1.1 Introduction
   1.1.1 What is Supramolecular Chemistry?
   1.1.2 History of Calix[n]arene

1.2 Functionalization of Calix[n]arene
   1.2.1 Upper Rim Modification
      1.2.1.1 Formylation
      1.2.1.2 Nitration
      1.2.1.3 Halogenation
      1.2.1.4 Sulphonation
      1.2.1.5 Azo Coupling
   1.2.2 Lower Rim Modification
      1.2.2.1 Etherification
      1.2.2.2 Esterification
   1.2.3 Bridged Calix[n]arene

1.3 Application of Calix[n]arene
   1.3.1 Fluorescent Sensor For Toxic Metal
      1.3.1.1 Calixarene based Fluorescence Sensors
         1.3.1.1.1 Calix[4]arene based PET systems
         1.3.1.1.2 Calix[4]arene based PCT systems
         1.3.1.1.3 Calix[4]arene based FRET systems
         1.3.1.1.4 Calix[4]arene based Excimer formation
         1.3.1.1.5 Literature Survey
1.3.2  Liquid Crystals

1.3.2.1  Calix[4]arene based Liquid Crystals

1.3.2.1.1  Literature Survey

1.3.3  Biological Activities

1.3.3.1  Anticancer Activity

1.3.3.2  Anti-Mycobacterial

1.3.3.3  Anti Bacterial Activity

1.3.3.4  Anti Fungal Activity

1.3.3.5  Anti Proliferative

1.3.3.6  Anti Viral Activity

1.3.3.7  Anti Malarial Activity

1.3.4  Staining

1.3.4.1  Introduction

1.3.4.2  Mechanisms of Staining (Principle)

1.4  Aim and Scope of the Work

1.5  Present Investigation

1.6  References
1.1 Introduction

Much of the inspiration and origin of supramolecular chemistry comes from the chemistry found in living biological systems. Sometimes incredibly complex, sometimes elegantly simple, nature has evolved an enormous amount of highly specific, hierarchical, selective and cooperative chemistry that enables living systems to maintain themselves in a dynamic equilibrium with their environment and to feed, respire, reproduce and respond to external stimuli. In biological chemistry, the supramolecular hosts are the receptor sites of enzymes, genes, antibodies of the immune system, and ionophores. The guests are substrates, inhibitors, co-factors, drugs or antigens. These components variously exhibit supramolecular properties such as molecular recognition, self-assembly, self-organisation, self-replication and kinetic and thermodynamic complementarity. The vast majority of these properties rely upon supramolecular interactions such as ion-ion interactions, ion-dipole interactions, dipole-dipole interactions, hydrogen bonding, cation-pi interactions, anion-pi interactions, pi-pi interactions, van der Waals forces and closed shell interactions [1].

1.1.1 What is supramolecular chemistry?

Supramolecular chemistry has been defined by one of its leading proponents, Jean-Marie Lehn, who won the nobel prize for his work in this area in 1987, as the ‘chemistry of molecular assemblies and of the intermolecular bond’. More colloquially this may be expressed as ‘chemistry beyond the molecule’, other definitions include phrases such as ‘the chemistry of the non-covalent bond’ and ‘non-molecular chemistry’. Originally supramolecular chemistry was defined in terms of the non-covalent interaction between a ‘host’ and a ‘guest’ molecule [2].
Developments in macrocyclic chemistry have led to supramolecular chemistry, a field that has attracted increasing attention among researchers in various disciplines. Notably, the discoveries of new types of macrocyclic hosts have served as important milestones in the field. Researchers have explored the supramolecular chemistry of several classical macrocyclic hosts, including crown ethers, cyclodextrins, calixarenes, and cucurbiturils. Calixarenes represent a third generation of supramolecular hosts after cyclodextrins and crown ethers. Concave macrocyclic scaffolds such as calixarenes, cyclodextrins, cucurbiturils, resorcinarenes, or pillararenes are extensively exploited for the design of molecular receptors. Their selective functionalization is key either for appending a specific molecular recognition site, or the introduction of reporting, sensing, chiral, or water-soluble subunits, or for their grafting on solid materials. However, selective and efficient functionalization of macrocyclic multifunctional platforms is highly challenging. Indeed, control of the chemo-, regio-, stereo-, and iteroselectivity is required. Calix[n]arenes are cyclic oligomers obtained by the condensation of phenol and formaldehyde under basic conditions, and they occupy a privileged position in supramolecular chemistry. Calixarenes, macrocycles composed of phenolic units linked by methylene groups at the 2- and 6-positions, are among the most widely studied organic supramolecular hosts and have been described as having “(almost) unlimited possibilities” because they can be easily modified. They generally serve as simple scaffolds to build podand-like receptors, and the calixarene cavity is exploited only rarely. Cavity complexation is the iconic feature of macrocyclic hosts and is appealing for construction of functional supramolecular architectures. However, the inclusion capabilities of the cavities of unmodified calixarenes are not as good as those of other common macrocycles such as crown ethers, cyclodextrins, and cucurbiturils; extensive
Chapter - 1

Chemical modification of calixarenes is necessary to achieve efficient endo-complexation.

1.1.2 History of calix[n]arene

In 1872, Adolph von Bayer published the first results concerning the products obtained from the reaction of phenol with formaldehyde. In spite of numerous attempts von Bayer was unable to isolate or characterise the products from this reaction, describing them simply as a substance resembling cement [3]. Subsequently in 1894, Lederer and Manasse succeeded in isolating from a similar reaction mixture, as crystalline materials, ortho-hydroxymethyl phenol and para-hydroxymethyl phenol [4, 5]. The presence of both isomers arises from the similar activity in the reaction of both the ortho and para sites of phenol. Zinke and Ziegler in 1944 simplified this problem of reactivity by blocking the para-site and using para-tert-butylphenol. They proposed a cyclic structure for the formed product (Figure 1) [6].

