfering metal ions (50 μM) in CH_3CN.
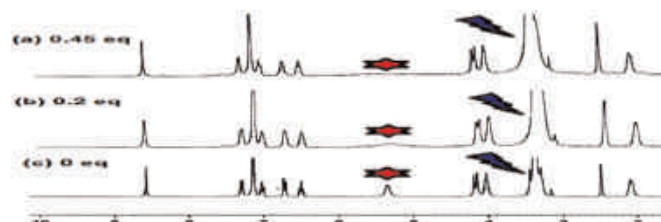

Fig. 9. Partial ^1H NMR spectra of sensor **3a** in DMSO-d_6 in the presence of (a) 0.45 eq Cu^{2+} (b) 0.2 eq Cu^{2+} and (c) in the absence of Cu^{2+}.

broadening of spectrum with loss of resolution (ESI Fig. S16). This could be attributed to the paramagnetic influence of copper ions at higher concentration [21]. However lower concentration of copper ions (−0.45 equivalents) gave presentable results but without stoichiometry. The stoichiometry of complexation of **3a** and copper ion, therefore, was determined by different methods e.g., Job's plot experiment and mass spectrometry as described earlier.

2.11. Effect of pH on sensing mechanism

The change in the intensity of fluorescence of **3a** could also be attributed to interaction of copper ions with amine function which could lead to a reduction in its conjugation or oxidation . This was examined by fluorescence measurements after addition of 43 μM solution of HCl which gave a new blue-shifted emission band at 313 nm on excitation at 293 nm while no change in intensity of emission band at 367 nm was noticed. This observation suggested that the new band formed on addition of HCl was probably due to protonation of the o-aminothiophenyl moiety (ESI Fig. S4). It was significant to note that addition of copper ions to the solution of receptor **3a** led to quenching of the emission band at 367 nm which got quenched while the emission band at 313 nm got enhanced. Cyclic voltammetric study of the Cu^{2+}-**3a** interaction indicated that the amino thiophenol moiety is not markedly affected by addition of one equivalent of copper ions. This observation revealed that the mechanism of interaction of copper ion with receptor **3a** is different from simple decrease in conjugation due to protonation. This conclusion is in consonance with other precedents which have observed inhibition of photoinduced electron transfer on interaction with copper ions [7d]. Further work is in progress to elucidate the exact mechanism of the recognition of copper ions.

3. Conclusion

In conclusion, we have synthesized three molecular receptors (**3a**, **3b** and **4**) and have examined their recognition characteristics by absorption and fluorescence spectroscopy. Copper ion has been determined to usher colorimetric and ratiometric changes in fluorescence spectrum of **3a**. **3b** with a longer spacer chain showed no change in fluorescence spectrum from that of **3a** indicating that calix[4]arene cavity plays an important role for efficient complexation with copper ions. Similarly another reference compound **4** with hydrophobic cavity without calix[4]arene unit exhibited only 25% quenching of fluorescence with copper ions indicating that the nature of interactions of **3a**, **3b** and **4** with Cu^{2+} ions is different. At this point of studies, it appears that compounds **3b** and **4** are poorer receptors than **3a** for efficient complexation with copper ions.

4. Experimental

4.1. General

All the reagents used in the study were purchased from Sigma-Aldrich or Merck and were chemically pure. The solvents were dried and distilled prior to use. HPLC grade solvents were used for UV and fluorescence measurements. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker DPX 300 instrument using tetramethylsilane (TMS) at 0.00 as an internal standard. Mass spectra were recorded on a Bruker Compass Data Analysis 4.0 Mass spectrometer while IR spectra were recorded on a Perkin Elmer FT-IR spectrometer in KBr disks. A Perkin Elmer Lambda 35 double beam spectrophotometer with variable bandwidth was used for

the acquisition of the UV–vis molecular absorbance. All the data were acquired using 1-cm^2 path length quartz cuvettes. Spectral response from appropriate blanks of reference solutions was subtracted before data analysis. Dilution correction was also carried out wherever necessary. Melting points were determined on an electrothermal melting point apparatus obtained from Toshcon–Toshniwal (India) and were uncorrected.

4.2. Synthesis of compound 2a

p-tert-Butylcalix[4]arene (2 g, 1 mmol) and K_2CO_3 (4.25 g, 10 eq) were taken in acetonitrile (50 mL) and 1,3-dibromopropane (1.24 g, 2 mmol) was added. The reaction mixture was refluxed until the starting materials had disappeared (as observed by TLC). On completion, the reaction mixture was filtered and the solvent was evaporated under reduced pressure. The remaining solid was extracted with chloroform and washed with water twice. The crude product was further re-crystalized by $CHCl_3$/MeOH. % yield=69; ^{1}H NMR spectrum: (300 MHz, $CDCl_3$): δ=1.00 (s, 18H, –$C(CH_3)_3$, 1.25 (s, 18H, –$C(CH_3)_3$, 2.50 (m, 4H, –OCH_2–CH_2–CH_2Br), 3.35 (d, 4H, J=12 Hz, ArCH_2Ar), 4.0 (t, 4H, J=5.6 Hz, –OCH_2–CH_2–CH_2Br), 4.09 (t, 4H, J=6.5 Hz, –OCH_2–CH_2CH_2Br), 4.26 (d, 4H, J=12 Hz, ArCH_2Ar), 6.86 (s, 4H, ArHcalix), 7.03 (s, 4H, ArHcalix), 7.68 (s, D_2O exchangeable, 2H, ArOH). ^{13}C NMR (75 MHz, $CDCl_3$): δ=29.8, 30.5, 31.2, 31.3, 32.9, 33.5, 72.8, 124.6, 125.1, 127, 132.3, 141.8, 148.4, 150.1. MS-FAB m/z for $C_{50}H_{66}Br_2O_4$ is 889.8 [M$^+$].

4.3. Synthesis of compound 2b

p-tert-Butylcalix[4]arene (2 g, 1 mmol) and K_2CO_3 (4.25 g, 10 eq) were taken in acetonitrile (50 mL) and 1,6-dibromohexane (1.24 g, 2 mmol) was added to it. The reaction mixture was refluxed until the starting materials disappeared (as observed by TLC). On completion, the reaction mixture was filtered and the solvent evaporated under reduced pressure. The residue was extracted by chloroform and washed twice with water. The crude product was purified by coloumn chromatography by using chloroform/hexane as the eluent, which gave a transparent liquid; % yield=55; ^{1}H NMR (300 MHz, DMSO-d$_6$): 1.10 (s, 18H, –$C(CH_3)_3$), 1.17 (s, 18H, –$C(CH_3)_3$), 1.58 (m, 4H, –$CH_2(CH_2)_2Br$), 1.73 (m, 4H, –$O(CH_2)_2CH_2$–), 1.91 (m, 4H, –CH_2CH_2Br), 2.02 (m, 4H, –OCH_2CH_2–), 3.38 (d, 4H, J=13 Hz, ArCH_2Ar), 3.53 (t, 4H, J=6 Hz, –CH_2Br), 3.93 (bs, 4H, –OCH_2–), 4.20 (d, 4H, J=13 Hz, ArCH_2Ar), 7.05–7.14 (bs, 8H, ArH), 8.57 (s, 2H, –OH, D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO-d6): δ=24.4, 27.3, 29.1, 30.7, 31.2, 33.3, 33.7, 34.4, 38.6, 75.8, 124.9, 125.3, 127.3, 132.9, 141.16, 146.8, 149.5, 150.1.

4.4. Synthesis of compound 3a

To a suspension of NaH (0.107 g, 4.48 mmol) in DMF (50 mL) was added o-aminothiophenol (0.42 g, 3.36 mmol) at 0 °C. The reaction mixture was stirred for 15 min and tetrakis-(p-tert-butyl) 26,28-bis (propyloxybromo)calix[4]arene (1 g, 1.12 mmol)**2a** was added to the reaction mixture. On completion of the reaction, the solvent was removed under reduced pressure and the reaction mixture was extracted with $CHCl_3$. After removal of the solvent, the crude product was subjected to column chromatography by using ethyl acetate/hexane (2:1) as the eluent. The separated compound could be re-crystallized from methanol–water to give thin creamish crystals. % yield=65, m.p. 75–77 °C, UV (λ_{max}, CH_3CN): 293 nm, IR (KBr pellet, cm^{-1}): 3343, 2954, 1607, 1497, 1361, 1310, 780; ^{1}H NMR (300 MHz, DMSO-d$_6$): 1.119 (s, 18H, –$C(CH_3)_3$), 1.24 (s, 18H, –$C(CH_3)_3$), 2.08 (m, 4H, –$OCH_2CH_2CH_2$), 3.29 (t, 4H, SCH_2), 3.43 (d, 4H, J=12.3 Hz, ArCH_2Ar), 4.02 (t, 4H, J=6.2 Hz, –OCH_2), 4.19 (d, 4H, J=12.3 Hz, ArCH_2Ar), 5.3 (s, 4H, NH_2, D_2O exchangeable), 6.5 (t, 2H, J=7.2 Hz, (aminothiophenol)ArH), 6.7 (d, 2H, J=7.8 Hz, (aminothiophenol)

ArH), 7.02 (t, 2H, $J=7.5$ Hz, (aminothiophenol)ArH), 7.28 (s, 8H, ArH), 7.3 (d, 2H, $J=7.2$ Hz, –(aminothiophenol)ArH), 8.57 (s, 2H, –OH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$) δ in ppm): 150.2, 149.1, 148, 146.5, 140.9, 135.5, 132.3, 129.1, 127.2, 125, 124.6, 117.9, 114.4, 73.7, 33.5, 33.3, 31.3, 31.2, 30.5, 29.31; HRMS (ESI-MS) m/z: calculated for $C_{62}H_{79}N_2O_4S_2$ is 979.5480, found 979.5476 $[M+H]^+$.

