Chapter-1

Introduction and review of literature

Abstract

This chapter deals with a brief introduction of the supramolecular chemistry and review of literature about different fluorescent probes based on various scaffolds like rhodamine/fluorescein, (thia)calix[4]arene, naphthalimide, dansyl, anthraquinone, coumarin and BODIPY for the detection of various types of biological and environmental important analytes such as Fe^{3+}, Zn^{2+}, CN^- and H_2S. The objectives of present work are defined on the basis of these reports.

1.1 Introduction and review of literature

J. M. Lehn defined a term supramolecular chemistry[1] as the "chemistry beyond molecule" which focuses on chemical systems formed by association of two or more species held together by weak and non-covalent interactions such as dispersion interactions, electrostatic interactions, hydrogen bonding and solvophobic effect.[2] Supramolecular chemistry is recognised as emergent field of reseach according to which this chemistry is shown to have top-down or bottom-up emergences. The top-down emergence is attributed to scope due to implication of supramolecular science and the bottom-up is related to hierarchy, opening the world of nanochemistry and nanomaterials. Both of these emergences constitute supramolecular chemistry as supramolecular science. Earlier inspiration of constructing supramolecular species comes from the natural molecules like lipid bilayers, proteins, oligonucleotides and DNA double helix.[3] During last few decades, there has been significant development in the field of supramolecular chemistry including molecular recognition, self-organisation, ensembles, molecular devices, medicine, catalysis, green chemistry and nanochemistry.[4] Out all of these, molecular recognition is the key component which aims at the design and synthesis of molecular receptors which mimic nature's specific interactions towards various guest species by non-covalent interactions.[5] Enzymes, receptors, antibodies, cells, membranes, carriers and channels are some of the biological examples depending upon the molecular recognition chemistry.[6] Taking into account the importance of natural receptors in biology, medicine, chemistry and environmental studies, a variety of synthetic organic receptors like crown ethers,[7] spherands,[8] cryptands,[9] porphyrins,[10] calixarenes,[11] and thiacalixarenes[12] have been developed which are used as molecular receptors. For a molecular host to be effective, the synthesis of its basic molecular scaffold should be easy which undergoes chemical modification with desired recognition behaviour. The self-assembly,[13] template synthesis[14] and self-sorting[15] are some of concepts which made supramolecular synthesis a powerful technique to build up large and complex architecture from simple building blocks having well-designed binding sites. Some of functional supramolecules like supramolecular catalysts, molecular elevators, valves, springs, molecular switches and molecular logic gates were developed based upon these concepts.[16] Some of the methods like flame photometry, atomic absorption spectroscopy and ion selective electrodes have been used to sense various analytes.[17] However, expensive instruments and large amount of sample is required in

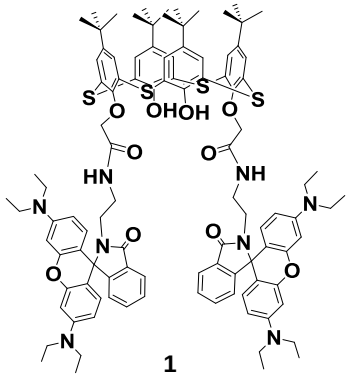
these methods which allow no continuous monitoring whereas fluorescence spectroscopy methods have advantages owing to their sensitivity, selectivity and real time detection with fast response time along with the sensing of biological important species *in vitro* and *in vivo*.[18] A fluorescent sensing system usually comprises of two integrated components i.e. ionophore and fluorophore, which can be independent or covalently linked in one molecule.[19] The binding of an ionophore with guest species changes the photophysical characteristics of fluorophore *via* different mechanisms and such change gives a signal whether by fluorescence quenching or enhancement which indicates the binding of guest species. The designing of fluorescent probes involves two approaches: classical and competitive approach. In classical approach, fluorophore is covalent linked to the receptor and interaction of guest species to the receptor induces the change in fluorescence of fluorophore.[20] Another approach is competitive approach termed as chemosensing ensemble method which involves the dissociation of fluorophore-receptor ensemble upon the addition of suitable competitive guest species which is able to interact selectively with the receptor resulting in the change in fluorescence of fluorophore.[21] Thus, the designing and development of fluorescent probes which selectively recognise biologically and environmentally important species has increased the interest from both the supramolecular chemisty and analytical point of view.[22]

Thus, keeping in view the significance of fluorescent probes in supramolecular chemistry, in the present investigation we have designed and synthesized fluorescent receptors based on thiacalix[4]arene and rhodamine/fluorescein as basic molecular scaffolds and studied their recognition behaviour toward different analytes such as Fe^{3+}, Zn^{2+}, CN^- and H_2S. Our approach involves the use of (1) rhodamine scaffolds having imine linkages for the selective sensing of Fe^{3+} ions; (2) incorporation of different types of ligating sites and fluorogenic moieties on thiacalix[4]arene scaffold for the desired recognition/logic behaviour; (3) different combinations of fluorescein appended fluorophore to develop H_2S selective fluorescent probes. The results of our findings have been divided into five chapters. Before proceeding to our results, a brief review of literature about rhodamine based chemosensors for the recognition of Fe^{3+}, thiacalix[4]arene based fluorogenic sensors for the detection of Zn^{2+}, fluorescent chemosensors for the sensing of CN^- and fluorescent probes for H_2S detection is discussed below.

1.2 Rhodamine based chemosensors for the selective recognition of Fe^{3+} ions

Among the numerous classes of fluorescent dyes, rhodamine dyes have been extensively employed in the designing of fluorescent chemosensors owing to their excellent photophysical properties such as high absorption coefficient, high photostability, high fluorescence quantum yield and relatively long emission wavelength.[23] Basically, rhodamine in its closed spirolactam form is colorless and non-fluorescent whereas its ring-opened form generates pink color along with the strong fluorescence emission.[24] In general, rhodamine spirolactam undergoes red color change with strong fluorescence emission in acidic medium by activation of carbonyl group in spirolactam form. In a similar manner, appropriate ligand on rhodamine spirolactam can cause color as well as fluorescence change in the presence of metal ions. A great interest in the development of chemosensors based on spiro-ring opening of rhodamine has been witnessed in recent years and a large number of chemosensors based on rhodamine have been reported in the literature for the sensing of Fe^{3+} ions. A brief review on rhodamine based chemosensors for the selective recognition of Fe^{3+} ions is discussed below:

Yamato and coworkers reported a rhodamine based thiacalix[4]arene chemosensor **1** which showed fluorescence enhancement in presence of Fe^{3+} and Cr^{3+} ions in the aqueous ethanolic



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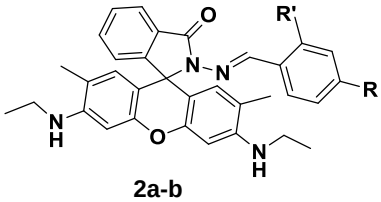
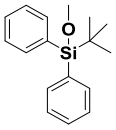
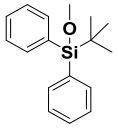


2a-b

2a: R'= H, R=



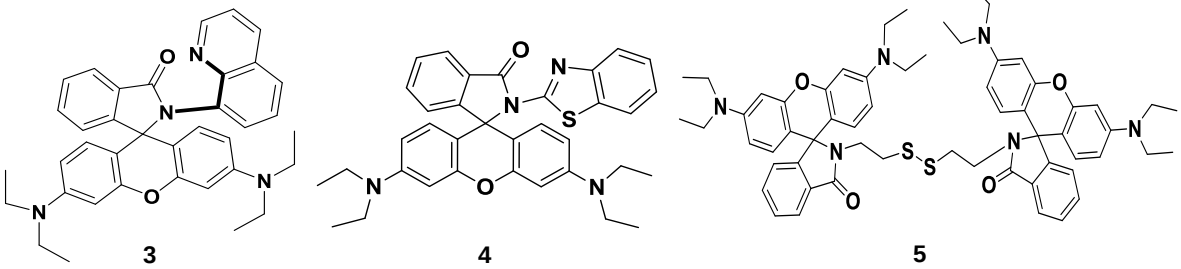
2b: R'= R=



solution with high sensitivity ascribed to the reversible spiro-ring opening mechanism of rhodamine moiety in the presence of Fe^{3+} and Cr^{3+}.[25] 1:1 stoichiometry was observed for the binding of Fe^{3+} or Cr^{3+} with receptor **1**. The response with Fe^{3+} or Cr^{3+} was unaffected by the addition of other interfering metal ions. The receptor **1** was also used for biomedical and environmental applications.

Bhattacharya and coworkers synthesized rhodamine based compounds **2a-b** bearing O-silyl protected mono- and di-hydoxy benzaldehyde groups for ppb level monitoring of Fe^{3+} in aqueous medium at physiological pH = 7.4.[26] The presence of Hg^{2+} induced the "turn-on" fluorescence response due to the spirolactam ring opening of rhodamine resulting in generation of intense pink colour with bright green fluorescence emission. The cleavage of O-silyl bond occurred upon the addition of F^- ions to the solutions of probes **2a-b** which resulted in deep yellow colour with yellow fluorescence emission. The initial chemodosimetric reaction of probes **2a-b** with F^- ions did not affect the detection of Hg^{2+}. The stoichiometries of interaction of F^- ions for **2a** and **2b** were found to be 1:1 and 2:1 which clearly indicates the presence of one and two silyl protecting groups in **2a** and **2b**, respectively. Two different mechanisms of interactions were operated which were confirmed from ^1H NMR, mass spectrometry and IR studies. Furthermore, the probes were practically utilized for the estimation of fluoride content in toothpastes and for the detection of Hg^{2+} ions in real life water samples without any potential interference.

Qian and coworkers reported Fe^{3+} responsive rhodamine based fluorescent sensor **3**.[27] The addition of 0.5 equiv of Fe^{3+} ions resulted in the 50-folds emission enhancement in CH_3CN. The 2:1 binding mode of **3**:Fe^{3+} was proposed from Job's plot as well as 1D and 2D COSY H-H experiments. The FEF (Fluorescenct Enhancement Factors) of 50-folds was achieved at 590 nm



3 **4** **5**

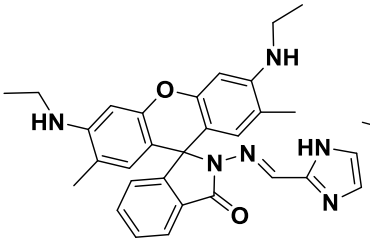
upon the addition of 60 equiv of Fe^{3+} ions in CH_3CN-HEPES (1:1, v/v) solution and the limit of detection was found to be 3.2×10^{-7} M in MeCN. Fluorescent microscopic imaging experiments demonstrated that the **3** could be successfully used as bioimaging agent for monitoring Fe^{3+} in living cells.

Yin *et al.* developed a fluorescent chemosensor **4** based on rhodamine-aminobenzothiazole conjugate which displayed strong Fe^{3+} selective orange fluorescence with a pink colour switch in

methanol.[28] The addition of 1.2 equiv of Fe^{3+} in methanol induced 193-folds fluorescence enhancement at 580 nm. The decrease in fluorescence upon addition of ethylenediamine to the mixture of **4** and Fe^{3+} methanolic solution implied the reversible binding between **4** and Fe^{3+}. DFT calculation method was used to demonstrate the binding mechanism of **4** for metal ions.