![Figure 1: Structure of calix[4]arene.](image)

In 1957, the first report of a biomedical use of the calixarenes was published by Cornforth [7], in which he described the anti-tubercular properties of ethylene glycol derivatives of calixarenes. At that time, the exact structural nature of the molecules remained undetermined. During the 1980s, a series of NMR studies by Gutsche
allowed both the macrocyclic nature of the calixarenes and the presence of cycles containing four, six and eight phenolic units to be determined [8]. It was also Gutsche who first proposed the trivial name “calixarenes” for these molecules, by structural analogy with the form of the ancient Greek Calix crater vases. Given the complexity of the IUPAC terminology for the calixarenes, the name has remained and is now in general usage. The simplified nomenclature of the calixarenes uses \([n]\) to denote the number of phenolic units in the macrocycle, thus calix[4]arene contains four units. The nature and position of substituents on the aromatic rings are given by sequential numeration and the appropriate term for the function is placed before the term calix[n]arene. Hydroxyl substitution follows again sequential numeration with generally the substituent name placed after calix[n]arene. In Figure 2 are given the formulae and numeration for calix[4]arene, calix[6]arene and calix[8]arene.

**Figure 2**: Types of calix[n]arene
1.2 Functionalization of calix[n]arene

In the past four decades, extensive attention has been devoted to the chemical modification of calixarenes, which has been used to profoundly alter the chemical and supramolecular properties of the parent macrocycles.

The transformations are usually performed by:

I. The functionalization at the para positions of the aromatic rings *ie.* the upper rim.

II. The functionalization at the phenolic hydroxyls *ie.* the lower rim.

III. Modification at bridge carbon atom

1.2.1 Upper Rim Modification

Modification of the upper rim of calixarenes involves substitution at the position para to the phenolic oxygen [9]. The tert-butyl group can be removed by an AlCl$_3$ catalyzed reaction in the presence of an acceptor such as phenol or toluene [10]. After removal of the tert-butyl group electrophilic substituent can be added to the para position. Substitution at this position includes halogenations [11-12], nitration [13], sulfonation [14], sulfochlorination [15], acylation [16], chloromethylation [17], aminomethylation [18-19] and diazo coupling [20]. Nitration [21] and sulfonation [22] have also been carried out by ipso-substitution of the tert-butyl groups can also be obtained. Once these substituent’s have been introduced they can undergo further reactions such as reduction of the nitro groups [23], aryl-aryl Suzuki coupling [24] to name a few examples.

1.2.1.1 Formylation

Thiacalix[4]arene immobilized in the cone conformation undergoes a direct Gross formylation reaction (Cl$_2$CH-O-CH$_3$/SnCl$_4$/CH$_2$Cl$_2$) to give the upper-rim formylated thiacalixarene [25]. Albeit using excess of the formylation agent and various reaction temperatures, only one formyl group is introduced into the meta position of the

1.2.1.2 Nitration

Proximally dietherified calix[4]arene at lower rim was selectively nitrated to give mononitrocalix[4]arene, dinitrocalix[4]arene, and trinitrocalix[4]arene, respectively, under different reaction conditions (Figure 3). These three nitrocalix[4]arenes could be purified in good yield just by recrystallization, which provided a concise approach to inherently chiral calixarene. It was disclosed by crystal structure of trinitrocalix[4]arene that R-enantiomer and S-enantiomer of it could form a self-included dimer by NO$_2$–HAr hydrogen bond and CH$_3$–Pi interaction in the cavity of the calixarene [28]. Lhotak et al. [29] described novel protection/deprotection method leading to the regioselective ipso-nitration of calix[4]arenes. Nitration of thiacalix[4]arene, immobilized in the 1,3-alternate conformation, leads regioselectively to meta-substituted products. Depending on the reaction conditions, mono- and dinitro-derivatives can be isolated in acceptable yields. This unique substitution pattern is inaccessible in classical calixarene chemistry, and yields inherently chiral compounds, which makes thiacalixarenes very attractive as building blocks or molecular scaffolds [30]. Chawla et al. [31] Prepared p-nitrocalix[n]arene methyl ethers via ipso-nitration and studied crystal structure of tetramethoxytetra-p-nitrocalix[4]arene. It has been determined that amongst different nitration procedures
adopted (AlCl₃/KNO₃/HNO₃/CH₃COOH, HNO₃/(CH₃CO)₂O, cerium(IV)ammonium nitrate/CH₃COOH), ipso-nitration with CH₃COOH/HNO₃ gives best yields of p-nitrocalixarenes. An efficient one step one pot synthesis of p-nitrocalix(n)arenes by ipso nitration of p-tert-butylcalix(n)arenes is also described [32].

Figure 3 : Selective ipso nitration of 1,2-dipropyl etherified calix[4]arene

1.2.1.3 Halogenation

Halogenation of thiacalix[4]arenes immobilized in the cone and in the 1,3-alternate conformations revealed substantially different reactivity of these conformations. While the 1,3-alternate afforded only meta substitution, the halogenations of the cone conformation yielded a mixture of two regioisomers located at the meta or para positions [33]. Quantum chemical calculations support the finding that the regioselectivity can be controlled by utilizing kinetic or thermodynamic conditions,
which is unprecedented in calixarene chemistry. As halogen-substituted thiacalix[4]arenes are potentially useful synthetic intermediates, the unique opportunity to functionalize the basic thiacalix[4]arene skeleton at two different positions was achieved. Kalchenko et al. [34] described synthesis and structure of new tetrahydroxythiacalix[4]arenes, existing in the cone conformation and possessing reactive bromide, chloromethyl or diorganylphosphoryl groups on the upper rim of the macrocycle. The molecular structure of tetrakis(diisopropoxyphosphorylmethyl) thiacalix[4]arene was examined by X-ray crystallography. The utility of the compound was demonstrated by the synthesis of cone-shaped tetraphosphoryl derivatives, promising receptors for metal cations or organic molecules. Chawla et al. [35] studied bromination of p-tert-butylcalix[n]arenes under different conditions (Figure 4) which can be used for the preparation of ring or methylene bridge substituted products as identified and confirmed by physical and chemical data.