4.5. Synthesis of compound 3b

This compound **3b** was synthesized by using the same method as adopted for **3a** except that *tetrakis*-(*p*-*tert*-butyl) 26,28-bis(hexyloxybromo)calix[4]arene **2b** (0.25 mmol) was taken instead of *tetrakis*-(*p*-*tert*-butyl) 26,28-bis(propyloxybromo)calix[4]arene **2a**. % yield = 61; ^{1}H NMR (300 MHz, $CDCl_3$): 0.96 (s, 18H, –C(CH$_3$)$_3$), 0.99 (s, 18H, –C(CH$_3$)$_3$), 1.42–1.18 (m, 16H, –(CH$_2$)$_4$CH$_2$Br), 3.06–3.21 (m, 8H, SCH$_2$, ArCH$_2$Ar), 3.86 (b, 4H, –OCH$_2$), 4.01 (s, 4H, –NH$_2$, D_2O exchangeable), 4.28 (d, 4H, $J=12$ Hz, ArCH$_2$Ar), 6.66 (t, 4H, $J=9$ Hz, (aminothiophenol)ArH), 6.82 (t, 4H, $J=6$ Hz, (aminothiophenol) ArH), 6.91 (d, 2H, $J=9$ Hz, (aminothiophenol)ArH), 7.08–6.9 (m, 8H, ArH-calix), 7.47 (d, 2H, $J=7.0$ Hz, –(aminothiophenol)ArH), 7.57 (s, 2H, –OH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=26.1$, 27.8, 28.4, 29.1, 29.3, 29.9, 31.9, 38.4, 74.7, 115.7, 121.2, 123.2, 123.6, 124.3, 125.8, 128.5, 131.3, 133.7, 144.9, 148.2, 150.7.

4.6. Synthesis of reference compound 4

4 was synthesized by using the reaction protocol described for **3** except that 1,4-dibromobutane (0.250 g, 0.25 mMol) was taken instead of *tetrakis*-(*p*-*tert*-butyl) 26,28-bis(propyloxybromo)calix [4]arene **2a**. % yield = 72; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=1.63$ (bs, 4H, –CH$_2$–CH$_2$), 2.69 (bs, 4H, CH$_2$–SAr), 4.29 (bs, 4H, NH$_2$, D_2O exchangeable), 6.70 (m, 4H, –ArH), 7.09 (t, 2H, $J=6$ Hz, ArH), 7.30 (d, 2H, $J=9$ Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=28$, 33.8, 114.4, 117.3, 118, 129.1, 135.3, 147.7; HRMS (ESI-MS) m/z: calculated for $C_{16}H_{21}N_2S_2$ is 305.1179, found 305.1141 $[M+H]^+$.

4.7. Solutions for UV–vis spectroscopic measurements

Spectroscopic grade dry CH_3CN was used as the solvent for experiments on spectral titration. The solution of receptor **3a** (4.0×10^{-5} M) and different perchlorate salts of cations (1×10^{-3} M) such as Na^+, Li^+, Cs^+, Ag^+, Mn^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+} were prepared in dry CH_3CN. The volume of the solution of **3a** used in the UV–vis measurements was 2.2 mL. The absorbance was measured from 200 to 800 nm on addition of the solution of different cations to the solution of receptor **3a** in dry CH_3CN.

4.8. Solutions for fluorescence spectroscopic measurements

Different solutions of metal ions (1×10^{-3} M) in dry CH_3CN were introduced in small portions to the receptor **3a** (4.0×10^{-5} M) in dry CH_3CN and fluorescence spectra were recorded from 300 to 600 nm at room temperature. The volume of the solution of receptor **3a** used each time in the fluorescence measurements was 2.0 mL. The receptor was excited at 293 nm and the emitted maximum was observed at 367 nm with a small hump at 313 nm.

4.9. Solutions for ^{1}H NMR spectroscopic measurements

The ^{1}H NMR titrations were performed at 298 K by using the solution of receptor **3a** (0.02 M) in DMSO-d_6 (400 μL) which was titrated by adding 25 μL solution of perchlorate salt of copper ions (0.1 M). The chemical shift changes for receptor **3a** were monitored.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at 10.1016/j.jlumin.2015.03.027.

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Metallo-supramolecular complex of 1,3-di{bis(2-hydroxynaphthyl)}-2-aminothiophenylcalix[4]arene for the detection of L-histidine using secondary interactions

Priyanka Munjal[a,b,*], H.M. Chawla[a,b]

[a] Chemistry and Toxicology Division, LNJN-National Institute of Criminology and Forensic Science, Rohini, Sector-3, New Delhi 110085, India
[b] Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, India

ABSTRACT

The 1,3-di{bis(2-hydroxynaphthyl)}-2-aminothiophenylcalix [4]arene **(L1)** has been synthesized and characterized by nmr, mass, uv- vis, fluorescence spectroscopy. The *in-situ* mixing of receptor **L1** and copper ions in 1:1 pattern forms metallo supramolecular complex **[L1-Cu2+]** which recognizes L-histidine from among other essential amino acids in the emission spectrum. It seems that receptor **L1** act as a primary sensor for cation recognition and as a secondary sensor for L-histidine.

1. Introduction

Metallo supramolecular complexes have been synthesized *in-situ* by mixing of supramolecular ligand and metal ions as guest moiety where the non-covalent interactions such as hydrogen bonding, electrostatic, hydrophobic interactions *etc* cling to these metallo supramolecular complexes. Further these primary interactions are extended for the recognition of different analytes such as cations, anions and neutral molecules that have been attracted as a keen attention towards researchers [1]. Recognition of bio molecules is a complex mechanism as compared to recognition of inorganic salts like cations and anions. Therefore the secondary interactions provided by metallo supramolecular complexes solve the problems to some extent. For example, Chan et al. has reported supramolecular complex for coumarin-Cu²⁺ for detection of L-histidine [2,3]. Similarly, Rao et al. has also developed new turn on fluorescent assay for cysteine by using secondary interactions of [calix[4]arene-Ag⁺] supramolecular complex. Prompted by this recent literature reports, in connection with our continuous research interest, fluorescent sensors are developed for bio molecules by using metallo-supramolecular complex of calix[4]arene derivative with copper ions [4].

Molecular recognition of bio-molecules such as amines, amino acids, peptides, proteins and carbohydrates have gained more attention in supramolecular chemistry. Among all, amino acids play an important role in the biological processes. Besides building blocks of proteins and polypeptides, amino-acids regulates key metabolism pathways which

are necessary for growth, maintenance, reproduction and immunity [5].

Several methods have been developed for amino acids sensors such as liquid chromatography, capillary electrophoresis, electrochemistry, colorimetry, raman scattering and fluorometry. Alternatively, the fluorescent methods offered high sensitivity and selectivity in the quantitative analysis [6]. Therefore, much interest has been shown in synthesizing artificial receptors of new fluorescent probes for amino acids. Among all naturally occurring amino acids; particularly, L-histidine has received considerable attention due to its fundamental role in biological environment [7–10]. L-histidine is not only an essential amino acid, but also found to be a powerful blood vessel dilator in gastric acid secretion and involved neurotransmission inside central nervous system [11–15]. It is also important in the growth and repair of tissues. Moreover L-histidine can act as an antioxidant as it protect against the radiation by helping in the removal of heavy metals from the body. Recently it was found that deficiency of L-histidine could be attributes to chronic kidney disease, nerve deafness and rheumatoid arthritis [16–22].

L-histidine can act as a tridentate ligand for transition metal ion. A recent discovery shows that a complex of Cu²⁺-L-his was found in human blood and is used for the treatment for Menkes disease (neurodegenerative disease) [23–29]. Therefore it is necessary to synthesize a molecular receptor for L-histidine.

Calix[4]arene are well known host to encapsulate various inorganic guests inside their hydrophobic cavity and their unique three dimensional structure which provides rigidity as well as flexibility is also used
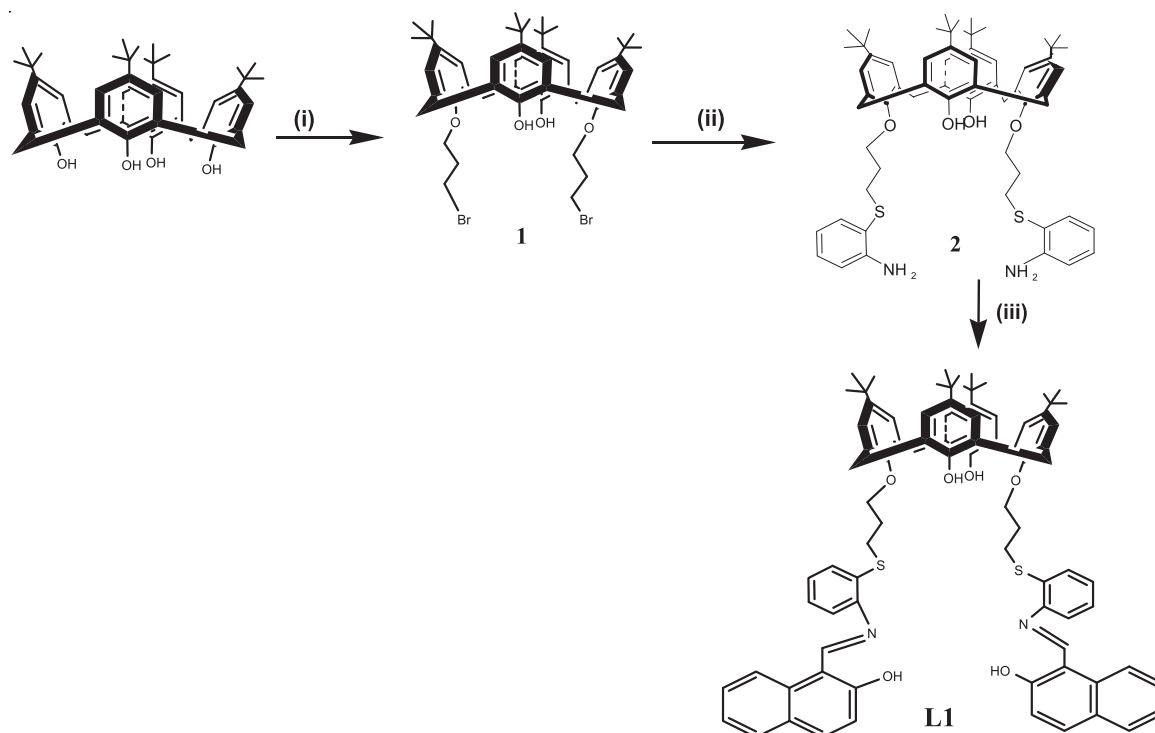
Scheme 1. *Reagents and conditions*: (i) 1,3 dibromopropane, CH$_3$CN, K$_2$CO$_3$, reflux; (ii) NaH, DMF, TBAHSO$_4^-$, o-aminothiophenol, stirring (iii) 2-hydroxynaphthaldehyde; Zn(ClO$_4$)$_2$, CH$_3$CN:CHCl$_3$ (9:1) stirring.

for bio-molecular sensing [30–38]. As part of our interest in developing newer methods for the sensing of ions and bio-molecules. Herein, we report a 2-hydroxy naphthylcalix[4]arene for sequential detection of copper ions by primary interactions and l- histidine by secondary interactions. L- histidine competitively binds with copper ions in the **[L1-Cu^{2+}]** supramolecular complex by forming a more stable complex of Cu^{2+}-L-histidine.