Li and Li *et al.* synthesized rhodamine based compound **5** as off-on chemosensor for Fe^{3+}.[29] The addition of 20 equiv. of Fe^{3+} increases the fluorescence intensity by 60-folds. The fluorescence respose of chemosensor toward Fe^{3+} was fast (less than 2 min) and pH independent in neutral condition. The fluorescence changes are specific toward Fe^{3+} ions in the presence of other competitive ions which made it to be used for biomedical applications. Moreover, living cell imaging experiment was carried out to monitor Fe^{3+} in living cells.

Chellappa *et al.* synthesized rhodamine based chemosensors **6** and **7** by incorporating 2-formyl imidazole units to rhodamine 6G fluorophores *via* imine linkages.[30] Both of the probes exhibited high sensitivity and selectivity toward Fe^{3+} ions in aqueous solution. The binding of



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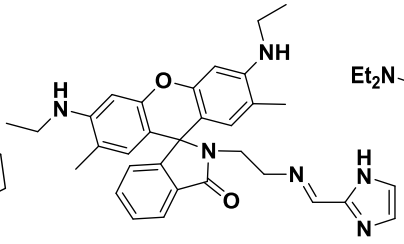

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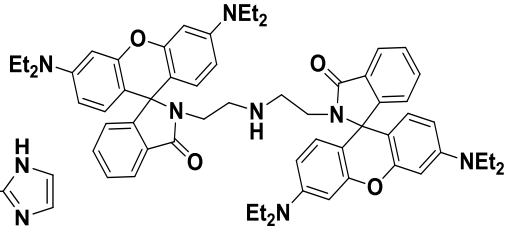

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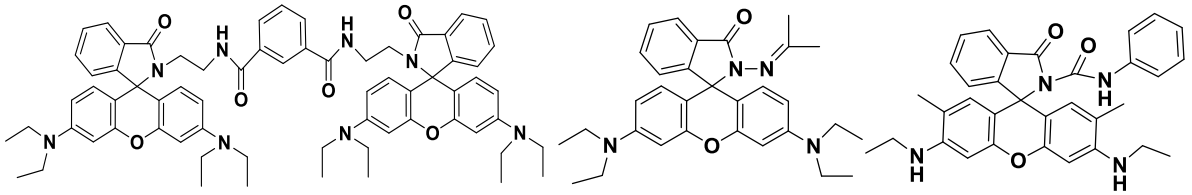
Fe^{3+} showed turn-on fluorescence behaviour with color change from colorless to pink. 1:1 binding mode was proposed from the Job's plot and ESI-MS studies. Confocal fluorescence imaging studies showed that these probes are widely applicable for *in-vivo* imaging of Fe^{3+}.

Tong and coworkers developed a fluorescent chemosensor **8** using rhodmine as a fluorophore for the selective detection of Fe^{3+} ions over other common coexistent metal ions in ethanol and Tris-HCl buffer (pH = 7.15).[31] The moderate binding of receptor **8** towards Fe^{3+} in aqueous media was the main limitation which hindered its usefulness in biochemical applications. 1:1 stoichiometry was observed for the binding of Fe^{3+} to probe **8**. The space effect of large rhodamine units in probe **8** restricts the 1:2 binding mode between Fe^{3+} and **8**.

Chen *et al.* synthesized bis(rhodamine)-based fluorescent probe **9** which exhibited high selectivity for the detection of Fe^{3+} over other commonly coexistent metal ions in ethanol-water

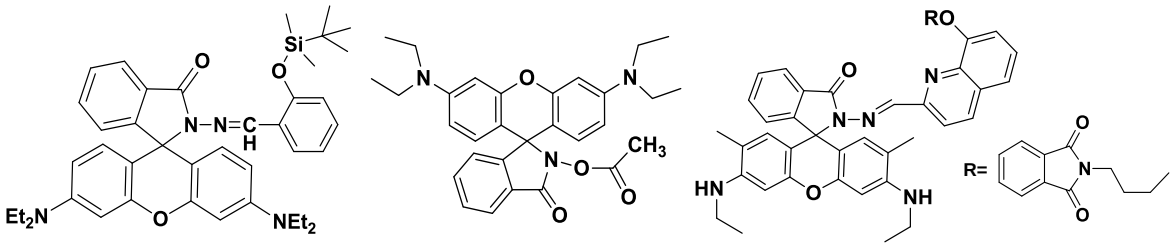
and aqueous Tris-HCl buffer.[32] The addition of Fe^{3+} ions led to the significant fluorescence enhancement within the range 500-600 nm due to opening of the spirolactam ring of the compound. The main limitation of probe **9** is its moderate binding towards Fe^{3+} which restricts its use in biochemical applications.



9 **10** **11**

Huang *et al.* reported a fluorescent chemosensor **10** as a turn-on sensor for Fe^{3+} ion.[33] Furthermore, fluorescence microscopy experiments showed that the probe **10** could be employed for imaging of Fe^{3+} ions in living cells.

Hu *et al.* developed a rhodamine 6G phenylurea conjugate **11** for the detection of acetate ions in H_2O-CH_3CN (1:1, v/v) with remarkable change in fluorescence intensity. The addition of Fe^{3+} ions also resulted in the clear color change from pink to colorless.[34] The background anions showed no interference with the acetate ions. The probe based on metal complexes showed selective detection of acetate ions over dihydrogenphosphate and fluoride ions in an aqueous medium. They have also synthesized a rhodamine B hydrazine and 2-*tert*-butyldimethyl silyloxy benzaldehyde conjugate derivative (RTSB) **12** for the selective detection of Fe^{3+} in H_2O/CH_3CN (v/v, 1:1) solution.[35] The addition of Fe^{3+} showed remarkable fluorescence enhancement with obvious red-shift. 1:1 binding stoichiometry was confirmed for **12**-Fe^{3+} complex from the Job's plot. Moreover, the addition of CN^- ions to **12**-Fe^{3+} resulted in the quenching of fluorescence at 581 nm with blue shift. The other background anions showed no interference with the detection of CN^- ions suggesting the **12**-Fe^{3+} complex could be employed as a "naked eye" sensor for CN^-. The reversibility experiment indicated that **12**-Fe^{3+} complex could be reused for the detection of CN^- ions with proper treatment. The limit of detection for CN^- was found to be $7.2x10^{-8}$ M. An acetyl rhodamine-hydroxamate based fluorescent chemosensor **13** exhibited excellent selectivity and sensitivity toward Fe^{3+} ions over other metal ions in CH_3CN-H_2O (1:1, v/v) solution.[36] The addition of Fe^{3+} ions resulted in the dramatic enhancement of both absorbance and fluorescence intensities and also colour change from colourless to red. No interference with the detection of

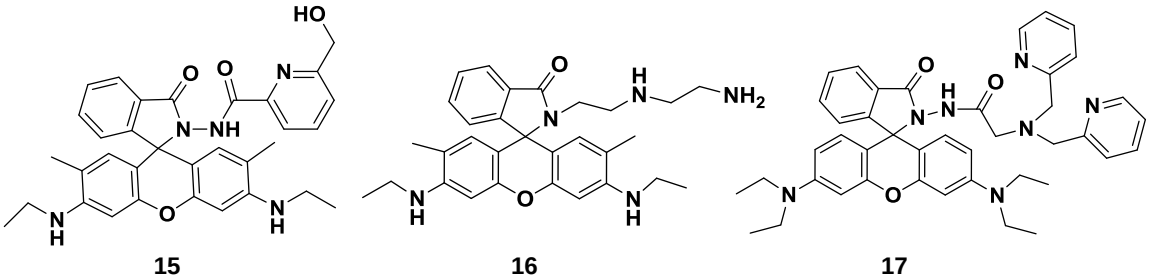


12 **13** **14**

Fe^{3+} has been observed upon the addition of other background metal ions. The binding stoichiometry of **13** and Fe^{3+} was estimated to be 1:1 using Job's plot method. The addition of acetate ion to **13**-Fe^{3+} complex verified the reversible interaction of **13** and Fe^{3+}. Confocal laser scanning microscopy experiments showed the use of probe **13** for the detection of Fe^{3+} in living cells.

Zeng *et al.* developed a chemosensor **14** with quinoline moiety bound to rhodamine 6G hydrazide.[37] The probe exhibited yellow fluorscence upon the addition of Fe^{3+} with quantum yield of 0.42 and 189-folds fluorescence enhancement at 559 nm in EtOH-H_2O (3:7, v/v) solution. 1:1 binding mode was supported by Job's plot. The reversible response of probe **14** towards Fe^{3+} has been confirmed from EDTA titration. Moreover, biological imaging and micro computed tomography (MCT) studies demonstrated the use of probe **14** for monitoring Fe^{3+} in living cells.

Goswami *et al.* developed a probe **15** from rhodamine-6G and 6-(hydroxymethyl) picolinohydrazide for the detection of Fe^{3+}.[38] The structure of probe was confirmed from its X-ray study. The probe showed high selectivity for Fe^{3+} ions over other interfering heavy and transition metal ions. The addition of Fe^{3+} induced green-yellow fluorescence with colorimetric change from colorless to pink. 116- and 23-folds enhancement in absorbance and fluorescence emission intensity was observed upon the addition of Fe^{3+} in CH_3CN-H_2O (1:1, v/v, 25°C)



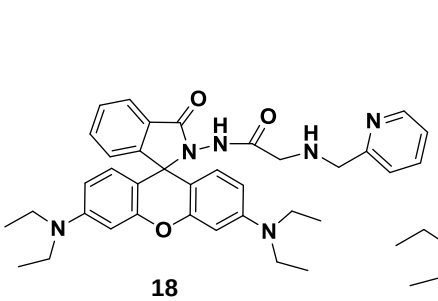
15 **16** **17**

solution at neutral pH (pH = 7.2). The turn-on fluorescence activity has been observed upon the addition of Fe^{3+} with visible colour change due to the ring opening of spirolactam framework to the open chain amide form of the probe. Furthermore, for practical application, probe showed excellent performance in "dip stick" test. The limit of detection was found to be in the 10^{-8} M range.

Mao *et al.* reported a fluorescent probe **16** using rhodamine as a fluorophore.[39] The structure alterations in the recognition unit made the probe selective for Fe^{3+} ions whereas other cations did not show any significant change in fluorescence spectra. 1:2 binding mode has been confirmed for Fe^{3+} and **16**.

Meng and co-workers synthesized rhodamine based fluorescent probe **17** which exhibited excellent selectivity toward Fe^{3+} in aqueous solution containing 1% methanol.[40] The probe **17** did not interfere with Cr^{3+} during Fe^{3+} detection. The results sugested that incorporation of another chelating moiety to the binding site results in the selective fluorescence enhancement for Fe^{3+} over other interfering ions. Moreover, confocal fluorescence imaging studies showed that **17** was cell permeable and suitable for monitoring intracellular Fe^{3+} in living cells with low cytotoxicity.

Meng and Liu coworkers synthesized rhodamine-based fluorescent probe **18** which showed selective real-time and "turn-on" fluorocolorimetric response in the presence of Fe^{3+} in water containing less than 1% organic cosolvent.[41] The addition of other competitive metal ions showed negligible effect on the fluorescence spectra of probe **18** even at higher concentrations and slight response has been observed with Cr^{3+} ions. The dramatic changes in both the colour and fluorescence has been observed for Fe^{3+} ions due to complexation of **18** with Fe^{3+} *via* 1:1 binding mode. Moreover, bioimaging investigations confirmed the cell permeablility of **18**, making it suitable for monitoring intracellular Fe^{3+} in living cells with low cytotoxicity.