![Bromination of calix[4]arene](image)

**Figure 4**: Bromination of calix[4]arene
1.2.1.4 Sulphonation

Chawla et al. [36] prepared calix[n]arene sulphonic acids (n= 4, 6, 8) by direct ipso substitution of respective p-tert-butylcalix[n]arenes and their methyl ethers. Shinkai et al. [37] prepared calixarene-p-sulphonates (tetramer, hexamer, and octamer) (75-88% yield) and used to give p-nitrocalixarenes (15-22% yields based on the starting calixarenes). The novel nitration has two advantages: (i) calixarenes are protected from nitric acid oxidation by the electron-withdrawing sulphonate groups and (ii) the difference in the water-solubility of the p-nitrocalixarenes from the sulphonate-containing, lower nitration products, allows ready isolation of the former. The p-nitrocalixarenes thus obtained have potential as intermediates in the synthesis of a variety of functionalised calixarenes. Association equilibrium constants and thermodynamic parameters (i.e., $\Delta H$ and $\Delta S$) for complexation between N,N-dimethylindoaniline and p-sulphonated calix[n]arenes in aqueous solution have been determined by means of UV–VIS spectroscopy. Equilibrium constants corresponding to calix[n]arene-p-sulfonates increase in the order $[4] < [6] \approx [8]$. $\Delta H$ and $\Delta S$ values, which are interpreted in terms of the importance of the various interactions responsible for complexation, lead to a linear $\Delta H - T\Delta S$ relationship. Such a linear correlation is interpreted in terms of the extent of calixarene conformational change and of host and guest desolvation involved in the complexation process [38]. The sulfonatocalixarenes are important in their own right as water soluble compounds but can also serve as intermediates for additional functionalization, generally by conversion to a sulfonamide. Treatment of p-sulfonatocalixarenes with SOCl$_2$, yields the chlorosulfonyl compound [39] (Figure 5).
1.2.1.5 Azo coupling

Deligoz et al. [40] described synthesis, extraction and chromogenic properties of calix[4]arenes carrying phenylazo and amido groups on their upper and lower rims respectively. Novel azocalix[4]arene amides and some of their telomers have been synthesised and characterised by spectroscopic methods as well as elemental analysis techniques. The colour changes of the resulting solutions can be observed by the ‘naked eye’. Metal extraction abilities of compounds have been investigated. Telomer structures of azocalix[n]arenes exhibited higher extraction rates compared to those of their monomers. And also studied the preparation of azocalix[4]arene anthracenate derivatives that could form complex with Hg$^{2+}$ in which four diazo coupled and one anthracenate calixarene moieties are united in one molecule. Furthermore, the anthracenate ligands and their mercury complex, both have solvent dependent UV/vis spectra (solvatochromicity) which can be suitable for a variety of ICT based “on-off” or “off-on” type of chemosensor materials [41]. Menon et al. [42] prepared series of azocalix[4]arene dyes by linking 2,4-di-chloroaniline, 2,4,5-tri-chloroaniline, 2,4-di-nitroaniline, 2-nitro p-toluidin, 4-nitro o-toluidin, 5-nitro o-toluidin and sulfanilic acid, to calix[4]arene through a diazo-coupling reaction (Figure 6). The absorption properties of the synthesized dyes were studied and the application of the water
soluble dyes on cotton and wool was investigated. Solvent based inks were investigated and the fastness properties of formulated inks were also discussed.

![Chemical Reaction Diagram]

Figure 6: o-, m- and p-substituted azocalix[4]arene derivatives

1.2.2 Lower rim Modification

The lower rim of calix[4]arenes has been subjected to fewer modification, but the applications of calix[4]arenes substituted at the lower rim are far greater than those substituted at the upper rim. The phenolic hydroxyl groups at the lower rim of the calixarenes represent an excellent reactive function for the introduction of groups which modify the shape and the complexing properties of these molecules. Preliminary work on the lower rim of calixarenes was started with alkylation and acylation reactions which have been reviewed extensively in text books [43-45] and review articles [46-48]. The lower rim can also be easily modified with heterocyclic moieties or other organic molecules and an appropriate expansion of the cavity after substitution can be achieved. The research put forth over here shows that after the structural modification of calix[4]arene at the lower rim by etherification and esterification; further substitution on the lower rim is possible to design molecules for
specific applications like biological activity, liquid crystal etc. The lower rim modifications adopted for the present work are reviewed below:

### 1.2.2.1 Etherification

Calix[4]arenes react with alkyl halides and aqueous potassium hydroxide in the presence of PEG as phase transfer catalyst at room temperature to give the selectively distally dietherified product in excellent yields. While at the same reaction conditions, calix[8]arene give the fully etherified product [49]. Partial etherification of phenolic-OH groups of calix[4]arenes with various alkyl halides/tosylates and K$_2$CO$_3$ under microwave irradiation afforded 1,3-dialkoxy calix[4]arenes in their cone conformation only as predominant/sole product in good yields (71–85%). The protocol was found to be much superior to conventional heating both in terms of yield and reaction time. Some of the 1,3-dialkoxy calix[4]arenes were elaborated further to the syntheses of cesium selective calix[4]crown-6 ionophores [50].