2. Results and discussion

L1 was synthesized in three steps from p-*tert*-butyl calix[4]arene as given in Scheme 1. O-Aminothiophenylcalix[4]arene **2** was prepared by nucleophilic substitution reaction with **1** followed by condensation reaction with 2-hydroxy-naphthaldehyde by using zinc perchlorate as the catalyst to yield **L1**. All the intermediates and **L1** were characterized by ^{1}H NMR, ^{13}C NMR, HRMS and FTIR. In its ^{1}H NMR (CDCl$_3$) spectrum of **L1** exhibited peaks at 15.3 ppm and 9.21 ppm corresponding to hydroxyl and imine protons of naphthyl group and characteristic signals at 4.3 and 3.3 ppm which could be ascribed to ArCH$_2$-Ar protons indicating the cone conformation of obtained calix[4]arene (ESI Fig. 4). In its ^{13}C NMR spectrum the signals at 33.6 ppm were further confirmed the cone conformation of **L1**. It also exhibits strong IR absorptions at υ_{max} 3432, 1623 due to –OH str. and C=N str. Frequencies. HRMS gave *m/z* at 1287.6368 which confirmed the di-substitution of naphthyl moiety at calix[4]arene scaffold (ESI Fig. 6).

3. Studies of receptor L1 towards cation recognition through primary interactions

The colorimetric response of **L1** (25 μM) on addition of different cations was observed when perchlorate salts of different metal ions such as Cu^{2+}, Zn^{2+}, Ag$^+$, Hg^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Li$^+$, Na$^+$, Cs$^+$ in THF were added to the solution of **L1.** It was observed that **L1** showed a specific colorimetric response towards Cu^{2+} ions from other cations present and color get changed from colorless to light yellow in THF (shown in Fig. 2 inset).

The 25 μM solution of receptor **L1** in THF exhibited two maxima at 391 nm and 319 nm respectively with a small hump at 469 nm in the absorption spectrum. After colorimetric response, **L1** (25 μM) was explored by addition of perchlorate salts of cations in UV-vis absorption spectroscopy. Amongst eleven cations in THF (Cu^{2+}, Zn^{2+}, Ag$^+$, Hg^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Li$^+$, Na$^+$, Cs$^+$ as their perchlorate salts) only Cu^{2+} was found to cause bathocromic shifts in the absorption spectrum of **L1** as shown in Fig. 1. On gradual addition of Cu^{2+} in 25 μM solution of receptor **L1**, the absorption band appeared at 390 nm was found to be quenched with concurrent appearance of a new band at 470 nm, latter being responsible for the color change *i.e.* from colorless to light yellow as shown in Fig. 2. The clear isosbestic points at 350 nm and 420 nm indicate the formation of a complex. The stoichiometry of the complex formed between **L1** and Cu^{2+} was found to be 1:1 corroborated by Job's plot. (ESI Fig. 7)

The 25 μM solution of receptors **L1** in THF was excited at 402 nm,
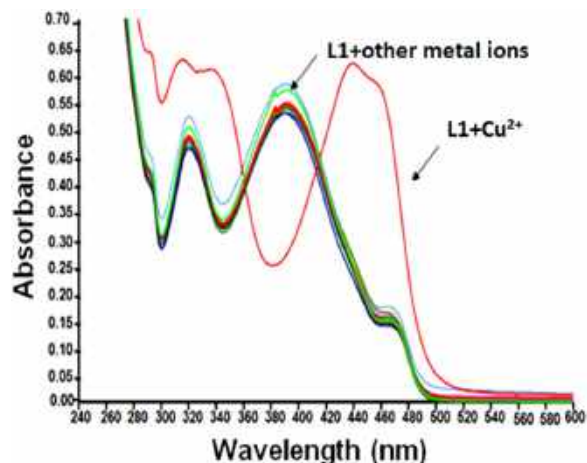
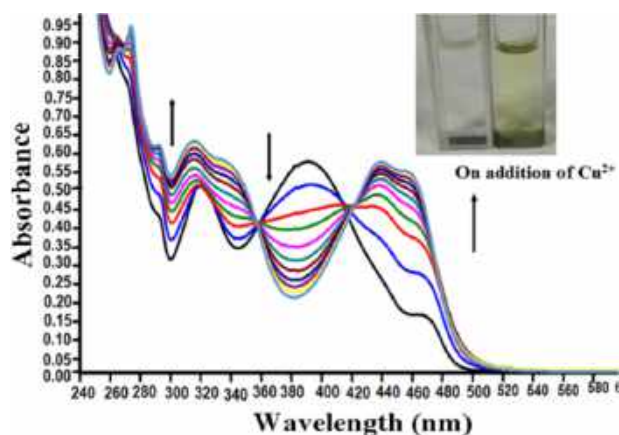**Fig. 1.** UV-vis spectra of **L1** (25 μM) upon addition of various cations (0.045 mM) in THF.

Fig. 2. UV-vis spectra of **L1** (25 μM) on addition of 0–2 equivalents Cu^{2+} in THF. Inset Colorimetric response of receptor **L1** towards copper ions in THF.
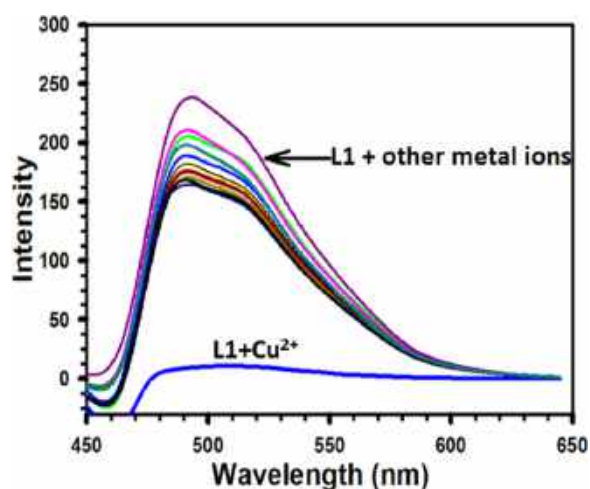

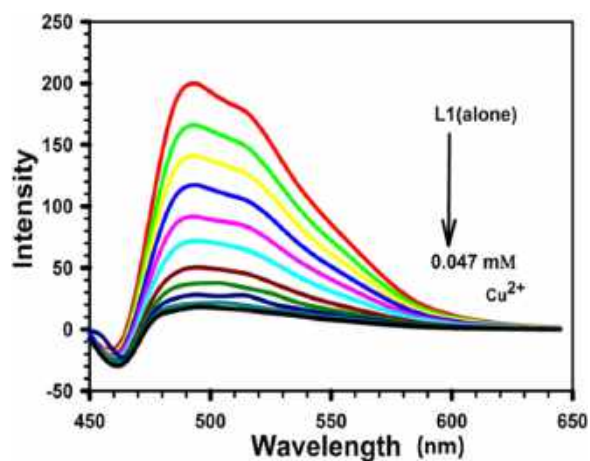**Fig. 3.** Fluorescence spectra of **L1** (25 μM) upon addition of cations (0.047 mM) on excitation at 402 nm in THF.

Fig. 4. Fluorescence spectra of **L1** (25 μM) upon the titration with 0–2 equivalents of Cu^{2+} when excited at 402 nm in THF.

the emission maxima was observed at 492 nm. The receptor **L1** was explored by performing fluorescence titrations with different perchlorate salts of cations. Upon addition of 1.8 equivalents of various cations in 25 μM solution of receptor **L1**, there was no change observed in the emission maxima except with Cu^{2+} ions. It was found that only Cu^{2+} has induced significant change in the emission maxima, where as
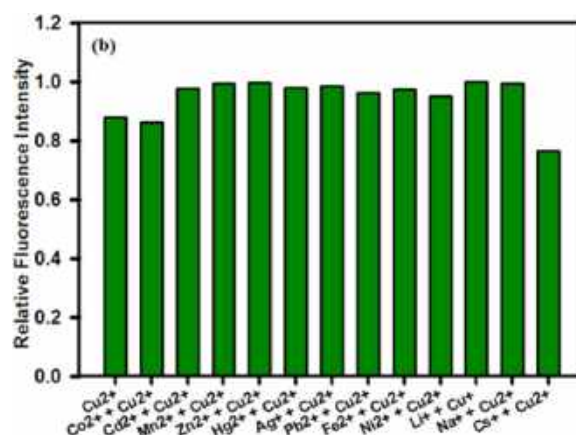

Fig. 5. Fluorescence spectra of **L1** (25 μM) in the presence of various cations (2equivalents) in THF in response to Cu^{2+} ions.

other cations showed negligible change as shown in Fig. 3. On gradual addition of Cu^{2+} (up to 1.8 eq), the emission maxima at 492 nm was quenched by 96 fold (Fig. 4). The binding constant of the receptor **L1** with Cu^{2+} was calculated by Benesi Hildebrand equation and K_a was found to be $3.0 \times 10^4 \, M^{-1}$ (ESI Fig. 8). The minimum concentration at which **L1** can detect Cu^{2+} was found to be 0.80 μM. (ESI Fig. 9) which is sufficiently below the limit of Cu^{2+} in drinking water set by the U. S. Environmental Protection Agency EPA (20 μM).