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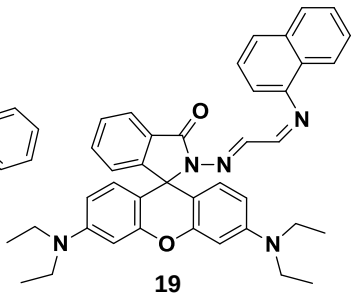

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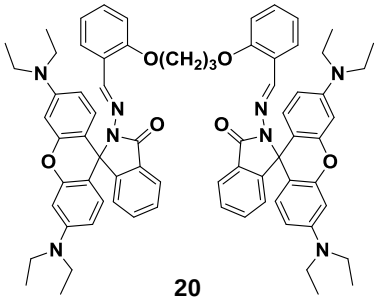

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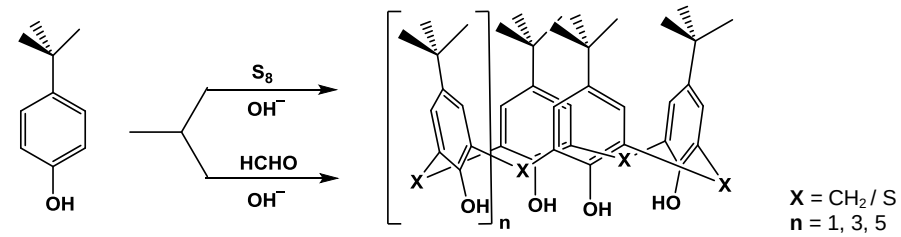
Wang and coworkers developed a fluorescent probe **19** based on rhodamine B which acted as fluorescent turn-on chemosensor for Fe^{3+}.[42] No significant colour change was observed with the addition of other metal ions revealing the potential use of receptor as a "naked eye" sensor for the detection of Fe^{3+} in real-time. The addition of other metal ions showed negligible change in absorption and fluorescence spectra except Al^{3+} and Cr^{3+} which showed mild effect as compared to Fe^{3+}. The addition of Fe^{3+} to **19** resulted in 260-folds enhancement in absorption intensity and 170-folds increase in fluorescence emission intensity. The addition of anions also showed no interference for the detection of Fe^{3+}. The minor interference was observed with SO_4^{2-} and OAc^- and a remakable interference with N_3^- and NO_2^- relative to other anions for the detection of Fe^{3+} These results indicated the selectivity of probe towards Fe^{3+} under various conditions.The binding stoichiometry of **19** and Fe^{3+} was confirmed to be 1:1 by Job's plot using the continuous variation method. The detection limit was estimated to be as low as 0.27 µM. In addition, the sensor was practically utilized for quantitative detection of Fe^{3+} in real samples.

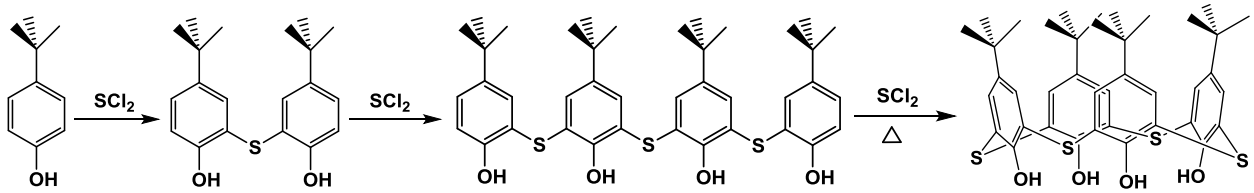
Thennarasu and Mandal coworkers reported the bis-rhodamine probe **20** which showed the fluorescence enhancement with intense pink colour change upon the complexation of Fe^{3+}.[43] The probe showed selective recognition of Fe^{3+} ions over other competitive metal ions. The spatial disposition of O-N-O donor atoms combined with a suitable spacer in bis-rhodamine analog has increased the sensitivity and selectivity for Fe^{3+} ions. The colour changed from pink to colourless upon the addition of EDTA to **20**-Fe^{3+} solution which confirmed the reversibility of complex formation. For practical applications, probe **20** was used for imaging of live fibroblast cells exposed to micro molar concentrations of Fe^{3+} ions in aqueous samples.

1.3 Thiacalix[4]arene based chemosensors for the detection of Zn^{2+} ions

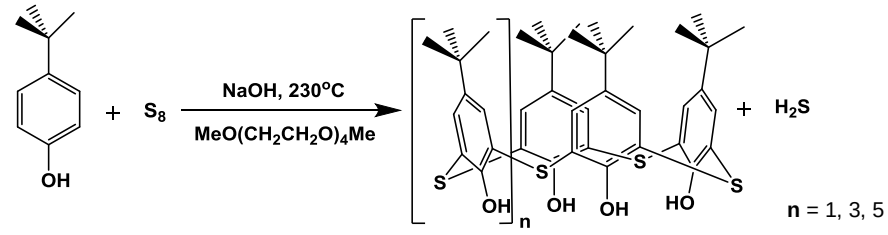
Calixarenes, the third generation supramolecular host compounds have been extensively used for designing fluorescent chemosensors due to their unique structural and chemical diversity.[44] The cyclic oligomer 'calixarenes' are formed under basic conditions by reacting *p-tert*-butylphenol and formaldehyde. Methylene bridges at O, O'-positions linked the phenolic moieties in calix[4]arenes. The reaction of *p-tert*-butylphenol and elemental sulfur (S_8) under basic conditions leads to the formation of thiacalix[4]arenes, where the phenolic units are linked through sulfur atoms instead of methylene groups of conventional calixarenes (**Scheme 1.1**). Ohba *et al.*[45] reported the first synthesis of *p-tert*-butylthiacalix[4]arene in poor yield through the



Scheme 1.1 General method of synthesis of calix[n]arenes and thiacalix[n]arenes



Scheme 1.2 Four steps synthesis of thiacalix[4]arene



Scheme 1.3 One step synthesis of thiacalix[n]arenes

tedious stepwise reaction of *p-tert*-butylphenol and SCl_2 (**Scheme 1.2**). Miyano *et al.*[46] reported one step and one pot synthesis of thiacalix[n]arenes (n = 1, 3, 5) *via* the reaction of *p-tert*-butylphenol, elemental sulfur (S_8) and sodium hydroxide in tetraethylene glycol dimethyl ether with the removal of H_2S leading to the formation of thiacalix[4]arene as a major product (54%) along with minor amount of thiacalix[6]arene and thiacalix[8]arene (**Scheme 1.3**). Both calixarene and thiacalixarene scaffolds have two rims, i.e. upper wider rim comprising of *para*-substituents and lower narrow rim comprising of phenolic moieties (**Figure 1.1A**). The four different conformations[47] are known for calixarenes and thiacalixarenes depending on the orientation of phenolic units with respect to each other, i.e. Partial cone' (uuud); 'Cone' (uuuu); '1,2-Alternate' (uudd) and '1,3-Alternate' (udud) (**Figure 1.1B**). The four hydroxyl groups interact *via* hydrogen bonding which makes the cone conformation more stable.[48] There is a dynamic equilibrium between all of its four conformations. Conformations can be locked in

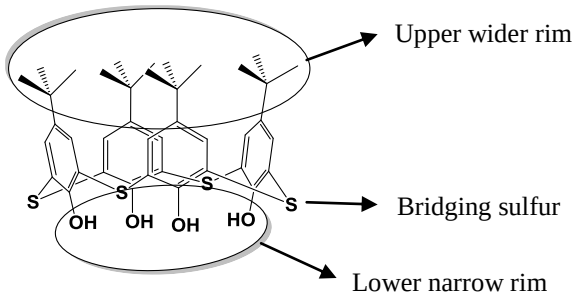
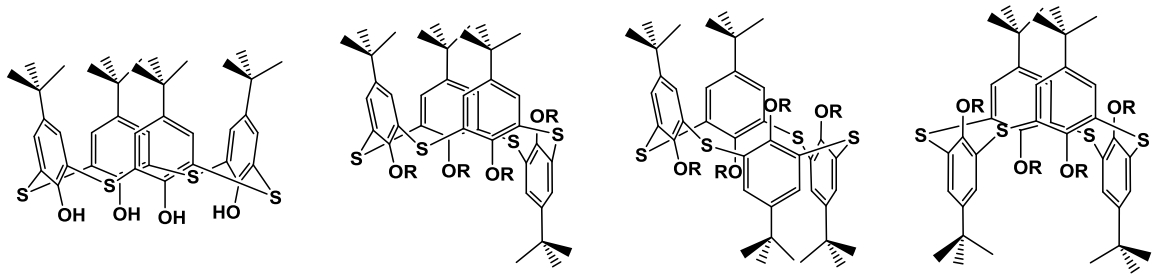


Figure 1.1A Structure of thiacalix[4]arene



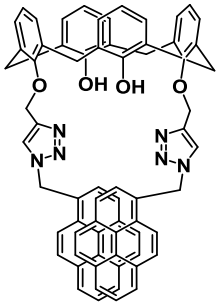
Cone (uuuu) Partial Cone (uuud) 1,2-alternate (uudd) 1,3-alternate (udud)

Figure 1.1B Different conformations of thiacalix[4]arene

place by replacing the hydroxyl groups with other substituents which increases the rotation barrier. Through the chemical modifications at the upper rim, lower rim and bridging sulfur atoms, a number of thiacalix[4]arene derivatives can be prepared for the selective detection of analytes. Thiacalix[4]arenes possess high complexation ability towards transition metals (due to additional sulfur-metal interactions) and the regio-/ stereoselective oxidation of sulfur bridges to sulfoxides or sulfones is the chemistry unseen in calixarenes.[49] Among the various derivatives of thiacalix[4]arenes of 1,3- alternate conformations, thiacalixcrowns are considered as important. A thiacalix[4]crown in 1,3- alternate conformation provide crown ether moiety for metal ion complexation with a potential for additional binding by cation- π interactions between the rotated benzene rings and a polyether complexed metal ion.[50] Many of the thiacalix[4]arene receptors have been reported in the literature for molecular recognition of various types of analytes such as cations, anions and neutral guest species. Among the various cations, zinc is recognized as the the most abundant divalent metal ion in the human body and is essential cofactor of various metabolic enzymes.[51] The detection of Zn^{2+} ions has become an important as free zinc in excess

can be toxic.[52] A brief review on fluorogenic receptors based on thiacalix[4]arene derivatives for Zn^{2+} detection is discussed below:

Kim *et al.* reported a chemosensor **21** based on calix[4]arene bearing triazole moieties which exhibited high selectivity for Zn^{2+} and Cd^{2+} ions in a ratiometric manner through an enhanced monomer emission and diminished excimer emission.[53]



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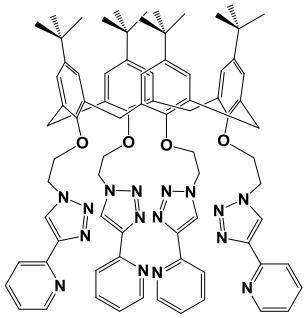