![Figure 7: Etherification of calix[4]arenes under microwave irradiation.](image-url)
1.2.2.2 Esterification

Calix[4]arenes reacted with acid chlorides at room temperature in the presence of triethylamine to give the selectively diametrical diesterified products in good to excellent yields. While at the same reaction conditions, calix[8]arenes gave the fully esterified products [51]. Deligoz et al. [52] have prepared a new family of azocalix[4]arene tetraester derivatives with the incorporation of ethyl ester units to azocalix[4]arene. Characterization of the synthesized azocalix[4]arenes was carried using elemental analyses, UV–vis, FT-IR and \(^{1}H\) NMR spectroscopic techniques.

Figure 8: Esterification of p-tert-butylcalix[4]arene
1.2.3 Bridged Calix[n]arenes

Lhotak et al. [53] described novel method for the intramolecular bridging of calix[4]arenes, based on the reductive coupling of dialdoximes. Reaction of the distal dialdoxime, immobilized in the cone conformation, using Zn/TiCl4 afforded a calixarene with a 1,2-diaminoethane bridge and possessing meso stereochemistry. Meso-Diamino-bridged calixarenes represent unique synthetic intermediates with possible application in the design of new receptors and also regioselective derivatization via an organomercury intermediate allowed for the introduction of carboxylic acid functionality into the meta position of the calix[4]arene skeleton. Intramolecular Friedel–Crafts cyclization led to a novel type of calixarene containing a ketone bridging moiety. Subsequent attack of the ketone by organometallic compounds occurred selectively from outside providing tertiary alcohols with the OH group oriented inside the cavity [54]. Azov et al. [55] studied the synthesis of several calix[4]arene derivatives with tetrathiafulvalene (TTF) bridges at the upper rim.

Figure 9: Various bridge calixarenes
1.3 Application of calix[n]arene

Literature survey reveals that calix[n]arenes have been used for a multitude of applications, e.g., in liquid crystal display [56], polymer synthesis [57], electrochemical sensors [58], optical sensors [59], chiral recognition devices [60], as HPLC stationary phases [61], in electroanalytical techniques such as voltammetry and potentiometry [62], chromatography [63], enzyme mimics [64], ion channels [65], self assembling monolayers [66], catalysis [67], ion selective electrodes [68], phase transfer catalysts [69], drug delivery [70], fluorescent sensors [71], metal ion sensors [72], DNA chip technology/biosensing technology, biotechnology, biology, drug discovery [73], liquid liquid extraction [74], liquid membrane transport [75] and solid lipid nanoparticles [76].

However, the most promising applications of calix[4]arenes identified for investigation in the present study are based on their binding ability for recognition of cations resulting in the application as fluorescent sensors, as liquid crystals and as medicinal agents and hence we have focused our literature survey on these applications. Moreover, we have also explored the possibilities of applying calix[4]arene and calix[4]resorcinarene dyes as staining agent which may open up an innovative field of application of calixarene chemistry.
1.3.1 Fluorescent Sensor For Toxic Metal

Fluorescent devices for the sensing and reporting of chemical species are currently of significant importance in chemistry, biology, and environmental science [77]. The design and synthesis of a sensitive and selective fluorescent sensor is a fundamental goal for organic and analytical chemists [78]. The development of practical fluorescent chemosensors for many heavy and transition metal (HTM) ions is still a challenge. First, many of HTM ions are known as fluorescence quenchers via enhanced spin-orbit coupling [79], or energy or electron transfer [80], and the quenching is not only disadvantageous for a high signal output upon complexation but also hampers temporal separation of spectrally similar complexes with time-resolved fluorometry. Second, the most common fluorescent probes undergo nonspecific quenching with HTM analytes, such as Hg$^{2+}$ and Cu$^{2+}$ [78b]. Third, although HTM ions are relatively easy to chelate and detect in organic solvents, they are rather difficult to recognize directly in aqueous environments due to their strong hydrations. This limitation needs to be addressed when designing an HTM sensor for biological and environmental applications [81].

Arsenic is a toxic metalloid found widely in nature [82]. The detection of which is of prime importance in the present context. The arsenic content of the Earth’s crust is 1.8 ppm; in the soil, it is 0.2–40 ppm with an average concentration of 5 ppm [83]. Volcanic materials contain arsenic at 20 ppm; the average concentration of arsenic in seawater is approximately 3 ppm [84]. Arsenic is released into the atmosphere mainly from the weathering of rocks, from the soil, and from plants; the estimated annual emission of arsenic into the atmosphere from the Earth’s surface is approximately $2.37 \times 10^7$ kg [85]. Methylation of arsenic by soil microbes generates volatile methylated arsenic species, having the general formula $(\mathrm{CH}_3)_n \mathrm{AsH}_{(3-n)}$ ($n = 1–3$), that
are released into the atmosphere [86]. Plants intake arsenic through their roots; after conversion, they also discharge the methylated arsenic species \((\text{CH}_3)_2\text{AsH}\) and \((\text{CH}_3)_3\text{As}\) into the air [87]. These compounds react with \(\text{O}_2\) to produce \((\text{CH}_3)_2\text{AsO}\) and \((\text{CH}_3)_2\text{As(OH)}\), which further react with ozone (\(\text{O}_3\)) and \(\text{N}_2\text{O}_4\) to generate \(\text{As}_4\text{O}_6\), which reenters the soil and is then converted back to \(\text{HAsO}_2\) [88]. Arsenic can flow into lakes, rivers and groundwater through the action of rain or wastewater discharge [89]. Arsenic can cause acute and chronic poisoning, including abdominal pain, bloody diarrhea, acute renal failure and neuropathy, muscle weakness, skin keratinization, pigmentation, and cancer (lung, liver, bladder, and skin cancer) [90]. In addition, arsine (\(\text{AsH}_3\)) gas, which is used commonly in the electronics industry and has a garlic flavor, readily induces hemolysis and then death through acute renal failure [91].