The selectivity of receptor **L1** towards Cu^{2+} even in the presence of different cations was determined by competitive experiment. When titration of receptor **L1** was carried out with one equivalent of copper ions in combination with same equivalent of other competitive metal ions, a slight change was observed in the emission spectra (Fig. 5). It was found that Cu^{2+} can replace all other biological important cations respectively.

The mass spectrum of receptor **L1** with Cu^{2+} was taken in high resolution mass spectroscopy and the molecular ion peak of free receptor **L1** was found at m/z 1287.6368 (M^+) (as shown in ESI Fig. 6). Titration of **L1** with Cu^{2+} has resulted in a 100% molecular ion peak at m/z 1350.5204 which corresponds to $[\textbf{L1} + \textbf{Cu}^{2+}]$ respectively, suggesting that the receptor **L1** formed a 1:1 complex with Cu^{2+} (shown ESI Fig. 11).

In ^1H NMR spectrum of receptor **L1** exhibited peaks at 9.21 and 15.30 ppm due to imine and hydroxyl protons. On addition of 1 eq of copper perchlorate ions, the peaks disappeared immediately as shown in ESI Fig. 12. These results has clearly indicated that copper ions form supramolecular complex with receptor **L1**.

In the IR spectrum, **L1** exhibited characteristic absorption bands at $3423 \, cm^{-1}$ and $1623 \, cm^{-1}$ which could be assigned to the hydroxyl υ (O-H) imine υ(-C=N-) functionalities respectively. In addition multiple sharp peaks at $2955 \, cm^{-1}$, $1197 \, cm^{-1}$ due to υ(C-$H_{aromatic}$) stretching and υ(-C-N)stretching were also observed in the spectrum. On addition of copper ions the resulted metallo-supramolecular complex $[\textbf{L1-Cu}^{2+}]$ exhibits peaks at $3443 \, cm^{-1}$ and $1631 \, cm^{-1}$ in IR spectrum. These results have indicated that peaks are broadened and shifted towards higher absorption region during complexation with copper ions as shown in ESI Fig. 13.

4. Studies of L1-Cu^{2+} complex towards amino acids through secondary interactions

The metallo-supramolecular complex $[\textbf{L1-Cu}^{2+}]$ was synthesized by mixing of 25 μM of receptor **L1** and 25 μM of Cu^{2+} *in-situ* by 1:1 in dry THF (Fig. 6). The formation of metallo-supramolecular complex has already been characterized above by NMR, UV-Vis, Mass and IR spectroscopy.

When *in-situ* (**L1** + Cu^{2+}) supramolecular complex was excited at
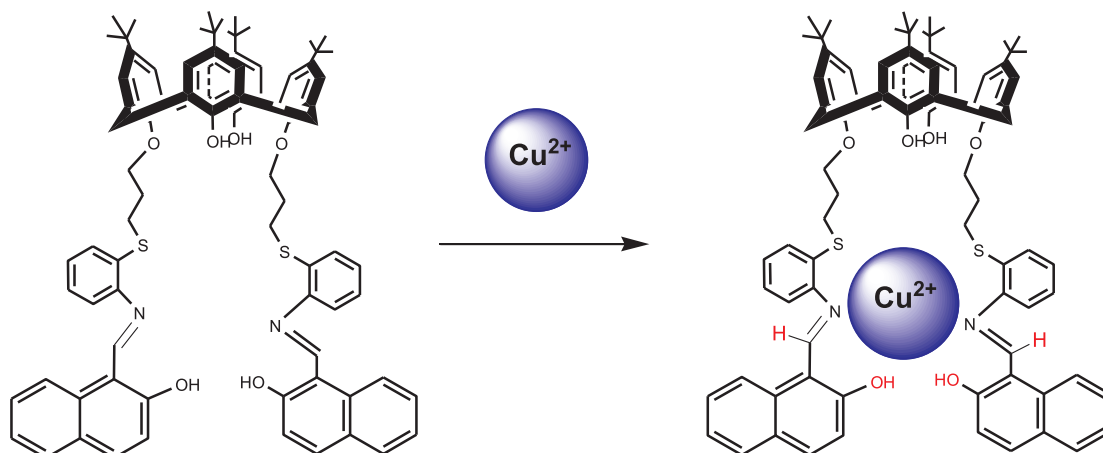
Metallo-Supramolecular complex

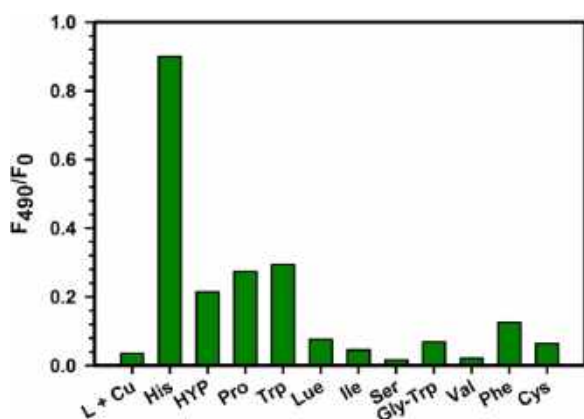**Fig. 6.** In-situ synthesis of metallo-supramolecular complex.

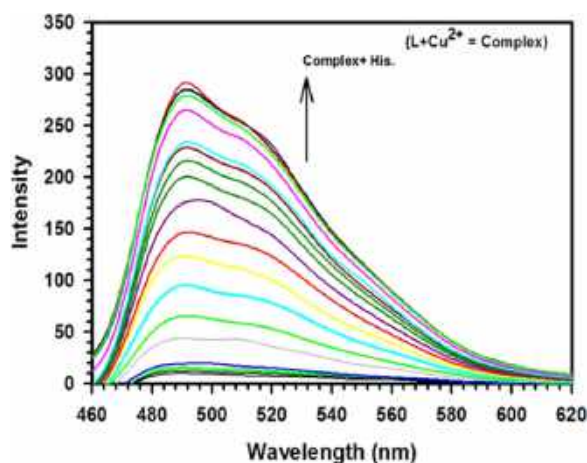**Fig. 7.** Fluorescence spectra of [**L1-Cu^{2+}**] with different amino acids in THF.

Fig. 8. Fluorescence spectra of [**L1-Cu^{2+}**] upon the titration with 0–18 equivalents of L-histidine when excited at 402 nm in THF.

402 nm, the weak emission band occurred in the range of 460–600 nm. This *in-situ* complex was titrated with all the amino acids such as histidine, hydroxy proline, proline, leucine, isoleucine, tryptophan, serine, glycyl tryptophan, valine, phenylalanine, and cysteine. Upon addition of 18 eq of various amino acids there was no such enhancement observed on emission maxima except with L-histidine (Fig. 7). Only marginal increase was observed with tryptophan and proline. It is

evident that only L-histidine induces significant change in emission maxima, where as other amino acids showed negligible variation. On gradual addition of L-histidine (up to 18 eq), the emission maxima at 492 nm was enhanced by 200 folds (Fig. 8), indicating that [**L1-Cu^{2+}**] could be applied as fluorescent OFF-ON sensor for L-histidine. This was exactly the reverse of titration when receptor **L1** was titrated with Cu^{2+}. The copper was removed from the complex by histidine to release free **L1** and thus the recognition of histidine by [**L1 + Cu^{2+}**] act as a secondary sensor.

The selectivity of [**L1 + Cu^{2+}**] towards L-histidine in the presence of different amino acids was studied by performing competitive experiments. When titration of receptor [**L1 + Cu^{2+}**] was carried out with L-histidine in combination with other competitive amino acids, a marginal effect occurred in emission spectra as shown in Fig. 9. This result indicates that [**L1 + Cu^{2+}**] can detect L-histidine with a high selectivity over other coexisting amino acids.