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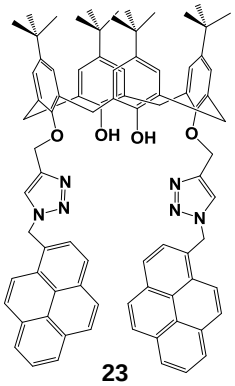


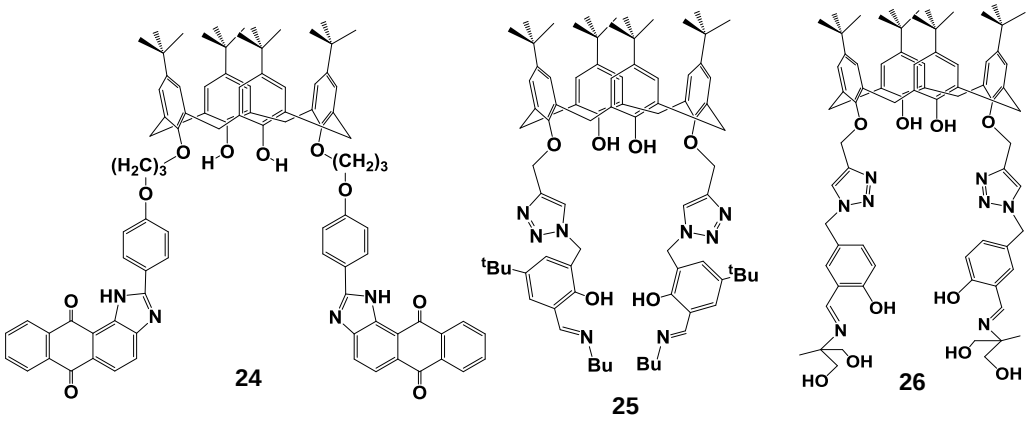
23

A calix[4]arene based sensor **22** bearing pyridin-2′-yl-1,2,3-triazole groups was reported by Souchon *et al*.[54] The addition of Zn^{2+} and Cd^{2+} ions to methanolic solution of probe **22** resulted in the decrease in fluorescence emission along with the formation of a new emission band and these changes attributed to the excimer formation. However the exact binding mode was not found, UV-Vis titrations showed the formation of ligand–metal complexes with both 1:1 and 1:2 stoichiometry.

Park and Zhu *et al.* independently developed a metal ion chemosensor **23** which exhibits a ratiometric response with the addition of Zn^{2+} or Cd^{2+} ions in CH_3CN, with quenching of pyrene excimer emission and increasing monomer emission.[55,56] The ^1H NMR studies showed that the pyrene moieties of probe **23** initially undergo π–π stacking, but on addition of metal ion into the triazole binding pocket resulted in their separation. The fluorescence response of probe **23** was further checked in the presence of other metal ions where the addition of Hg^{2+} and Cu^{2+} ions led to the decrease in fluorescence intensities of both monomer and excimer emissions.

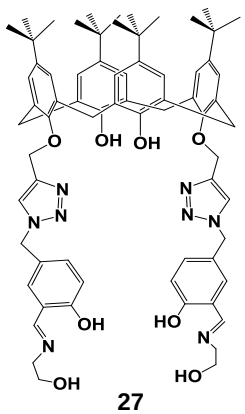
Chawla *et al.* reported an anthraquinonoidal calix[4]arene based receptor **24** for the preferential detection of zinc ion in preference to chemically similar cadmium ions and other metal ions.[57] The quenching in fluorescence emission intensity has been observed upon the addition of Zn^{2+} ions. The stoichiometry of **24**-Zn^{2+} complexation was found to be 1:1. The

binding of zinc ions with nitrogenous groups at the lower rim of calix[4]arene cavity in probe allows the spatial disposition of anthraquinonoid moieties resulting in the fluorescence changes.



24 **25** **26**

Rao *et al.* reported fluorescent sensor **25** which exhibits turn-on fluorescence response with Zn^{2+} ions in buffered aqueous methanol solution.[58] The addition of other competing metal ions did not affect the fluorescence response of probe **25** towards Zn^{2+} ions. The fluorescence of **25**–Zn^{2+} complex was unaffected upon the addition of blood serum and related albumin proteins. The coordination of zinc ion with imine and phenolate groups has been confirmed from the X-ray crystal structure of its Zn^{2+} complex. They have also synthesized calix[4]arene based sensors **26** and **27** with two potential binding sites: one bis-triazole binding pocket and other imino groups functionalised with hydroxymethyl groups.[59] Both the receptors **26** and **27** showed increase in fluorescence emission in the presence of Zn^{2+} over a range of divalent metal ions in MeOH. ^{1}H NMR studies revealed the binding of Zn^{2+} only at the Schiff base site. A carboxamidoquinoline based 1,3-di-conjugate of calix[4]arene **28** was also developed for the detection of Zn^{2+} ions.[60] The decrease in fluorescence emission at 410 nm and the appearance of new emission band at 490 nm with the nine-folds overall fluorescence enhancement was observed upon the addition of Zn^{2+} ions to the solution of **28**. The receptor **28** exhibited bluish green fluorescence change in the presence of Zn^{2+} ions whereas no such color change was observed upon the addition of other ions except Cu^{2+}, Fe^{2+} and Hg^{2+}. The triazole linked calix[4]arene conjugate **29** appended with o-imino phenol units provides a multiple sensing molecular tool for the detection of amino acids by the specific metal ions.[61] The fluorescence enhancement has been observed upon the addition of Zn^{2+} ions to the solution of **29**, whereas



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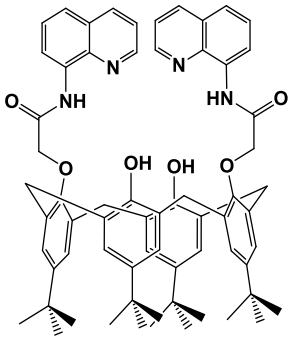

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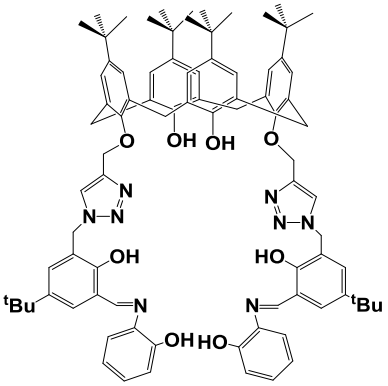
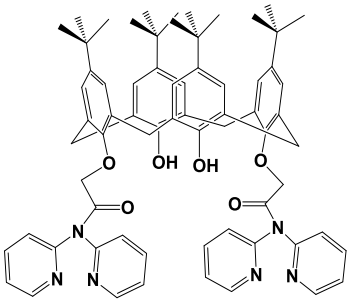


29

quenching in fluorescence was observed in the presence of Fe^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+} and Cu^{2+} ions. The probe **29** showed distinguishable absorbance and fluorescence changes in the presence of these metal ions with 1:2 stoichiometry of ligand to metal ions. The *in-situ* prepared metal complexes [Zn_2**29**], [Fe_2**29**], [Mn_2**29**], [Ni_2**29**], [Co_2**29**] and [Cu_2**29**] were further used for the detection of various amino acids *via* demetalation. The sensing behaviour showed the preferable binding of Cu^{2+} and Zn^{2+} with Cys and His, Mn^{2+} with Asp and Glu, Ni^{2+} with His and Asp, Fe^{2+} with none and Co^{2+} with Asp, Cys and His. An amide conjugate of calix[4]arene **30** linked with a bispyridyl group on each of the arms possessing two binding cores as it was determined from its



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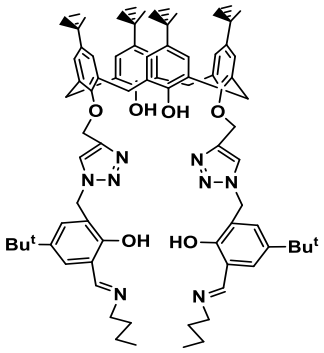

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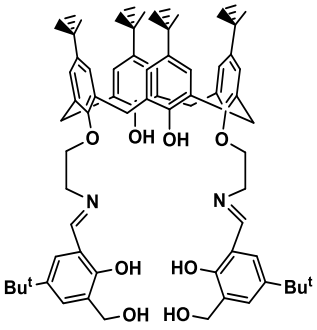
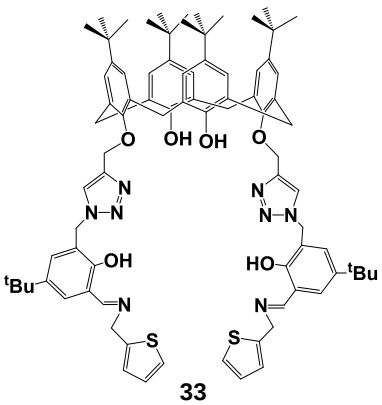


32

crystal structure.[62] The probe **30** showed switch-on and switch-off fluorescence response with the addition of Zn^{2+} and Ni^{2+} ions, respectively. A calix[4]arene based fluorescent chemosensor **31** appended with triazole moieties has also been synthesized for the selective monitoring of Zn^{2+} ions.[63] The chemosensor **31** has also been practically applied for the sensing of Zn^{2+} ions in blood serum milieu and albumins. They have also developed calix[4]arene based fluorescent receptor **32** bearing hydroxymethyl salicylyl imine groups.[64] The probe **32** exhibited sensitivity

towards both the Zn^{2+} and Cd^{2+} ions but its sensitivity is more toward Zn^{2+} as compared to Cd^{2+} ions. The probe **32** showed 10-folds higher binding towards Zn^{2+} ions in comparison to Cd^{2+} ions in methanol. Further the computational studies showed 1:1 complex formation of **32** using N_2O_2 core for zinc ions and N_2O_4 core for cadmium ions. The geometry around Zn^{2+} ion was best fitted to a trigonal bipyramidal with one vacancy and that around Cd^{2+} ion was best fitted to a capped octahedron with one vacant site. Furthermore, **32**-Zn^{2+} complex was used for the selective detection of phosphate species such as AMP, ADP, and ATP *via* fluorescence quenching. The probe **32** was able to detect the minimum concentration of $HPO4^{2-}$ as 426 ppb. INHIBIT logic gate was developed for receptor **32** based upon Zn^{2+} and HPO_4^{2-} ions as chemical inputs. A triazole linked calix[4]arene **33** bearing iminothiophenyl moeties exhibited high fluorescence emission in the presence of Zn^{2+} ions in aqueous buffer solution.[65] Further **33**-Zn^{2+} ensemble was used for the detection of phosphate based anions in general and in particular PPi anions among the different eighteen anions tested. The differences in microstructural features of probe **33**, its complexes with Zn^{2+} and PPi ions has been demonstrated through the transmission electron microscopy and atomic force microscopy experiments. Further the addition of Ca^{2+} ion led to the regeneration of fluorescent chemosensing ensemble **33**-Zn^{2+} which is due to the displacement of Zn^{2+} from {Zn·PPi} complex. The switch-on and off fluorescence behaviour of probe **33** in presence of Zn^{2+} and PPi ions were also demonstrated in HeLa cells. Moreover, they demonstrated reversible and reconfigurable "write-read-erase-read" logic property using Zn^{2+} and PPi ions as chemical inputs. The triazole-linked calix[4]arene conjugate **34** showed increase in fluorescence in the presence of Zn^{2+} due to inhibition of PET phenomenon.[66] **34**-Zn^{2+}