### 1.3.1.1 Calixarene Based Fluorescence Sensors

Cations and anions play an important role in a wide range of chemical reactions, including biological metabolism as well as many other processes. For the purpose of detection and quantitative determination of ions, much effort has been devoted to the development of appropriate chemosensors. The main issue in the design of any effective chemosensor is the association of a selective molecular recognition event with a physical signal highly sensitive to its occurrence. Changes in both the absorption and emission of light can be utilized as signals provided appropriate chromophores or fluorophores are available and two important classes of sensors are those of the optical and fluorimetric types. While spectrophotometry and fluorimetry are both relatively simple techniques which are rapidly performed, nondestructive and suited to multicomponent analysis, fluorimetry is commonly considered superior, principally because of its greater sensitivity. In general, for fluorimetric determination
of cations or anions, any sensor must include two components, an ionophore and a fluorophore, which can be independent species or covalently linked in one molecule. The ionophore is required for selective binding of the substrate, while the fluorophore provides the means of signaling this binding, whether by fluorescence enhancement or inhibition. The construction of sophisticated molecular sensors may be considered a basic exercise in macrocyclic chemistry since the fundamental requirements of high sensitivity and optimal selectivity can only be met by the combination of large molecular units, and calixarenes are well-recognized as appropriate such units [92-108].

Calix[4]arenes, in particular, have been prepared and characterized in all four possible conformations with an unexpected variety of substituents, one of the major purposes of these synthesis being the use of the product ligands in many areas of both cation and anion coordination chemistry. Molecular sensors conglomerate the properties of supramolecular receptors, selective for a given guest, with the ability to produce a measurable signal in response to binding. Optical signals reflecting changes in absorption or fluorescence are the most frequently exploited because of the simplicity and low cost of the methods required. Absorption, often seen in the visible region as obvious colour changes, can commonly provide immediate identification of an analyte, while fluorescence can provide ready detection even at nanomolar concentrations. Fluorescent chemosensors which combine such sensitivity with selectivity can, in the case of metal ions, for which calixarenes already have established uses, be particularly valuable in biological and environmental analysis. Suitably modified calixarene unit can be used as an ionophore and covalently linked photoactive organic molecules or poly pyridyl based metal complexes can act as fluorophore [109-117]. Upon binding of metal ion by ionophore the electronic
communication between the chromophores may take place in several ways, such as photoinduced electron transfer (PET) [118-124], photoinduced charge transfer (PCT) [125-132], fluorescence resonance energy transfer (FRET) [133-135] etc. A brief description of each category with the examples available in the literature is given below.

1.3.1.1 Calix[4]arene based PET Systems

In the simplest cases, emission of a photon, fluorescence, follows HOMO to LUMO excitation of an electron in a molecule. Where this emission is efficient, the molecule may be termed a fluorophore. Vibrational deactivation of the excited state prior to emission usually gives rise to a “Stokes shift” in that the wavelength of the emitted radiation is less than that of the exciting radiation. Various other interactions may also modify the emission process, and these are of considerable importance in regard to analytical applications of fluorescence. Thus, when a lone electron pair is located in an orbital of the fluorophore itself or an adjacent molecule and the energy of this orbital lies between those of the HOMO and LUMO, efficient electron transfer of one electron of the pair to the hole in the HOMO created by light absorption may occur, followed by transfer of the initially excited electron to the lone pair orbital. Such PET provides a mechanism for non-radiative deactivation of the excited state (Figure 10), leading to a decrease in emission intensity or “quenching” of the fluorescence.

![Figure 10: Mechanism of photo induced electron transfer (PET) for recognition of guest ions.](image-url)
1.3.1.1.2 Calix[4]arene based PCT Systems

Electronic excitation necessarily involves some degree of charge transfer, but in fluorophores containing both electron-withdrawing and electron-donating substituents, this charge transfer may occur over long distances and be associated with major dipole moment changes, making the process particularly sensitive to the microenvironment of the fluorophore. Thus, it can be expected that cations or anions in close interaction with the donor or the acceptor moiety will change the photophysical properties of the fluorophore.

Figure 11: Mechanism of photon charge transfer (PCT) for recognition of guest ions. Upon, for example, cation complexation of an electron donor group within a fluorophore, the electron-donating character of the donor group will be reduced. The resulting reduction of conjugation causes a blue shift of the absorption spectrum together with a decrease of the molar absorptivity. In contrast, metal ion binding to the acceptor group enhances its electron-withdrawing character, and the absorption spectrum is thus red-shifted with an increase in molar absorptivity. The fluorescence spectra should be shifted in the same direction as the absorption spectra, and in
addition to these shifts, changes in the quantum yields and lifetimes can be observed. All these photophysical effects are obviously dependent on the charge and the size of the cation, and therefore, some selectivity is expected.

### 1.3.1.1.3 Calix[4]arene based FRET Systems

FRET arises from an interaction between a pair of dissimilar fluorophores in which one acts as a donor of excited-state energy to the other (acceptor). This returns the donor to its electronic ground state, and emission may then occur from the acceptor center. FRET is influenced by three factors: the distance between the donor and the acceptor, the extent of spectral overlap between the donor emission and acceptor absorption spectrum and the relative orientation of the donor emission dipole moment and acceptor absorption moment.

### 1.3.1.1.4 Calix[4]arene based Excimer formation

Where aromatic rings are involved in weak interactions (such as $\pi$-stacking) which bring them within van der Waals contact distances, electronic excitation of one ring can cause an enhanced interaction with its neighbor, leading to what is termed an excited-state dimer or “excimer”. In other words, an excimer is a complex formed by the interaction of an excited fluorophore with another fluorophore in its ground state. Excimer emission typically provides a broad fluorescence band without vibrational structure, with the maximum shifted, in the case of most aromatic molecules, by about 6000 cm$^{-1}$ to lower energies compared to that of the uncomplexed (“monomer”) fluorophore emission. An excimer may also form from an excited monomer if the interaction develops within the lifetime of the latter. Thus, it is expected that excimers are more likely to be produced by relatively long-lived monomer excited states. Rates of fluorophore diffusion, especially in viscous solvents, are therefore another limit on excimer formation. Importantly, the separation and relative orientation of multiple
fluorophore units attached to ligands can be controlled by metal ion coordination, so that recognition of a cation can be monitored by the monomer:excimer fluorescence intensity ratio.