5. Plausible binding mechanism

Two types of mechanism has been suggested for the detection of analyte through metallo-supramolecular complexes [4] 1) by making new complex with metallosupramolecular complex; 2) by releasing metal ions as shown in the Fig. 10. According to first approach, guest moiety does not leave the recognition site on addition of analyte and emission spectrum, shows some new peak which differ from metallo-
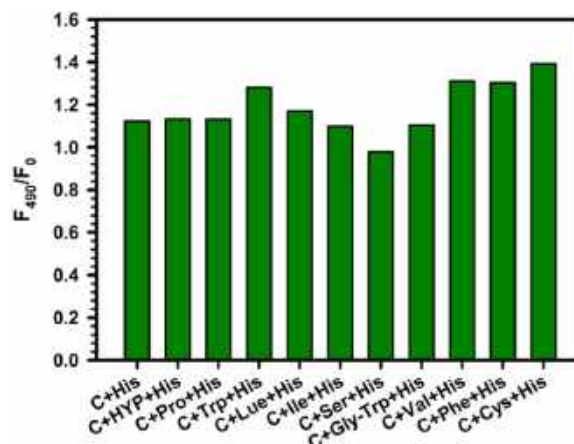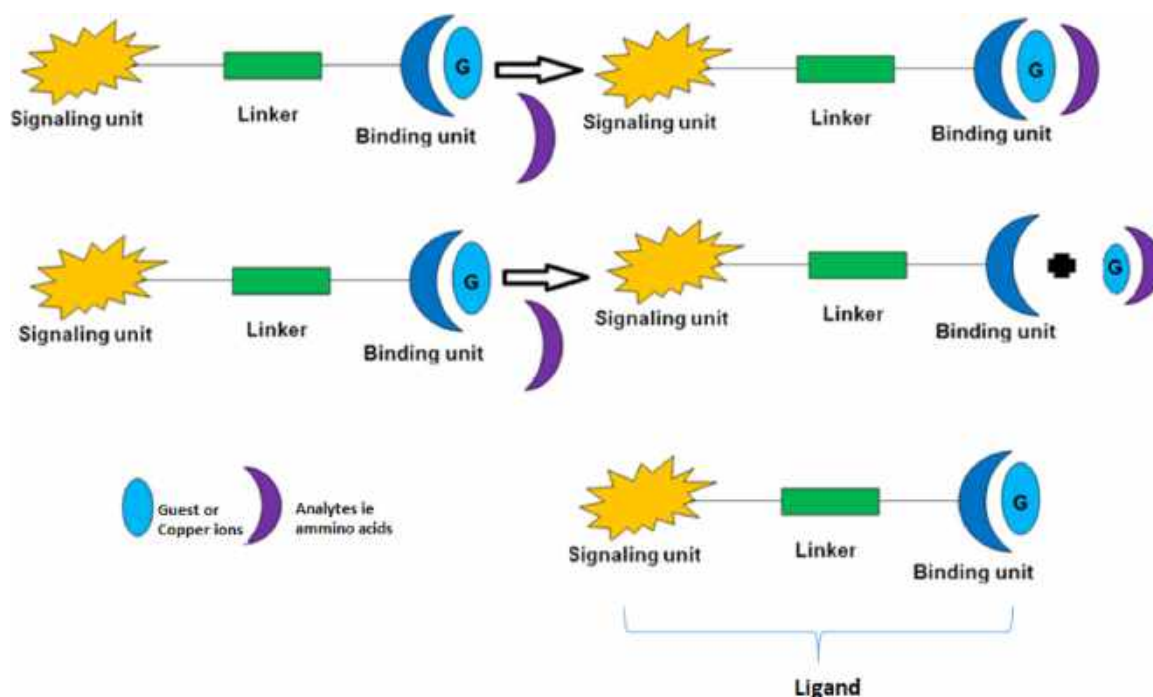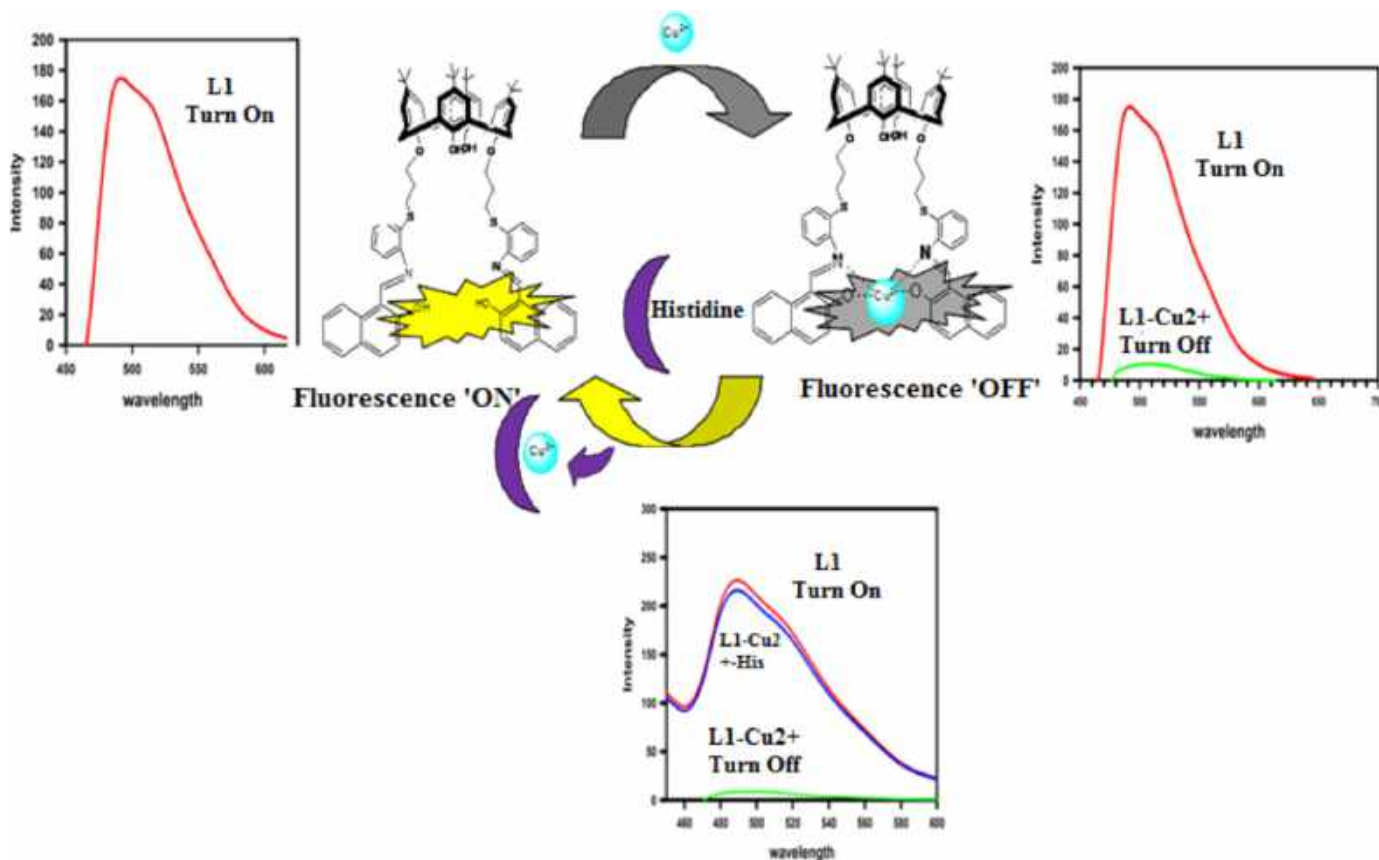

Fig. 9. Fluorescence spectra of [**L1-Cu^{2+}**] in the presence of various amino acids (18 equivalents) in THF in response to L-histidine.

Fig. 10. Two different approaches for the detection of analyte through metallo-supramolecular complex formation.

Fig. 11. Plausible binding mechanism of L-histidine through displacement of copper ions (inset shows emission spectrum of L1 and supramolecular complex).

supramolecular complex. But in second approach analyte has displaced the guest species from supramolecular complex and recover the spectrum of free ligand. In this direction, we explored the mechanism of detection of L-histidine by metallo-supramolecular complex [**L1-Cu²⁺**] by releasing copper ion or by making a new complex by identifying the

changes in emission spectroscopy. When we added copper ions, the fluorescence intensity of receptor **L1** has been quenched and represented as "turn off" sensor. On addition of L-histidine the emission spectrum of receptor **L1-Cu²⁺** has been regained their intensity and represented as "turn on" sensor as shown in Fig. 11. These results have
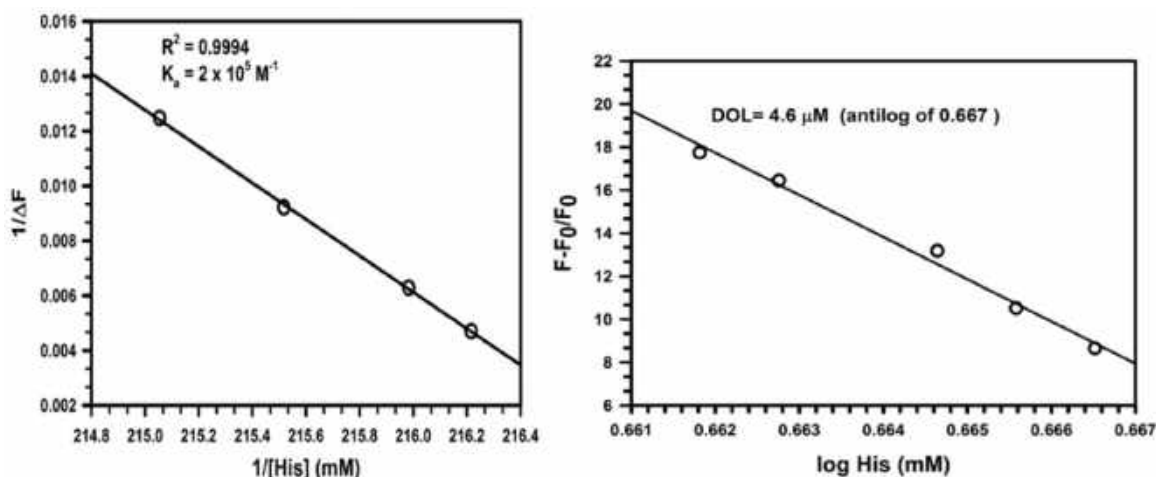
Fig. 12. (a) Benesi-Hildebrand analysis plots of **L1-Cu^{2+}** at different concentration of L-histidine; (b) A plot of F_0-F/F Vs log [His] to calculate detection of limit of **L1-Cu^{2+}** for L-histidine.

clearly indicated that L-histidine snatch copper ions from metallo-supramolecular complex [L1-Cu2 +] and recover the fluorescence intensity which was quenched on addition of Cu^{2+} ions. Therefore receptor **L1** behave as primary sensor for Copper ions and secondary sensor for L-histidine.

This property of fluorescent switch of L1 and L1-Cu^{2+} was successfully applied for quantitative sensing of copper ions and L-histidine. As shown in Fig. 12 the binding constant of receptor [**L1 + Cu^{2+}**] complex with L-histidine was calculated by Benesi Hildebrand equation, and the corresponding association constant K_a was found to be 2.18×10^5 M^{-1}. The minimum concentration at which [**L1 + Cu^{2+}**] could detect L-histidine was found to be 4.6 µM by plotting a graph between F-Fo/Fo vs log [L-histidine] (Fig. 12).