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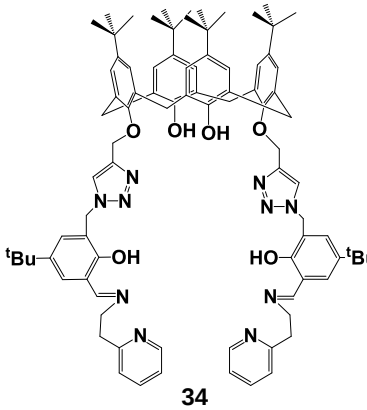

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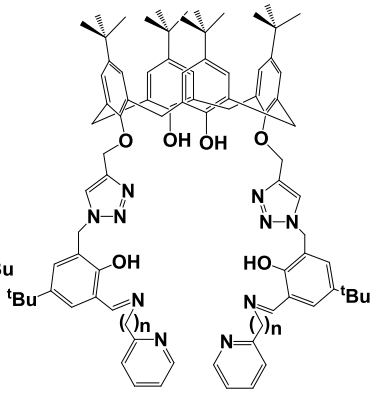


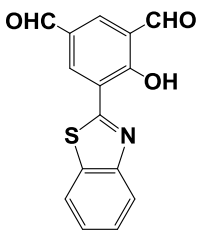
35a-b
35a: n = 1
35b: n = 2

ensemble was further used for the recognition of cysteine in proteins. The addition of cysteine caused the decrease in fluorescence intensity at 454 nm of the **34**-Zn^{2+} ensemble. No significant fluorescence change was observed with the addition of other competitive mercapto biomolecules such as cysteine, homocysteine, glutathione and mercaptopropionic acid. **34**-Zn^{2+} ensemble was also used for determining cysteine in blood serum samples, with a limit of detection found to be 846 ppb. The pyridyl based triazole linked two calix[4]arene conjugates **35a-b** showed switch-on fluorescence response in the presence of Zn^{2+} over the other 12 metal ions *viz.* Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Na^{+} and K^{+} tested.[67] The electronic properties of probes and their zinc complexes were demonstrated from TDDFT calculations. The zinc complexes of **35a-b** were found to be unaffected in the presence of 18 anions *viz.* F^{-}, Cl^{-}, Br^{-}, I^{-}, N_3^{-}, CO_3^{2-}, NO_2^{-}, NO_3^{-}, SCN^{-}, SO_4^{2-}, ClO_4^{-}, HSO_4^{-}, HCO_3^{-}, $H_2PO_4^{-}$, $P_2O_7^{4-}$, AMP, ADP and ATP studied and only the switch-off response has been observed upon the addition of phosphate ions, in particular to ATP and PPi. The unusual sensitivity and reactivity of **35b**-Zn^{2+} over **35a**-Zn^{2+} complex was observed due to the highly distorted geometry of **35b**-Zn^{2+}. **35b**-Zn^{2+} was able to detect PPi up to 278 ± 10 ppb. Further the receptors **35a-b** were used as fluorescence imaging agents in live cells.

1.4 Fluorescent chemosensors for the detection of CN^- ions

Anions are considered of the great importance in a wide range of industrial, biological and environmental applications. Among the various anions, cyanide is recognized as one of the most toxic anions which can cause the death of human beings even if present in small amount. Thus, there is a great need to develop efficient sensing system for cyanide ion detection from contaminant sources. Many of the chemosensors have been reported in the literature for the detection of cyanide ion. A brief review on fluorescent chemosensors for the CN^- ion detection is discussed below:

Goswami *et al.* developed a benzothiazole receptor **36** having aldehyde groups at *ortho* and *para* positions of OH group.[68] The receptor **36** showed selective ratiometric response upon the addition of CN^- ions ascribed to the nucleophilic addition of cyanide ion which hampered the excited state intramolecular proton transfer (ESIPT) phenomenon. The results were confirmed from DFT and TDFT calculations. The probe **36** showed high selective response with CN^- ions over OAc^-, F^- and other various anions.



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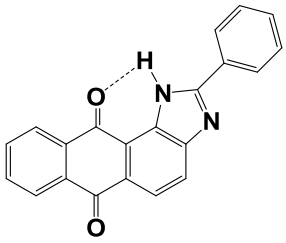

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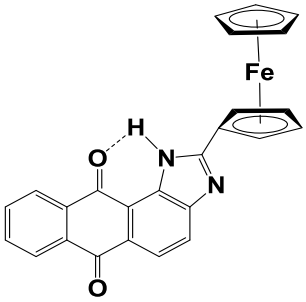
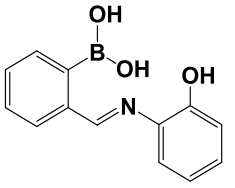


38

Das *et al.* developed anthraquinone based two fluorescent probes **37** and **38** appended with imidazole units which were capable of sensing CN^- ions with limit of detection as 006 and 0.078 ppm, respectively.[69] The decrease in absorbance at 393 nm and the increase in absorbance at 474 nm were observed upon the addition of CN^- to the solution of **37**. The probe **38** exhibited the increase in absorbance at 552 nm and the decrease in absorbance at 390 nm in the presence of CN^-. The addition of CN^- ions showed the ratiometric fluorescence change with both the probes. These spectral and color changes were attributed to the formation of corresponding hydrogen bonded adducts **37**-CN^- and **38**-CN^-.

Wu and coworkers developed a boronic acid based receptor **39** which showed hypsochromic shift in absorbance spectrum in the presence of CN^- ions and the color of solution changed from yellow to colorless.[70] The probe **39** exhibited fluorescence emission at 540 nm which is attributed to the interaction of B-N with a rigid five membered ring. The quenching in emission at 540 nm was observed in the presence of CN^- ions attributed to the nuclephilic attack of CN^- at imine group of the receptor **39**.



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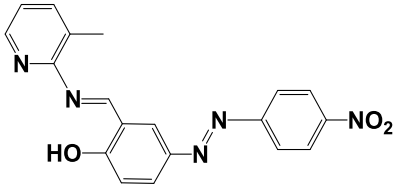

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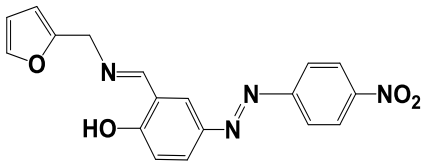
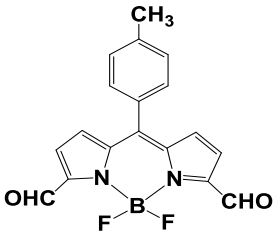


41

Singh *et al.* reported two azo linked chemosensors **40** and **41** which displayed high selectivity towards CN^- and less selectivity for AcO^- ions with immediate color change (light pink for AcO^- and red for CN^-).[71] The anion binding mechanism *via* deprotonation process was proved by NMR titration. The different anions did not interfere in the detection of CN^- ions

whereas only CN^- showed interference in the case of AcO^- ions due to its higher basic nature. The results showed ability of both **40** and **41** to act as better chemosensors for CN^- ions than AcO^- ions in the presence of other different competing anions.

Ravikanth and coworkers developed a 3,5-diformyl-borondipyrromethene **42** as chemodosimeter for the detection of CN^- ions.[72] The fluorescence quenching at 554 nm has been observed upon the addition of CN^- ions (0-2 equiv) to the solution of probe **42** in acetonitrile. The detection limit was found to be 3 ppm. The nucleophilic attack of CN^- occurred at carbonyl



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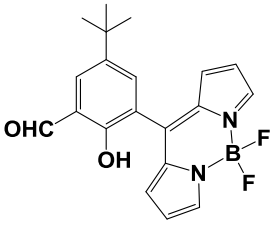

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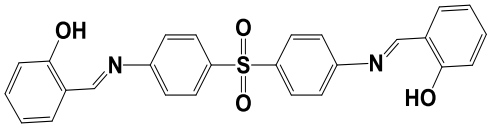

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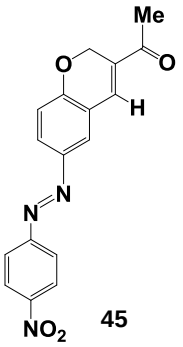
groups in **42** leading to the formation of cyanohydrin adduct which altered its electronic properties, reflected by its change in photophysical properties and the colour change observed was from red to blue visible under UV lamp. They have also reported meso-salicylaldehyde substituted BODIPY **43** as a chemodosimeter for the turn-off detection of CN^- ions.[73] The probe **43** exhibited a broad emission band at 520 nm in the absence of CN^- ions which is due to the intramolecular hydrogen bonding between formyl and hydroxyl groups which makes the system rigid and decreases the non-radiative decay process and hence, the molecule become fluorescent. The formyl group was activated for nucleophilic attack by the neighboring hydroxyl group. The nucleophilic addition of CN^- ions led to the conversion of aldehyde group to the cyanohydrin group accompanied by the changes in absorbance, fluorescence and electrochemical properties of probe **43**.

Shu and Chen coworkers reported a 4,4′-bis(2-hydroxybenzylidene amine) diphenyl sulfone **44** for the simultaneous detection of CN^- and F^- ions.[74] Upon the addition of CN^- ions to the solution of **44**, the decrease in fluorescence emission at 340 nm was observed along with the appearance of two new bands at 388 and 521 nm and the color of solution changed from colorless to purple. On the other hand, addition of F^- led to the appearance of band at 474 nm along with the color change from colorless to orange. No change in color and spectra was

observed in the presence of other anions. The sensing mechanism was confirmed from NMR experiment and DFT calculations.

Park *et al.* developed an azo dye based colorimetric probe **45** bearing masked phenol at the *para* position of azo group which upon the addition of CN^- ions showed ratiometric UV-vis response along with the colour change from colorless to dark violet due to formation of **45**-CN^- complex.[75] The addition of CN^- ions to the solution of **45** caused Michael addition to take place followed by [1,3]-sigmatropic rearrangement reaction which generates the stable free phenol, whose signal gets transduced to azo group affording dramatic color change. The time taken was five hours to get this reaction complete with second order rate constant ($k_2 = 6.5 \times 10^{-3}\ M^{-1}\ s^{-1}$) at room temperature.



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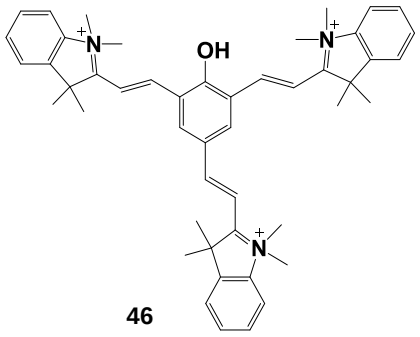

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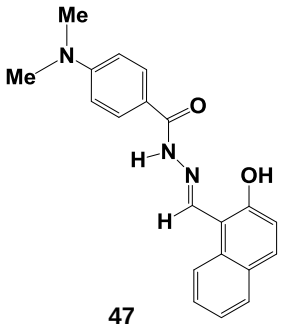


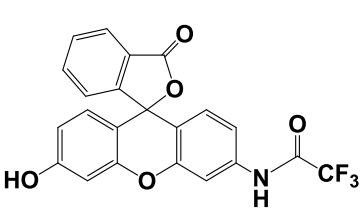
47

Yang *et al.* developed a receptor **46** possessing 2-hydroxy-1,3,5-benzenetricarbaldehyde and 1-methyl-2,3,3-trimethyl-3H-indolium moieties which showed turn-on fluorescence response with CN^- ions accompanied by blue-green color fluorescence change.[76] The π-conjugation between 2-hydroxy-1,3,5-benzenetricarbaldehyde and indolium was blocked by the nucleophilic addition of CN^- at C=N bond of indolium group resulting in the fluorescence change. The detection limit was found to be as low as 45 nmol/L using receptor **46**.