1.3.1.1.5 Literature survey

Figure 12: Some of the previously reported fluoroionophore based on calix[4]arene scaffold.

C. P. Rao et al. extensively studied [136-145] fluorescence behavior of calix[4]arene with different linking group. Figure 12: (A) an in situ prepared Zn$^{2+}$ complex of triazole linked imino thio phenyl conjugate of calix[4]arene, [ZnL], was demonstrated to be highly fluorescent in HEPES buffer solution. [ZnL] has been used as a chemosensing ensemble for the recognition of phosphates in general and pyrophosphates in particular among the eighteen different anions studied. In
accession, a reversible “write-read-erase-read” logic gate property of L has been demonstrated through a feedback loop in the presence of Zn\(^{2+}\) and PPi. (B) A N,N-dimethylamine ethyl imino appended triazole-linked calix[4]arene conjugate, L, has been synthesized and characterized, and its Cd\(^{2+}\) complex has been isolated and characterized. (C) The triazole linked o-imino phenol appended calix[4]arene conjugate (L) has been synthesized and characterized. The binding and recognition behavior of conjugate, L toward the transition metal ions, such as Mn\(^{2+}\), Fe\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\) and Zn\(^{2+}\) has been demonstrated using fluorescence, absorption and ESI-MS techniques. (D) Carboxamido quinoline appended calix[4]arene-1,3-di-conjugate (L) has been synthesized and characterized and its single crystal XRD structure has been established. L has been shown to act as selective ratiometric turn-on fluorescence sensor for Zn\(^{2+}\) up to a lowest concentration of 183±18 ppb (2.82 μM) with a nine-fold enhancement by exhibiting blue-green emission. (E) The structurally characterized lower rim 1, 3-di {4-antipyrine} amide conjugate of calix[4]arene (L) exhibits high selectivity toward Hg\(^{2+}\) among other biologically important metal ions, viz., Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\), Mg\(^{2+}\), Mn\(^{2+}\), Fe\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Cd\(^{2+}\), Hg\(^{2+}\), Pb\(^{2+}\) and Ag\(^{+}\) as studied by fluorescence, absorption, and ESI MS. L acts as a sensor for Hg\(^{2+}\) by switch-off fluorescence and exhibits a lowest detectable concentration of 1.87±0.1 ppm. (F) A calix[4]arene conjugate bearing salicylyl imine having dibenzyl moiety (L) has been synthesized and characterized, and its ability to recognize three most important essential elements of human system, viz., iron, copper, and zinc, has been addressed by colorimetry and fluorescence techniques. L acts as a sensor for Cu\(^{2+}\) and Fe\(^{2+}\) by exhibiting visual color change and for Zn\(^{2+}\) based on fluorescence spectroscopy. L shows a minimum detection limit of 3.96 and 4.51 ppm and 45 ppb, respectively, toward Fe\(^{2+}\), Cu\(^{2+}\) and Zn\(^{2+}\). The in situ prepared [ZnL] exhibits
phosphate sensing among 14 anions studied with a detection limit of 25 ppb. \( \text{(G)} \) A benzimidazole appended triazole linked 1, 3-diconjugate of calix[4]arene (L) has been synthesized and characterized. The conjugate L has been found to recognize Cu\(^{2+}\) among the thirteen different metal ions studied by exhibiting ratiometric fluorescence changes through newly generated excimer band at \( \sim 380 \) nm. Fluorescence off–on–off behavior has been clearly demonstrated on the basis of the binding variability of Cu\(^{2+}\) to L.

![Figure 13](image1.png)

**Figure 13** : Some of the previously reported fluoroionophore based on calix[4]arene scaffold.

Kim et al. [146-150] have comprehensively studied and reported various fluoroionphore for recognition of cations and anions with different fluorescence
mechanism. **Figure 13: (A)** A photoinduced charge transfer (PCT)-based 1,3-alternate calix[4]crown fluoroionophore containing two cation recognition sites, a crown ether ring and two facing pyreneamide groups, is synthesized. Upon addition of K\(^+\), Pb\(^{2+}\), or Cu\(^{2+}\), wavelength changes are observed in both the fluorescence and absorption spectra, but with different binding modes. With K\(^+\), fluorescence emissions of the ligand scarcely change, while addition of Pb\(^{2+}\) or Cu\(^{2+}\) produces a remarkable change in both the excimer and monomer emissions. The observed data indicate that the metal cation is encapsulated in the crown-5 ring for K\(^+\) and by the two facing amide groups in the latter case, which is verified by a metal ion exchange experiment. The wavelength shifts in both fluorescence and absorption spectra upon addition of Cu\(^{2+}\) show that, in contrast to Pb\(^{2+}\), Cu\(^{2+}\) interacts with the nitrogen atoms of the amide groups, resulting in a PCT mechanism. Calix[4]azacrown (B), bearing an anthracenyl unit, was reported to have a pronounced chelation enhanced fluorescence (CHEF) effect with Cs\(^+\), Rb\(^+\), and K\(^+\) ions. Compound (C) (Figure 13) bearing chromophores capable of two-photon absorption has been synthesized by Kim et al. 1,3-Alternate calix[4]arene-based fluorescent chemosensors bearing two-photon absorbing chromophores have been synthesized, and their sensing behaviors toward metal ions were investigated via absorption band shifts as well as one- and two-photon fluorescence changes. Free ligands absorb the light at 461 nm and weakly emit their fluorescence at 600 nm when excited by UV-vis radiation at 461 nm, but no two-photon excited fluorescence is emitted by excitation at 780 nm. Addition of an Al\(^{3+}\) or Pb\(^{2+}\) ion to a solution of the ligand causes a blue-shifted absorption and enhanced fluorescence due to a declined resonance energy transfer (RET) upon excitation by one- and two-photon processes. Anion sensing based on PCT has as yet been developed much less than that of cations, but recently, Kim et al. reported that with
two fluorescent pyrenes and with two coumarins can be used to selectively sense F⁻ ion (D). When excited at 346 nm, shows monomer and excimer emissions at 385 and 482 nm, respectively. The addition of F⁻ to compound E in CH₃CN induces a 54 nm red shift of the absorption band and a 12 nm blue shift of the excimer emission band with an enhancement of the fluorescence intensity, the latter shift being due to formation of a static excimer in the ground state. When compound E is excited at 335 nm, it exhibits an emission band at 420 nm, which is quenched upon complexation of F⁻ in CH₃CN to give rise to a weak band at 508 nm. The addition of F⁻ to compound E causes a decrease in emission intensity together with a red shift, which results from H-bonding followed by protonation.