6. Conclusion

In conclusion, we have developed a new 1,3-di(bis-2-hydroxynapthalene) 2-aminothiophenylcalix[4]arene **L1** for highly sensitive and selective detection L-histidine through Supramolecular complex of [**L1-Cu^{2+}**]. Interaction of Cu^{2+} with receptor **L1** quenches the fluorescence emission by 96%. The fluorescence spectrum of [**L1 + Cu^{2+}**] supramolecular complex was able to detect L-histidine exactly in a reverse manner as Cu^{2+} was added in receptor **L1**, suggesting that the release of receptor **L1** from copper complex. This system is simple in design and fast in operation and is more convenient and promising than the other methods. The detection of limit is lower than previous fluorescence method available for detection of L-histidine. Importantly, the protocols offers high selectivity for the detection of L-histidine among other amino acids. Therefore the present receptor **L1** can be utilized as a primary sensor for Cu^{2+} and secondary sensor for L-histidine.

7. Experiment

7.1. General

All the reagents used in the study were purchased from Sigma Aldrich or Merck and were considered chemically pure. The solvents were dried and distilled prior to use. HPLC grade solvents were used for UV and fluorescence measurements. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker DPX 300 instrument using tetramethylsilane (TMS) at 0.00 as an internal standard. Mass spectra were recorded on a Bruker Compass Data Analysis 4.0 Mass spectrometer while IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer in KBr disks. A Perkin Elmer Lambda 35 double beam spectrophotometer with variable band width was used for the acquisition of the uv-vis molecular absorbance. All the data were acquired using 1-cm^2 path length quartz cuvettes. Spectral response from appropriate blanks of reference solutions was subtracted before data analysis. Dilution correction was also carried out whenever found necessary. Melting points were determined on an electrothermal melting point apparatus obtained from Toshcon-Toshniwal (India).

7.2. Synthesis of compound 1

p-tert- Butylcalix[4]arene (2 g, 1 mmol) and K$_2$CO$_3$ (4.25 g, 10 eq) were taken in acetonitrile and 1,3 dibromopropane (1.24 g, 2 mmol) was added. The reaction mixture was refluxed until the starting materials disappeared (observed on TLC). On completion, the reaction mixture was filtered and the solvent was evaporated under reduced pressure. The remaining solid was extracted with chloroform and washed with water twice. The crude product was further re-crystalized by CHCl$_3$/MeOH. % yield 69; ^{1}H NMR spectrum: (300 MHz, CDCl$_3$): δ = 1.00 (s, 18H, -C(CH$_3$)$_3$, 1.25 (s, 18H, -C(CH$_3$)$_3$, 2.50 (m, 4H, -OCH$_2$-CH$_2$-CH$_2$Br), 3.35 (d, 4H, J = 12 Hz, ArCH$_2$Ar), 4.0 (t, 4H, J = 5.6 Hz, -OCH$_2$-CH$_2$-CH$_2$Br), 4.09 (t, 4H, J = 6.5 Hz, -OCH$_2$-CH$_2$CH$_2$Br), 4.26 (d, 4H, J = 12 Hz, ArCH$_2$Ar), 6.86 (s, 4H, ArHcalix), 7.03 (s, 4H, ArHcalix), 7.68 (s, D$_2$O exchangeable, 2H, ArOH). ^{13}C NMR (75 MHz, CDCl$_3$): δ = 29.8, 30.5, 31.2, 31.3, 32.9, 33.5, 72.8, 124.6, 125.1, 127, 132.3, 141.8, 148.4, 150.1. MS-FAB *m/z* for C$_{50}$H$_{66}$Br$_2$O$_4$ is 889.8 [M$^+$].

7.3. Synthesis of compound 2

To a suspension of NaH (0.107 g, 4.48 mmol) in DMF (50 mL) was added o-aminothiophenol (0.42 g, 3.36 mmol) at 0 °C. The reaction mixture was stirred for 15 min and tetrakis-(p-tert-butyl) 26,28-bis (propoxybromo)calix[4]arene (1 g, 1.12 mmol) was added to the reaction mixture. On completion of the reaction, the solvent was removed under reduced pressure and the reaction mixture was extracted with CHCl$_3$. After removal of the solvent, the crude product was subjected to column chromatography by using ethyl acetate/hexane (2:1) as the eluent. The separated compound was than re-crystallized with methanol-water to give thin creamish crystals. % yield = 65, m.p. 75–77 °C, UV (λ$_{max}$, CH$_3$CN): 293 nm, IR (KBr pellet, cm^{-1}): 3343, 2954, 1607, 1497, 1361, 1310, 780; ^{1}H NMR (300 MHz, DMSO-d_6):1.119 (s, 18H, -C(CH$_3$)$_3$), 1.24 (s, 18H, -C(CH$_3$)$_3$), 2.08 (m, 4H, -OCH$_2$CH$_2$CH$_2$), 3.29 (t, 4H, SCH$_2$), 3.43 (d, 4H, ArCH$_2$Ar), 4.02 (t, 4H, J = 6.2 Hz,-OCH$_2$), 4.19 (d, 4H, J = 12.3 Hz, ArCH$_2$Ar), 5.3 (s, 4H, NH$_2$, D$_2$O exchangeable), 6.5 (t, 2H, J = 7.2 Hz,

(aminothiophenol)ArH), 6.7 (d, 2H, $J = 7.8$ Hz, (aminothiophenol) ArH), 7.02 (t, 2H, $J = 7.5$ Hz, (aminothiophenol)ArH), 7.28 (s, 8 H, ArH), 7.3 (d, 2H, $J = 7.2$ Hz, -(aminothiophenol) ArH), 8.57 (s, 2H, -OH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$) δ in ppm): 150.2, 149.1, 148, 146.5, 140.9, 135.5, 132.3, 129.1, 127.2, 125, 124.6, 117.9, 114.4, 73.7, 33.5, 33.3, 31.3, 31.2, 30.5, 29.31; HRMS (ESI-MS) *m/z*: calculated for $C_{62}H_{79}N_2O_4S_2$ is 979.5480, found 979.5476 [M + H]$^+$.

7.4. Synthesis of 1,3-di{bis(2-hydroxynapthalene)}-2-aminothiophenylcalix[4]arene L1

This compound was synthesized by stirring 2-aminothiophenylcalix [4]arene **2** (500 mg, 0.50 mmol) along with 2-hydroxy naphthaldehyde (264 mg, 1.5 mmol) in the presence of 3–4 mg of Zinc perchlorate taken as a catalyst in $CH_3CN:CHCl_3$ (9:1) solvent mixture. The color of the solution immediately changes to yellow. On completion of the reaction, the solvent was evaporated and the crude oily product left was triturated with methanol to yield a yellow colored fine solid precipitate which was filtrated. The precipitate was re-crystallized from methanol/water to give a compound, m.p. 140–145 °C. It exhibits a strong IR absorption at υ max 3432 (-OH str.), 1623 (C=N str.). HRMS gave *m/z* as 1287.6368, calculated for $C_{84}H_{90}O_6N_2S_2$ [M +] is 1287.6313. ^1H NMR (CDCl3) δ 0.967 (s, 18H, -C(CH₃)₃); 1.278 (s, 18H, -C(CH₃)₃); 2.24 (m, 4H, (CH₂CH₂CH₂); 3.25 (d, 4H, ArCH₂Ar, $J = 12.9$ Hz); 3.395 (t, 4H, -SCH₂, $J = 13.8$ Hz); 4.066 (t, 4H, -OCH₂, $J = 11.1$ Hz); 4.3 (d, 4H, ArCH₂Ar, $J = 12.9$ Hz); 6.78 (s, 4H, ArH-calix); 7.005 (s, 4H ArH-calix); 7.04 (d, 2H, ArH-aminothiophenyl); 7.197 (bm,4H, ArH-naphthalene); 7.29 (t, 2H, ArH-aminothiophenyl); 7.441 (t, 2H, ArH-aminothiophenyl); 7.52 (d,4H,ArH-naphthalene); 7.63 (d, 2H, ArH-naphthalene); 7.72 (d, 2H, ArH-naphthalene); 7.9 (d, 2H, ArH-naphthalene, $J = 8.7$ Hz); 8.849 (s, 2H, -OH-calix, D_2O exchangeable); 9.21 (s, 2H, CH=N); 15.31 (s, 2H, -OH-naphthalene, D_2O exchangeable) ^{13}C NMR data is δ 29.46, 30.81, 30.98, 3.64, 33.76, 33.93, 74.40; 76.54, 76.97, 77.39, 109.10, 117.34, 119.07,121.82, 123.39, 125.04, 125.50, 126.82, 127.32, 127.65, 127.85, 129.21, 129.86, 132,74, 136.31, 141.53, 149.47, 150.52, 154.27.

7.5. UV-vis and fluorescence experiments

The spectroscopic grade THF was used as the solvent for titration experiments which were carried out in 10 mM quartz cuvettes at 25 °C. The molecular receptors **L1** was dissolved in THF to obtain a concentration of 25 μM stock solution, cations as their perchlorate salts (1 mM) in THF were added to the 25 μM host solution and used for the titration experiments.

7.6. Amino acids experiments

The spectroscopic grade THF was used as the solvent for titration experiment. The amino acids were dissolved in 500 μl of water and diluted with THF to obtain a concentration of 10 mM. The solution used in these titrations were prepared *in-situ* by mixing **L1** (25 μM) and Cu^{2+} in a 1:1 ratio.

Acknowledgement

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.jlumin.2018.06.056.

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A new metallo-supramolecular sensor for recognition of sulfide ions

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Har Mohindra Chawla *, Preeti Goel, Priyanka Munjal

Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, India

A B S T R A C T

A hydroxynaphthyl hydrazine linked calix[4]arene derivative (**4**) has been designed, synthesized and evaluated for multi ion recognition. **4** not only recognizes copper ions selectively through a change in color, UV–Vis and fluorescence spectrum but the resultant metallo-supramolecular complex (**4**·Cu^{2+}) emulates specific recognition of S^{2-} ions as well.