Guo *et al.* reported a fluorescent probe **47** bearing 2-hydroxy-1-naphthaldehyde hydrazone moiety for the sensing of cyanide ions.[77] The addition of CN^- ions to the solution of **47** caused the fluorescence enhancement at 460 and 495 nm where the former band corresponds to excited state charge transfer (CT) for the fluorophore 4-(N,N-dimethylamino)benzamide and the latter corresponds to naphtholate anion. The probe **47** showed selectivity towards CN^- ions over other different anions due to nucleophilic nature of CN^- and activation of imine group by neighboring hydroxyl proton through intramolecular hydrogen bonding. The specific reaction resulted in the

fluorescence enhancement and the color change occurs from colorless to yellow. They have also developed a rhodafluor-based probe **48** linked with mono-trifluoroacetylamino unit[78] which showed selective fluorescence enhancement at 520 nm in the presence of CN^- ions over other different anions attributed to the spirolactone ring-opening induced by the cyanide ions. The detection limit for CN^- was calculated to be 2.66×10^{-8} M.

Chao *et al.* developed a fluorescent probe **49** by ethylene bridging of an electron donor N-ethylcarbazole-3-formaldehyde and an electron acceptor 1,1,2-trimethyl-3-ethyl-1H-benz[e]indolium iodide.[79] The addition of CN^- ions led to the decrease in absorbance at 503 nm



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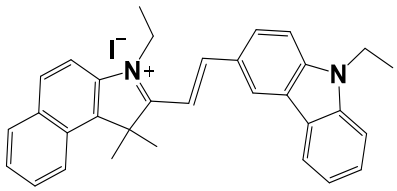

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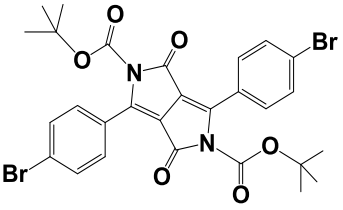

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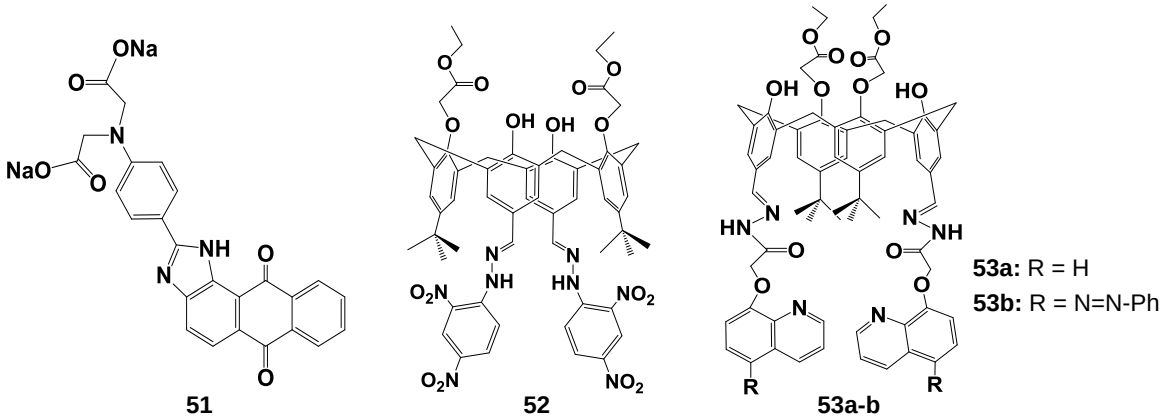
and increase in broad band at 250-300 nm along with the color change to crimson. The quenching in fluorescence emission at 595 nm was observed with the increasing emission at 440 nm which is due to the electron withdrawing nature of benzo[e]indolium group which allows the nucleophilic addition of CN^- at imino group. The detection limit for CN^- was found to be 0.23 μM.

Jang and coworkers reported a diketopyrrolopyrrole **50** which exhibited absorbance band at 442 nm and fluorescence emission at 512 nm.[80] The nucleophilic addition of CN^- at the electron deficient carbonyl carbons in probe **50** led to the decrease in emission at 512 nm along with the color change from green to red. The detection limit for CN^- was found to be in micromolar range.

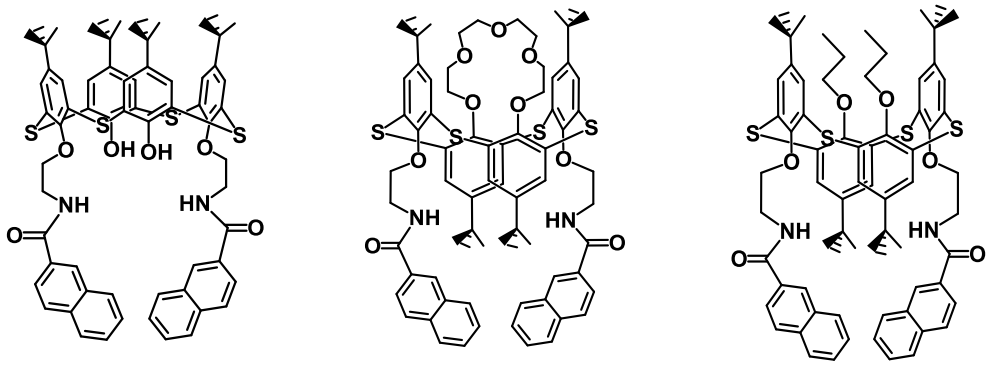
Bhattacharya and coworkers reported anthra[1,2-d]-imidazole-6,11-dione based fluorescent probe **51** for the selective detection of F^- and CN^- ions.[81] The addition of F^- and CN^- ions caused the red shifting in absorbance band of **51** by 120 and 80 nm, respectively attributed to the intramolecular charge transfer (ICT) process and the color change observed was from light yellow to red with CN^- and from light yellow to dark blue with F^-. The probe exhibited ratiometric detection of CN^- in aqueous medium with red shifting in absorbance band from 464



51 **52** **53a-b**

nm to 504 nm.

Chawla *et al.* reported a new calix[4]arene based receptor **52** for the detection of CN- ions.[82] Upon the addition of different anions ($H_2PO_4^-$, HSO_4^-, SCN^-, CN^-, AcO^-, F^-, Cl^-, Br^-, I^-) to the solution of probe **52**, intramolecular charge transfer band at 407 nm disaapeared and only the new emission band formation occurs at 473 nm in the presence of CN^- ions along with the color change from light orange to red-wine. The probe **52** showed high selectivity towards CN^- ions due to its low solvation energy (ΔH_{hyd} = -67 kJ mol^{-1}). They have also developed calix[4]arene based two fluorescent probes **53a-b** linked with quinolone moieties,[83] out of which 90% fluorescence quenching has been observed at 405 nm upon the addition of 2 equiv. of Cu^{2+} ions into the solution of receptor **53a** whereas only 8% quenching was observed with the receptor **53b** which is due to the presence of electron withdrawing azo group. The broadening of spectrum has been observed along with the shoulder peak at 450 nm upon the addition of 10 equiv. of CN^- ions into the solution of **53a**-Cu^{2+} complex which is due to the adduct formation between CN^- and **53a**-Cu^{2+}.



54 **55** **56**

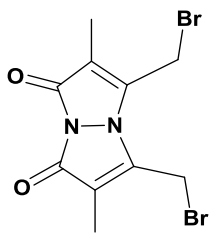
Kumar *et al.* reported thiacalix[4]arene based sensors **54-56** appended with naphthyl units which showed selective response with CN^- ions through hydrogen bonding and displacement approach.[84] The addition of CN^- ions to the solution of receptor **54** led to the fluorescence quenching at 350 nm whereas its addition upto 500 equiv to the solutions of receptors **55** and **56** showed ratiometric fluorescence behaviour with decreasing emission at 350 nm and increasing emission at 454 nm. This fluorescence change was attributed to the photoinduced electron transfer (PET) phenomenon from CN^- to naphthalene group. Further the *'in-situ'* prepared iron complexes of **54-56** are used for the selective fluorescence enhancement of CN^- ions. The stoichiometries of all these iron complexes were found to be 1:1 from Job's plot. Fluorescence "on-off" switching has been observed with the chemical inputs Fe^{3+} and CN^- ions which mimics the operation of exclusive-NOR (XNOR) gate.

1.5 Fluorescent probes for the selective detection of H_2S

H_2S is a colorless noxious gas having characteristic of unpleasant rotten egg smell. H_2S is third gasotransmitter besides nitric oxide and carbon monoxide.[85] H_2S is produced endogenously both from enzymatic and non-enzymatic reactions. Hydrogen sulfide is endogenously biosynthesized by three enzymes: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST).[86] These enzymes exhibit different expression in different tissues which suggests the importance of H_2S in cardiovascular, respiratory, circulatory, urinary and nervous systems. Hydrogen sulfide is also known by the name of mitochondrial toxin as it works as a weak acid (pK_{a1} = 7.04 and pK_{a2} = 11.96)[87] in aqueous solution which gets in equilibrium with HS^- at physiological pH and then inhibits the function of cytochrome oxidase which give rise to histotoxic hypoxia.[88] Abnormal level of H_2S results in the diseases like Down's syndrome,[89] Alzheimer's disease,[90] diabetes[91] and liver cirrhosis.[92] The toxic and corrosive nature of H_2S gas can lead to the damaging of pipelines and catalysts and also the ceramic membranes which are used for syngas separations.[93] The extereme toxicity of H_2S in human body is due to its good reducing ability and high lipid solubility even at low levels.[94] The various traditional methods such as electrochemical analysis,[95] gas chromatography,[96] colorimetry[97] and metal-induced sulfide precipitation[98] have been developed for the detection of H_2S but they often have low temporal resolution and leads to the destruction of cells or tissues. Thus, these methods are not suitable for endogenous H_2S detection in real

time. Fluorescence imaging technique is highly desirable in comparison to traditional methods for H_2S detection due to its high biocompatibility and real-time spatial imaging. At the molecular level, H_2S acts as both good reducing agent as well as good nucleophile and is considered as a better nucleophile than thiols in physiological media since it has pKa value of 7.0 in aqueous solution whereas thiols have higher pKa values of ~8.58.[99] Many of the reaction based fluorescent probes have been reported in literature[100] for the detection of H_2S which offer high spatiotemporal resolution and greater live-cell compatibility than that of traditional methods. Three strategies involved in the development of these reaction based probes include nucleophilic attack, azido or nitro group reduction and CuS precipitation. Some of the recent representative examples of fluorescent probes for H_2S detection are reviewed below.