Menon and co-workers [151] studied Figure 14 : (A) novel structurally simple calix[4]arene appended 8-amidoquinoline linked conjugate which has been used as turn-on fluorescence probe for Zn²⁺ and turn off for F⁻. Moreover, this probe has been applied for Zn²⁺ detection in blood serum upto 8.7 µM and fluoride upto 22 nM in waste water sample by emission spectra. (B) Fluorescence switch on-off-on receptor constructed on quinoline allied calix[4]arene was synthesized for selective recognition of Cu²⁺ from blood serum and F⁻ from industrial waste water. The detection limit of synthesized receptor was found to be 4.16 nM for Cu²⁺ and 2.15 nM for F⁻ [152]. (C) A highly efficient PET switch on-off-on fluorescence receptor was reported based on calix[4]arene for selective recognition of Cd²⁺ and Sr²⁺. The detection limit of synthesized receptor was found to be 0.94 pM for Cd²⁺ and 1.04 pM for Sr²⁺. Moreover, this probe has been applied for recognition Cd²⁺ and Sr²⁺ from waste water [153]. (D) A novel photoinduced electron transfer (PET) based substituted calix[4] arene fluoroionophore has been used for the selective recognition of tryptophan (L-Trp) and histidine (L-His) by emission spectroscopy. The detection limit of the
synthesized receptor was found to be 0.00826 nM for L-Trp and 0.00158 nM for L-His. Moreover, this probe has been applied for the recognition of L-Trp and L-His from blood serum [154]. (E) A pyrenyl linked calix[4]arene fluroionophore has been synthesized and used as a ditopic chemosensing ensemble for Fe$^{3+}$ and H2PO$^4$ using emission spectra. The detection limit of the synthesized receptor was found to be 0.88 pM for Fe$^{3+}$ and 1.11 pM for H2PO$^4$. Moreover, this probe has been applied for recognition of Fe$^{3+}$ in blood serum and H2PO$^4$ in waste water [155].

![Figure 14: Some of the previously reported fluroionophore based on calix[4]arene scaffold by our research group.](image.png)
1.3.2 Liquid Crystals

Liquid crystals (LCs) are self-assembled dynamic functional soft materials which possess both order and mobility at molecular, supramolecular and macroscopic levels [156–158]. Liquid crystals beautifully demonstrate the powerful general organization principle of matter by maximizing the interaction energy and minimizing the excluded volume. LCs exhibit extreme sensitivity to small external perturbations such as electric and magnetic fields, and surface effects, which is the basis for their information display applications [159]. However, high-tech high-performance ‘hard’ materials like Kevlar which act as energy-absorbing bullet-proof guards used by defence personnel and law enforcing agents, have been obtained from ‘soft’ liquid-crystalline materials [160]. Liquid crystals are prototype self-assembling materials since they involve almost all kinds of supramolecular interactions such as van der Waals interaction, dipolar and quadrupolar interactions, charge transfer and p–p interaction, metal coordination, hydrogen bonding etc [159]. The domain of liquid crystals spans across multiple disciplines of pure and applied science including bioscience and materials science [159].

The liquid-crystalline state of matter exists between the crystalline solid and amorphous liquid states. For this reason LCs are referred to as intermediate phases or mesophases. However, the LC states are true thermodynamic stable states of matter like solids, liquids and gases. Hence LCs are also often called as the fourth state of matter. Liquid crystals share the anisotropic (direction dependent) properties of crystalline solids and fluid properties of isotropic liquids. The constituents of the mesophases are called mesogens and the mesogens can be organic, inorganic and organometallics in nature [159]. Molecular shape, microsegregation of incompatible parts, specific molecular interactions, self-assembly and self-organization are
important factors that drive the formation of various LC phases in matter. Although there are various ways of classifying LCs depending on molecular features (i.e. calamitic, discotic and bent-core) and supramolecular assemblies (i.e. nematic, smectic, columnar etc.); the most commonly used classification is based on how the liquid-crystalline phases have been obtained, i.e. thermotropic and lyotropic phases. Thermotropic LC phases are obtained by the effect of temperature on pure compounds or a mixture of compounds either by heating the crystalline solid or by cooling an isotropic liquid. On the other hand, lyotropic liquid-crystalline (LLC) phases are achieved by dissolving amphiphilic compounds in suitable solvents over a range of concentrations and temperature.