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During recent years, there has been an upsurge in research activity in the design, synthesis and evaluation of molecular receptors for anions.[1] This is partly due to the academic challenges involved and the utility of anion recognition in numerous biological, chemical and environmental processes.[2] Simple anions like fluoride, cyanide, sulfide, arsenate, phosphate, nitrate, chloride, sulfate, chlorate, and chromate are often considered as critical anions due to their importance in drinking water and consequent onset of diseases when they are present in higher concentrations.[3] The studies are also very useful for effluent treatments and in ensuring a green environment. Besides mentioned inorganic anions, molecular receptors are also being investigated for the recognition of organic anions like citrate, tartrate and aliphatic and aromatic carboxylates.[4]

Major difficulties encountered in the design of molecular receptors for anions[5] are due to their larger size and charge spread in comparison to those in cations. Their shape (which may vary from linear to octahedral), smaller window for manipulation of pH of the medium as well as stiff competition from electron donor solvents further complicate investigations on receptors for anions. The approaches used for the design of target molecular receptors have been based upon complementarity of charge, shape, size or through the electron deficient nature of the functional group appendages. In this connection, the use of metal ions with expandable valence shells (e.g., use of ferrocene, boron trifluoride residues attached to the organic molecular scaffolds)[6] have proven useful

for recognition of anions. Recently secondary supramolecular forces have been deployed for anion recognition.[7,8,19]

Molecular scaffolds generally employed for the target purpose are hydrophobic cavity containing organic macrocycles or pseudo molecular macrocycles such as calixarenes,[10] resorcinarenes,[11] cyclodextrins,[12] steroids,[13] molecular tweezers,[14] and clefts[15] which provide templates for assembling efficient platforms for anion recognition.

In the family of active anions, sulfide is known to strongly interfere in numerous biological processes. Continuous exposure to sulfide can cause gradual and cumulative damage that include loss of consciousness, irritation of mucous membranes, and suffocation.[16,17] Once protonated, sulfide ions become more toxic and caustic. Some of the strategies evolved for recognition of S^{2-} at low concentrations include spectroscopic and electrochemical titrations,[18] metal anion affinity,[7–9,19] ion chromatography,[20] and chemo luminescence measurements.[21] In this Letter we have focused our attention to the design of reversible sensors for sulfide ions by using its known affinity for copper ions to make a stable copper sulfide complex.

The synthesis and evaluation of a molecular Schiff base molecular probe **4** reveals that it is not only capable of detecting copper ion but the formed supramolecular metalloreceptor **4·Cu^{2+}** could be further used for selective and specific detection of sulfide ions from among various related anions via colorimetric and fluorescence protocols.

Calix[4]arene with a naphthalene moiety attached at the lower rim was synthesized by adopting a reaction sequence given in Scheme 1.[22,23] Bis(4-formylphenylpropyloxy)-*p-tert*-butylcalix[4]arene (**3**) was prepared by the method reported earlier.[24] It was

* Corresponding author. Tel.: +91 11 26591517; fax: +91 11 26581 102.
  E-mail addresses: hmchawla@chemistry.iitd.ac.in, hmchawla@chemistry.iitd.ernet.in (H.M. Chawla).
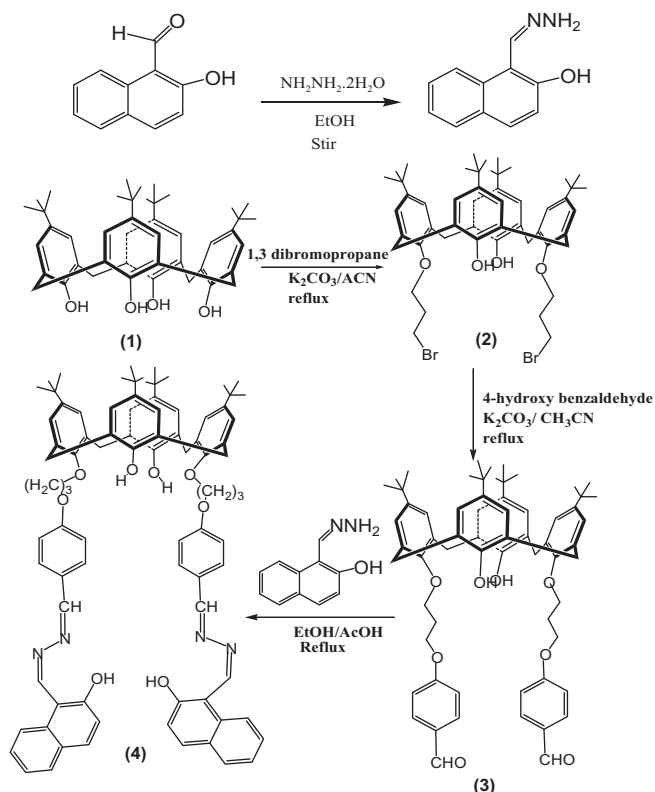
Scheme 1. Synthesis of molecular receptor **4**.

refluxed with 2-hydroxy-1-naphthaldehyde hydrazone in the presence of glacial acetic acid in ethanol to yield a product which when washed with methanol gave a novel calixarene derivative **4** in good yield (83%). The identity of all the intermediates and the target molecular receptor **4** was established by IR, ^{1}H and ^{13}C NMR as well as by ESI-MS analysis (ESI, Fig. S1). The synthesized molecular receptor **4** showed a $>$C=N— signal at 1603 cm^{-1} and a sharp pair of doublets at a δ 3.36 and δ 4.29 for axial and equatorial protons, respectively, in the ^{1}H NMR spectrum. A distinct signal at δ 31.02 for the methylene carbons in its ^{13}C NMR spectrum revealed its symmetric cone conformation for the calix[4]arene scaffold. It was further confirmed by observing D$_2$O exchangeable singlets at δ 13.03 which could be assigned to the –OH protons. Non deuterable singlets at δ 9.69 and 8.29 for the azo-methine proton (–N=CH) confirmed the depicted structure for **4** (Scheme 1).

The sensing ability of naphthalene based molecular receptor **4** for cations was examined in CH$_3$CN by naked eye color change, UV–Vis absorption and fluorescence spectroscopy.

On gradual addition of metal cations such as Li$^+$, Na$^+$, K$^+$, Cs$^+$, Ag$^+$, Ca^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+}, Fe^{2+}, Ni^{2+}, Zn^{2+}, and Cu^{2+} as their perchlorate salts in CH$_3$CN, a color change from light yellow to dark yellow was observed only in the presence of copper ions. ESI-MS of the **4** showed a molecular–ion peak [M+Na]$^+$ at *m/z* 1331.5334 and at *m/z* 1371.7741 (ESI, Fig. S5) due to the presence of [**4**+Cu^{2+}]. Other metal ions did not produce any noticeable change in color even on adding higher concentrations.

Following our observations based on color change, cation sensing ability of receptor **4** in CH$_3$CN was further monitored by UV–Vis absorption spectroscopy. UV–Vis spectra of **4** was characterized by two peaks (337 nm and 405 nm) with $\varepsilon = 6.4 \times 10^4$ M^{-1} cm^{-1}. Addition of different cations as perchlorates, led to no change in the absorption spectra of **4** except copper perchlorate.

On addition of Cu^{2+} ions, there was considerable change in the absorption spectra of synthesized calixarene derivative. To further

comprehend the Cu^{2+} binding nature of **4**, quantitative UV–Vis titration of **4** (20 µM) was carried out with increasing equivalents of Cu^{2+} ions (0–1.45 equiv). It was determined that gradual addition of Cu^{2+} ions to the receptor **4** led to a decrease and a shift in absorbance at 405 nm to 430 nm (Fig. 1) accompanied by a color change of the solution of receptor.

Fluorescence emission spectra of **4** (20 µm) in CH$_3$CN was recorded after excitation at 337 nm. It was determined that on addition of Cu^{2+} ions a prompt change in the intensity of fluorescence emission of **4** was observed to yield more than 90% quenching of the emission maxima at 505 nm. To learn more about the properties of **4**–Cu^{2+} complex formation, a titration of the receptor was performed with increasing concentration of Cu^{2+} ions. The fluorescence intensity of a 20 µM solution of **4** decreased with an increase in the concentration of copper perchlorate to reach saturation levels after addition of 1.5 equiv of Cu^{2+} ions (Fig. 2). Though analysis of the vibronic spectrum is outside the scope of this study, it appears that the fluorophore gets excited to its S$_1$ state in the absence of metal ions to result in higher intensity emission band due to S$_1 \rightarrow$ S$_0$ state. However in the presence of copper ions, specific complexation leads to intersystem cross over from S$_1$ to T$_1$ state to usher a much decreased intensity of fluorescence and deactivation by bimolecular non radiative processes. Analysis of the infrared spectrum of the compound and its complex with Cu^{2+} reveals that a significant shift (26 cm^{-1}) in ν(OH) of the
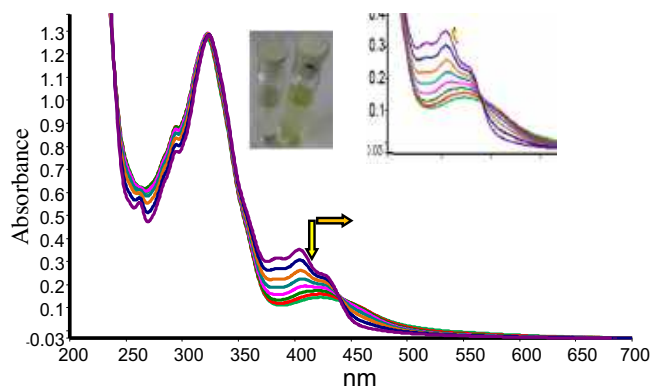
Figure 1. Change in the UV–Vis spectra of **4** (20 µM) upon addition of Cu^{2+} (0–28 µM) in CH$_3$CN; inset: color change upon addition of Cu^{2+} to the solution of molecular receptor **4**.
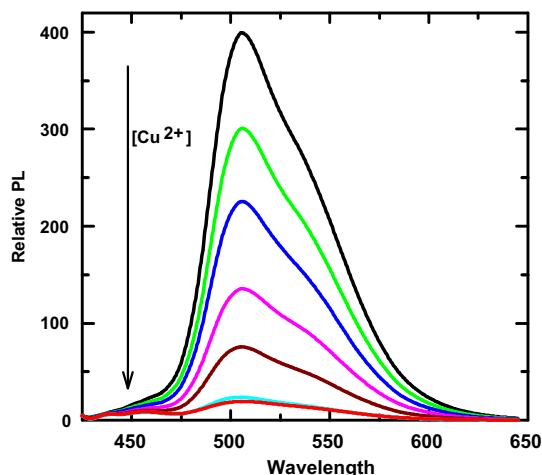
Figure 2. Quenching in the fluorescence intensity of **4** (20 µM) in CH$_3$CN in the presence of Cu^{2+} (0–29 µM).

naphthalene moiety (3436–3410 cm^{-1}), may be due to its interaction with copper ions which weakens the OH bonds. These conclusions are in consonance with literature precedents.