Pluth *et al.* synthesized dibromobimane based probe **57** which showed "turn-on" fluorescence response with H_2S.[101] The probe **57** was used for sulfide quantification with limit of detection as low as 0.6 pM. The initial nucleophilic attack of H_2S led to the formation of thiol intermediate which further undergoes intramolecular reaction to give the bimane thioether as fluorescent product. The probe **57** was also able to react with other thiols with α- or β-hydrogens, if present to give the same fluorescent product. Thus, the receptor **57** was used for sensitive detection of H_2S and caution was taken for its use in biological samples in the presence



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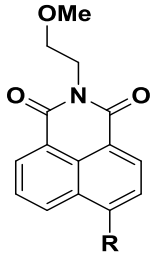
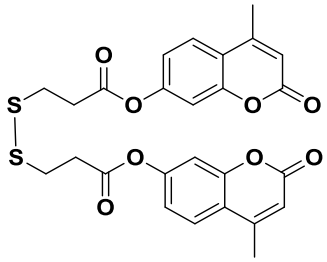


58a: R = NO_2
58b: R = N_3

58a-b

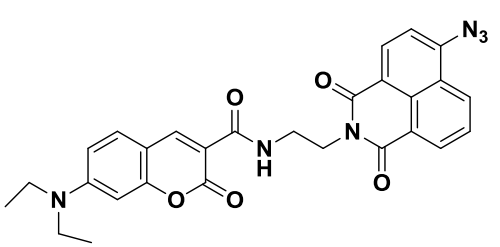


59

of other sulfhydryl species. They have also reported fluorescent probes **58a-b** which exhibited selective detection of H_2S over glutathione, cysteine and other reactive oxygen, nitrogen and sulfur species.[102] The 15-folds and 60-folds fluorescence enhancement was observed for **58a** and **58b** in response to H_2S with completion of reaction in 90 min and 45 min, respectively. After 60 min incubation of H_2S, the detection limits of **58a** and **58b** for H_2S were calculated to be 5-10 μM and 1-5 μM, respectively. The probes were also applied for the visualisation of H_2S in living cells.

Zhou and Zhang coworkers developed lysosome-targetable probe **59** for the selective detection of H_2S.[103] The addition of H_2S led to the 15-folds fluorescence enhancement at 453 nm which gets saturated at 30 equiv of H_2S. The reduction of disulfide bonds led to the generation of nucleophilic sulfhydryl groups which further undergoes intramolecular cyclization by cleaving the neighbouring ester groups resulting in the formation of fluorescent product. No reaction was observed upon the addition of other biological thiols. The reaction of **59** towards H_2S showed bright blue fluorescence by naked eye and thus selective for H_2S. A good linear correlation between fluorescence emission intensity at 453 nm and concentration of H_2S in 0-300 µM range was observed. The limit of detection was found to be 7.9 x 10^{-7} M. The probe **59** was used for detection of endogenous and exogenous H_2S by *in-vivo* imaging of HeLa cells, C. elegans and D. melanogaster. The probe **59** was employed for tracing the accumulation of lysosomes in D. melanogaster and lysosomal injury in C. elegans (SRP-6 nulls) with a high resolution.

Lin and coworkers synthesized ratiometric fluorescent probe **60** for the detection of H_2S based on the FRET strategy.[104] The addition of NaHS to probe solution resulted in the decrease in fluorescence of coumarin at 474 nm with appearance of new fluorescence emission band at 534 nm corresponding to naphthalimide moiety. The FRET phenomenon from coumarin to naphthalimide unit was induced by ICT effect in the presence of H_2S due to reduction of azide group of **60** to amine unit. The fluorescence intensity ratio (I_{534}/I_{474}) showed 8.7-folds enhancement. A good linear relationship between fluorescence emission intensity ratio (I_{534}/I_{474}) and H_2S concentration in 1.0 µM to 30 µM range was ascribed suggesting the potential use of



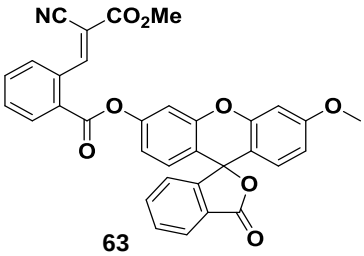
60 **61** **62**

probe for quantitative determination of H_2S. The small increase in intensity ratio was observed for other biological thiols such as cysteine and glutathione at 5mM while the large increase observed upon the addition of 20 µM of H_2S suggesting the selectivity of probe towards H_2S over other biological thiols. Furthermore, the low cytotoxicity of probe **60** made it suitable for

studies in living cells. The probe **60** was employed for monitoring exogenous and endogenous H_2S in living cells.

Zheng and Cui coworkers reported a fluorescent "turn-on" probe **61** for H_2S based on the PET phenomenon.[105] The probe showed 17-folds fluorescence enhancement at 655 nm with red fluorescence emission in the presence of 50 µM of NaHS. The sensing mechanism was proposed on the basis of thiolysis of dinitrophenyl ether moiety generating the fluorescent product along with the colour change from purple to red. The time-dependent fluorescence studies have also been done with 10 equiv of NaHS which showed the gradual increase in fluorescence with increase in reaction time. The detection limit of **61** for H_2S was found to be 2.7 x 10^{-7} M. The addition of glutathione at 20 mM showed small fluorescence enhancement by 3-folds and small interference with H_2S detection. The probe **61** showed high selectivity for H_2S over other competitive biological relevant analytes. The probe **61** was potentially used in the fluorescence fluorescence imaging of H_2S in living cells.

Xian and coworkers synthesized Michael-acceptor based fluorescent chemosensors **62** and **63** for the detection of H_2S.[106] The cyanoacrylate based probe **63** showed better reactivity and fast response for H_2S as compared to benzylidenemalonate based sensor **62**. The treatment of 5 µM probes **62** and **63** with 100 µM of NaHS led to the 11-folds and 160-folds turn-on fluorescence response. No significant fluorescence enhancement was observed upon the treatment of esterase to probes **62** and **63** for 2 h suggesting the stability of both probes towards esterase. The addition of other biological thiols such as cysteine and glutathione showed no change in fluorescence spectra of **62** and **63**. The detection limit for both of these probes was calculated to be ~1 µM.



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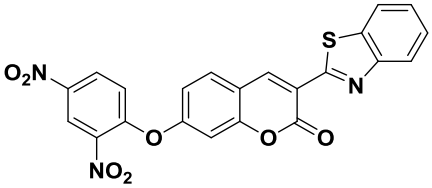

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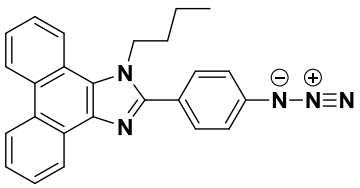


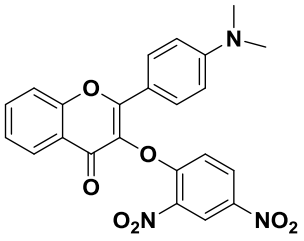
65

Yuan *et al.* reported fluorescent "turn-on" chemosensor **64** for the detection of H_2S based on photoinduced electron transfer and intramolecular charge transfer mechanism.[107] A strong

fluorescence emission peak was observed at 491 nm in the presence of H_2S with 185-folds enhancement which was ascribed to the thiolysis of dinitrophenyl ether group in probe **64**. The fluorescence emission of probe **64** changed from dark to green colour upon the addition of H_2S and the limit of detection was evaluated to be 0.42 μM. Other biologically relevant analytes did not show any interference in the detection of H_2S showing the specificity of dinitrophenylether group to react with H_2S in buffer solution at pH 7.4. The probe **64** was used for monitoring endogenous and exogenous H_2S in cells. Two photon fluorescence microscopy was also applied for detection of H_2S in living tissues.

Lin and coworkers developed fluorescenct receptor **65** appended with phenanthroimidazole unit for H_2S.[108] The receptor **65** showed turn-on fluorescence behaviour with H_2S and 20-folds fluorescence enhancement was observed with 5 equiv of NaHS. The probe **65** was converted into amine *via* H_2S mediated reduction of azido group of phenanthroimidazole based compound. The detection limit was found to be 8.79 x 10^{-7} M. The probe **65** showed selectivity towards H_2S over other biological tested species including cysteine and glutathione. The probe **65** was used for monitoring variations of H_2S levels in living cells.

Feng and coworkers synthesized 3-hydroxy-flavone based visible light excitable ESIPT probe **66** for the rapid and selective sensing of H_2S in aqueous solution.[109] Initially, the ESIPT phenomenon was inhibited by protection of hydroxy group in probe **66** with dinitrophenylether moiety. The addition of H_2S led to the thiolysis of dinitrophenylether which "turns on" the ESIPT phenomenon. The probe **66** was further used in biological serum and in simulated waste water samples for the detection of H_2S.



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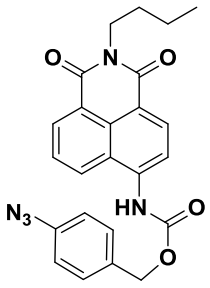

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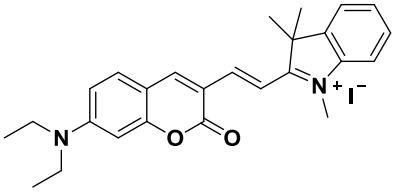


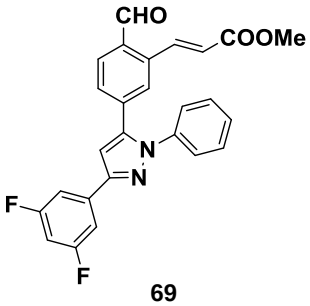
68

Qian, Liu and Zhao coworkers developed a naphthalimide-azide based probe **67** for the ratiometric sensing of H_2S.[110] The probe **67** was used for endogenous H_2S imaging in living cells and also for the detection of sulfide in human plasma. The reaction of H_2S with probe **67** showed

the visible colour change from pale yellow to bright yellow along with fluorescence emission colour change from blue to green. The probe was used for the measurement of sulfide levels in hippocampus of mouse models of LPS-induced neuroinflammation related diseases. The results showed the decreased level of endogenous H_2S which led to the generation of neurodegenerative diseases.

Yin and Huo coworkers synthesized a colorimetric and fluorometric probe **68** containing a conjugated indole-coumarin skeleton for HS^- detection based on nucleophilic addition reaction.[111] The addition of HS^- to probe **68** in CH_3OH-HEPES (10 mM, pH 7.4) buffer solution led to the blue shifting of absorption from 572 nm to 405 nm with change in colour of solution from violet to light yellowish. The fluorescence emission showed the blue shifting of emission band from 645 nm to 475 nm with colour change from purple red to green in the presence of HS^-. Furthermore, the probe **68** was able to detect H_2S in living cells.

Qian *et al.* synthesized chemosensors **69** and **70** for the effective *in vitro* and *in vivo* monitoring of H_2S.[112] The probe **69** undergoes blue shift of emission maximum from 428 to 391 nm upon the addition of Na_2S along with greater than 10-folds increase in fluorescence emission intensity. On excitation at 465 nm, greater than 13-folds increase in emission intensity at 510 nm was observed for the probe **70**. The probes **69** and **70** showed excellent selectivity for H_2S over other biological thiols. The sensor **69** exhibited 50-100 folds selectivity for H_2S over other tested thiols. For the probe **70**, 150-folds and 260-folds more selectivity was known for H_2S than for glutathione and cysteine, respectively. Both the probes upon reaction with H_2S showed colour change visible to naked eye. Furthermore, the probes were used for imaging of H_2S in HeLa cells.



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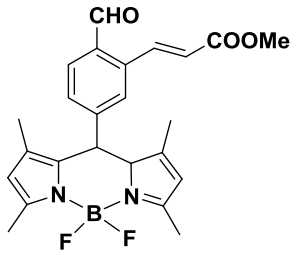

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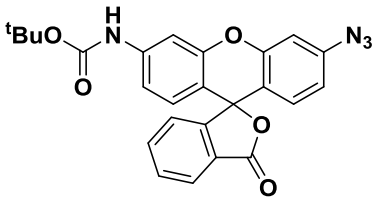
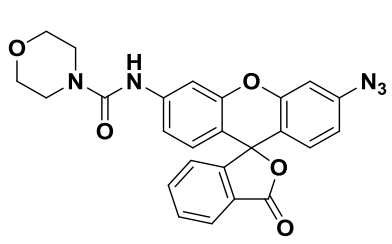


71

Chang and coworkers synthesized rhodamine based fluorescent chemosensors **71** and **72** which showed turn-on fluorescence response with H_2S ascribed to the H_2S-mediated reduction of

azides to amines.[113] Both the probes exhibited high selectivity for H_2S over other biological relevant oxygen, nitrogen and sulfur species. The selective detection of H_2S has been observed in water and live cells. The probes **71** and **72** showed *in-vitro* detection limit of H_2S in 5-10 µM range.



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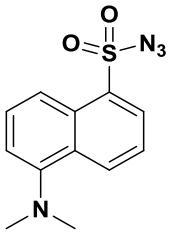

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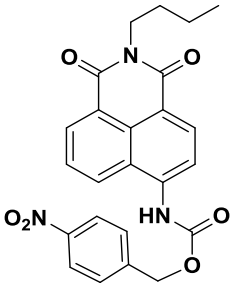


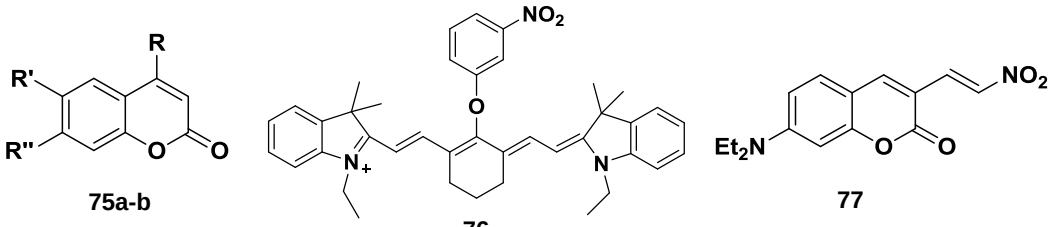
74

Wang and coworkers developed a dansyl based reduction sensitive fluorescent probe **73** for the sensing of hydrogen sulfide in aqueous solution.[114] The probe **73** showed selective response for hydrogen sulfide over other 18 anions tested with detection limit of 1 µM in buffer/tween and 5 µM in bovine serum. The linear relationship between fluorescence emission intensity and hydrogen sulfide concentration was observed in buffer/tween as well as in bovine serum. The probe **73** was further applied for the detection of H_2S in blood using C57BL6/J mouse model.

Zhang et al. reported 1,8-naphthalimide-based fluorescent probe **74** for the selective detection of hydogen sulfide.[115] The ratiometric response has been observed upon the addition of Na_2S to probe **74** solution along with fluorescence emission colour change from blue to green. The reaction of Na_2S with probe **74** got completed within 40 minutes. The fluorescence emission intensity showed good linear relationship against concentration of sulfide in PBS buffer and bovine serum. Furthermore, the probe **74** was applied for measuring endogenous hydrogen sulfide in mouse hippocampus.

Tang and coworkers developed coumarin-based chemosensors **75a-b** for the detection of hydrogen sulfide.[116] The probe **75b** exhibited sensitive and selective turn-on fluorescence response in the presence of hydrogen sulfide over other biological relevant species such as ROS, RNS, RSS and anions. The linear correlation was observed between turn-on fluorescence emission intensity and sulfide concentration in PBS buffer and fetal bovine serum. The probe was further utilized for the detection of H_2S in living cells.

Chen and coworkers reported cyanine based fluorescent probe **76** which exhibited turn-on



75a-b

75a: R = H, R' = N_3, R'' = H
75b: R = CH_3, R' = H, R'' = N_3

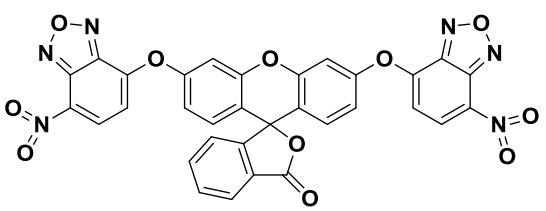
76

77

detection for hydrogen sulfide in aqueous solution and living cells.[117] The blue shifting of λ_{max} occurred from 809 nm to 789 nm in the fluorescence emission spectrum of probe **76** upon reaction with H_2S which is ascribed to the conversion of electron withdrawing nitro unit to electron donating amino unit. Hence the probe **76** undergo nitro group reduction to amine in response to H_2S. Furthermore, confocal microscopy results suggested that the probe **76** was able to detect H_2S level changes in living cells.

Li and Yu coworkers synthesized a reaction-based ratiometric fluorescent chemosensor **77** which can selectively detect H_2S.[118] The incremental addition of H_2S resulted in the decrease of emission intensity at 602 nm with concomitant increase at 482 nm with blue shift of 120 nm. The colour change observed upon the H_2S-mediated reduction of nitro group to amino group was from red to green and the emission gets saturated after 45 min. The fluorescence intensity increase observed was 4765 times upon the complete conversion into amine.

Yi and coworkers reported NBD-based colorimetric and fluorescent "turn-on" probes **78** and **79** for the sensing of H_2S in aqueous buffer at physiological pH value.[119] The probe on reaction with H_2S showed thiolysis of NBD (7-nitro-1,2,3-benzoxadiazole) ether bond. Upon treatment



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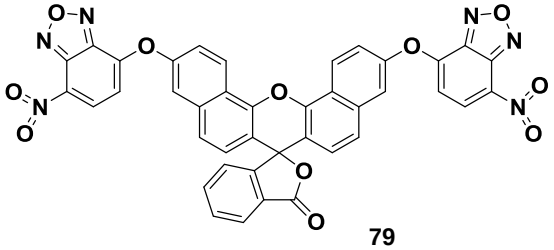
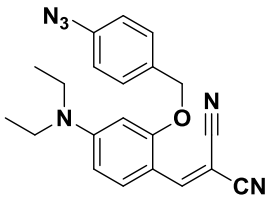


79

of H_2S at pH 7.4, the receptor **78** showed 1000-folds fluorescence enhancement and 77-folds increase with receptor **79**. The addition of H_2S to receptors **78** and **79** showed the green and yellow fluorescence, respectively which indicate the selective visualization of H_2S with naked eye. The small increase in fluorescence was observed with cysteine and glutathione at 1 mM and

other tested species showed no significant response. The receptor **79** acts as near-infrared (NIR) fluorophore for the detection of H_2S at physiological pH. The less photodamage to biological samples and minimal interference from background autofluorescence in living systems made the NIR probe suitable for biological applications.

Talukdar and coworkers reported a benzylidenemalononitrile based fluorescent receptor **80** which was capable of detecting H_2S over other biological thiols, ROS, reducing agents and other biological nucleophiles.[120] The addition of Na_2S induced the reduction of azide to amine group



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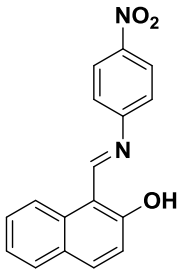

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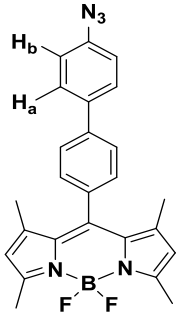

82

followed by a cascade reaction leading to the formation of iminocoumarin fluorophore which was demonstrated from HPLC titration, FTIR and mass specrtal analysis. The treatment of probe with Na_2S showed 31-folds fluorescence enhancement along with the change of fluorescence emission from faint orange to strong blue and the detection limit was found to be 169 nM. Live cell imaging studies were further used to demonstrate the applicability of probe for monitoring H_2S.

Kumar and coworkers reported a d-PET coupled ESIPT based chemosensor **81** for the selective sensing of H_2S among the other sulfur species such as glutathione and cysteine, reactive oxygen species and anions.[121] The reaction of H_2S with probe **81** led to the remarkable fluorescence enhancement at 462 nm with the appearance of blue coloured fluorescence emission which is due to the trammeling of d-PET mechanism. The time dependent fluorescence studies of **81** with H_2S showed 35-folds fluorescence enhancement in 20 min. The test-strip method was also employed indicating the practical applicability of probe **81** for instant visualization of traces of H_2S. Furthermore, the probe **81**was utilized for *in vitro* detection of H_2S in prostate cancer (PC3) cell lines. They have also developed a bodipy based fluorescent chemosensor **82** which selectively detects H_2S.[122] The probe **82** showed quenching of fluorescence at 515 nm upon the

addition of H_2S which is attributed to the photoinduced electro transfer (PeT) from nitrogen atoms to photoexcited bodipy unit. The addition of 60 μM H_2S led to the 80% quenching of fluorescence within 20 minutes. The detection limit for H_2S was calculated to be 35 nM. The probe **82** was further utilized for monitoring H_2S induced apoptosis in living cells which showed the increase in decay time of fluorescent product which is due to restriction of rotation in viscous intracellular matrix.

1.6 Observations drawn from literature:

From the above review of literature, the following conclusions were drawn:

- Rhodamine fluorophores based on xanthene family can act as a promising structure scaffolds for the designing of Fe^{3+} selective fluorescent probes due to their excellent photophysical properties which can induce color and fluorescence change in the presence of analyte species by opening the spirolactam ring and are successfully employed in the fields like biology and medicine.
- Thiacalixarene scaffold is a unique host which can be modified by grafting different chelating agents on upper and lower rims and due to its wide possibility of modification, this is considered as efficient platform for creation of pre-organized structures and supramolecular ensembles.
- Thiacalixarenes are efficient ionophores due to the presence of electron rich sulphur bridges which can bind to transition metal ions and are considered to have better complexation capability than that of classical calixarenes.
- The chemically modified thiacalix[4]arene derivatives can act as an efficient hosts which can encapsulate various types of guest species such as zinc and cyanide ions, either by changing the nature and number of binding sites or by controlling their conformations.
- In addition, scaffolds like naphthalimide, coumarin, fluorescein and BODIPY etc. appended with different recognition sites can be used for the sensing of various types of metal ions, anions and other biological relevant sulfur species.

1.7 Objectives of present work

Based upon the above observations from literature, in the present investigation we have designed and synthesized fluorescent receptors based on thiacalix[4]arene and rhodamine/fluorescein scaffold having different binding sites for sensing of various

biological and environmental important analytes. The results of our findings have been divided into following different chapters.

Chapter 2: Rhodamine based chemosensors for the selective recognition of Fe^{3+} ions

Chapter 3: A **t**hiacalix[4]crown based chemosensor for Zn^{2+} and $H_2PO_4^-$: sequential logic operations at the molecular level

Chapter 4: A new **t**hiacalix[4]arene-fluorescein based probe for detection of CN^- and Cu^{2+} ions and construction of a sequential logic circuit

Chapter 5: Fluorescent probes for the selective detection of H_2S

1.8 References

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