The influence of other metal ions on the fluorescence signal of the molecular probe was evaluated. It was observed that the addition of other metal ions did not elicit any change in the fluorescence intensity of the molecular receptor, thereby revealing its high selectivity for Cu^{2+} ion (ESI, Fig. S2). In addition, presence of various competing metal ions hardly had any effect in the detection of copper ions (Fig. 3). All these observations suggest that it is a useful probe for recognition of Cu^{2+} ions with high selectivity.

Job's plot revealed the stoichiometry between **4** and Cu^{2+} ions as 1:1 (ESI, Fig. S3). Based on 1:1 binding mode, the binding constant[25] was calculated to be 5×10^5 M^{-1} from the UV–Vis titration data.

The optical properties of the supramolecular complex, **4**–Cu^{2+} were further explored to determine possible selective detection of anions. It was observed that from among different anions such as F$^-$, Cl$^-$, Br$^-$, I$^-$, S^{2-}, H$_2$PO$_4^-$, HSO$_4^-$· and AcO$^-$ (added as their tetrabutylammonium salts) only addition of S^{2-} to the solution containing **4**–Cu^{2+} complex gave a discernible spectral change (Fig. 4). Moreover, the original color of **4** was also restored only on addition of S^{2-}.

Fluorescence titration experiments on **4**–Cu^{2+} complex with different amounts of S^{2-} was performed (Fig. 5) to reveal that the **4**–Cu^{2+} displayed a stepwise fluorescence enhancement on

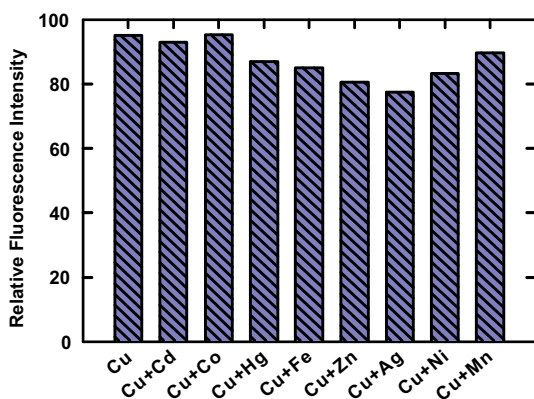**Figure 5.** Changes in fluorescence spectrum of **4**–Cu^{2+} solution (48 μM) upon addition of S^{2-} (0–145 μM).

addition of 145 μM of sulfide ions. Both intensity and shape of the initial emission spectrum of compound **4** could be restored probably due to S^{2-} induced Cu^{2+} sequestering. These results showed that **4**–Cu^{2+} has a good sensing property for S^{2-}. No difference in the fluorescence intensity was observed in our experiments when other anions were added.

The competitive experiments were performed by adding sulfide and equimolar concentrations of other anions (F$^-$, Cl$^-$, Br$^-$, I$^-$, AcO$^-$, HSO$_4^-$ and H$_2$PO$_4^-$) to **4**·Cu^{2+}. It was observed that different coexisting anions have almost no influence on the fluorescence intensity and detection of S^{2-} was very specific (Fig. 6).

The mass spectrum of the **4**–Cu^{2+} system was studied in the presence of S^{2-} gave a peak at *m/z* 1347.8261. This could be attributed to free **4** [M+K]$^+$ thereby establishing that sulfide anion is sensed through copper extraction from the **4**·Cu^{2+} complex.

It was also observed that the fluorescence intensity at 505 nm has an excellent linear relationship with the concentration of S^{2-} ion up to 67 μM. The LOD for sulfide ions could be calculated as 1.58 μM (ESI, Fig. S4) by using LOD$_{[S^{2-}]}$ = 3 × s_o/*m* where s_o represents the standard deviation and *m* represents the slope of relative fluorescence intensity versus [S^{2-}] in the aforementioned range.[26]

These results reveal that **4**–Cu^{2+} complex may be used as the platform for selective sensing of S^{2-}. It appears that stability of Cu^{2+} ion inside the **4**–Cu^{2+} complex can in principle be maintained in such a way that the disruption of the supramolecular complex

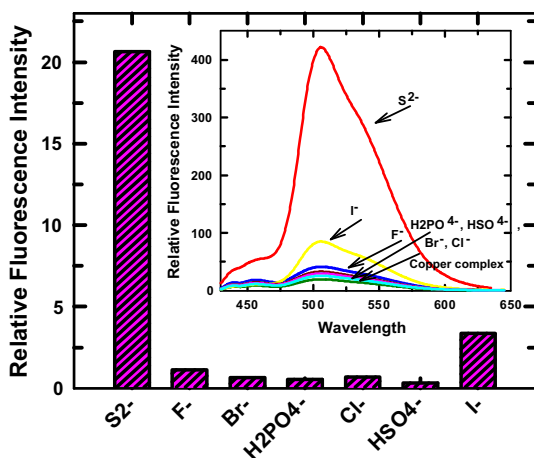**Figure 3.** Comparative study of molecular receptor **4** in CH$_3$CN in presence of 3.5 equiv of various metal ions.

Figure 4. Relative change in the fluorescence intensity upon addition of various anions to the solution of **4**–Cu^{2+} (48 μM) in CH$_3$CN.

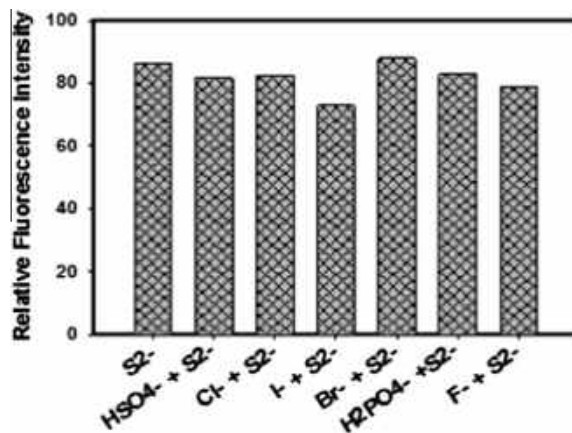**Figure 6.** Selectivity of **4**·Cu^{2+} for S^{2-} over other anions.

takes place selectively and exclusively in the presence of sulfide ions.

In summary we have achieved a copper ion responsive molecular probe that can be deployed for sensitive and selective recognition of sulfide ions through absorption spectral and fluorescence measurements.

Acknowledgements

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.12.057.

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22. *Procedure for the synthesis of* **4**: To a solution of calix[4]arene derivative (**3**) in ethanol was added 2-hydroxy napthaldehyde hydrazone and a catalytic amount of acetic acid. The reaction mixture was refluxed for 24 h. After completion of the reaction (TLC), the precipitate was filtered and washed with water. The structure of **4** was confirmed by ^{1}H NMR and ^{13}C NMR spectra as well as ESI-MS analysis.
23. *Analytical data for* **4**: Light yellow solid; Melting point: 324 °C (decomposed); UV (λ_{max}, ACN): 337 nm. IR (KBr pellet, cm^{-1}): 3436, 1678, 1603, 752;^{1}H NMR (300 MHz, CDCl$_3$, δ in ppm): 13.03 (s, 2H, D$_2$O exchangeable, OH),9.69 (s, 2H, CH=N), 8.29 (s, 2H, N = CH), 8.20 (d, 2H, ArH_{nap}), 7.91 (d, 2H, ArH_{nap}), 7.82 (d, 2H, ArH_{nap}), 7.73 (t, 2H, ArH_{nap}), 7.42 (t, 2H, ArH_{nap}), 7.62 (d, 4H, ArH), 6.87 (d, 4H, ArH), 7.08 (s, 2H, ArH), 6.91 (d+s, 6H, ArH), 4.31 (t, 4H, −OCH_2−CH$_2$−CH$_2$−), 4.24 (d, 4H, ArCH_2Ar), 4.13 (t, 4H, −OCH$_2$−CH$_2$−CH$_2$−), 3.38 (d, 4H, ArCH_2Ar), 2.36 (qu, 4H, −OCH$_2$−CH_2−CH$_2$), 1.27 (s, 18H, −C(CH_3)$_3$), 1.04 (s, 18H, −C(CH_3)$_3$); ^{13}C NMR (75 MHz, DMSO-d_6, δ in ppm): 161.06, 160.80, 150.69, 149.42, 147.18, 141.67, 137.86, 135.07, 132.80, 130.43, 128.25, 127.71, 125.68, 12.88, 120.03, 119.16, 72.92, 64.86, 34.02, 33.83, 31.05; HRMS (ESI-MS) *m/z*: calcd 1331.5384, found 1331.5334 (M+Na$^+$).
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