Liquid crystals are even more promising because of their important role in biology [161]. In a more general sense, the combination of order and mobility, as exhibited by liquid crystals, is a basic requirement for self-organization and structure formation in living systems. Major classes of biological compounds including lipids, proteins, carbohydrates and nucleic acids have been found to exist in various liquid crystalline phases in vivo as well as in vitro under well-defined conditions [161]. Their liquid-crystalline structure is believed to have a very important role in their biological functions and self-assembly processes [161]. Moreover, LCs possess various properties analogous to living cells such as amplification and transmission of information and properties [157]. Like living cells, they also respond to a large number of external stimuli such as light, heat, electric and magnetic fields as well as to the changes in chemical environment [159]. Furthermore, the existence of liquid-crystalline phases of large and small DNAs in vivo and in vitro has already been related to the significant role they would have played in the evolution of biological information in the prebiotic world [162].
Though liquid crystals are primarily known for their technological applications in liquid-crystal display (LCD) devices for information displays, nevertheless the ‘beyond display’ applications of liquid crystals are equally important and numerous. Liquid crystals can potentially be used as new functional materials for electron, ion, molecular transporting, sensory, catalytic, optical and bioactive materials [156]. Recently, their biomedical applications such as in controlled drug delivery, protein binding, phospholipid labeling and in microbe detection have been demonstrated [163]. Owing to their dynamic nature, photochemically, thermally or mechanically induced structure changes of liquid crystals can be used for the construction of stimuli-responsive multifunctional materials [156].

Liquid crystals are now playing a very significant role in nanoscience and nanotechnology [164–167]. Liquid-crystal nanoscience primarily deals with the synergetic relationship between liquid crystals and nanomaterials. Nanoscale particles do not induce significant distortions of LC phases. Therefore various nanomaterials have been dispersed and studied in LC medium to enhance the physical properties of LCs [165]. Moreover, alignment and self-assembly of nanomaterials themselves can be achieved in LC phases [164]. Liquid crystals act as tunable solvents for the dispersion of nanomaterials, and being anisotropic media, they provide a very good support for the self-assembly of nanomaterials into larger organized structures in multiple dimensions. Hence LC mediated self assembly can be efficiently used to organize different kinds of nanomaterials into soft and well-defined functional superstructures [165]. Furthermore, since the liquid-crystal medium responds to small external stimuli, the dispersed materials can be forced to follow the order of the host medium which elaborates the controlled dynamic self-assembly and (re)orientations of the dispersed nanomaterials. It is interesting to note that LC nanoscience provides
equal opportunity and credit to both thermotropic and lyotropic LCs, unlike materials science and life science which give preferences to the former and latter respectively. This deals with liquid-crystalline nanomaterials, nanomaterials in liquid crystals, as well as synthesis of nanomaterials using LCs as ‘templates’ or ‘precursors’.

Figure 15: Schematic design of crystal, liquid and liquid crystal

1.3.2.1 Calix[4]arene based Liquid crystals

Calixarenes are the third generation supramolecular platforms after crown ether and cyclodextrin [168,169]. Many researches were focused on syntheses and complexation properties of various calixarene derivatives. Some of calixarene-based liquid crystals were also studied by introducing the long alkyl chain on the upper or lower rim of calixarene skeleton [170-175]. Yang et al. reported the first example of calixarene-linked triphenylene dimers bridged by long alkyl chain [176].

Azo and ester functionalized materials are well known for their effectiveness in optoelectronic devices, food additives, indicators, photochemical molecular switches and therapeutic agents. Photochromic behaviour of azo compounds improves the activity of enzymes and polypeptides in biological system. There have been several
research articles in which calix[n]arene based LC are reported [177-187]. Previously our group have worked on crown ether and calix[4]arene based Liquid crystals [188]. Calix[4]arene being rigid core, it is easy to functionalize at hydrophilic hydroxyl groups with different linkage and various flexible aliphatic chains. It was found that calix[4]arene can act as “amorphous molecule” with bulky substituents on lower rim and was thus useful as a new positive type photoresist. Therefore, the introduction of long aliphatic side chains into calix[4]arene moiety would contribute “amorphous molecule” with LC properties. Calix[n]arene gives an epitome platform with unlimited freedom in regioselective and stereoselective derivatisation with easy modification at the upper rim and lower rim by means of non-covalent interaction, intermolecular forces and hydrogen bonding. These results motivated us for the extension of liquid crystal work and explore more mesogens in calix[4]arene system.

1.3.2.1.1 Literature survey

H. Guo et al. [189] have recently reported novel supramolecular liquid crystals based on mesomorphic properties of calix[4]arene-cholesterol derivatives. F. Yang et al. [190] synthesized and studied mesomorphic properties of all kinds of calixarene liquid crystals which have been discussed and reviewed. There are two methods to prepare calixarene liquid crystals. One way is to introduce the corresponding functional groups with long alkyl chains, which usually afford columnar liquid crystal with a rigid bowlic core. The organization of liquid crystals possesses interesting changes after the complexation of guests. Another way is to introduce triphenylene unit to obtain calixarenes bowlic column with triphenylene units as ancillary lateral columns. M. Warenghem et al. [191] have reported alignment of a nematic liquid crystal using substituted calixarene
Langmuir-blodgett films. K. Yonetake et al. [192] have reported new liquid crystals based on calixarenes. T. M. Swager et al. [193] have investigated rigid bowlic liquid crystals based on tungsten-oxo calix[4]arenes: host-guest effects and head-to-tail organization. J.F. Nierengarten et al. [194] have reported the synthesis and the liquid crystalline properties of a new class of conjugated derivatives that assembled four...

S. Kohmoto et al. [195] have synthesized octahomotetraoxacalix[4]arenes and concluded that encapsulation of functional guests by assembling may lead to the novel function reflecting the nature of inner guests. G. Shuling et al [196] have investigated liquid crystalline behavior and fluorescent property of calix[4]arene containing azobenzene photochromic group and found that the calix[4]arene skeleton was a good platform for conformation immobilization of azobenzene photochromic group and the formation of liquid crystalline materials. D. Chen et al. [197] have explored highly ordered smectic phases from polar calix[4]arene derivatives (Figure 16) It is important to find that liquid crystallinity can easily be induced by introducing the polarity or enhancing the polarizability of the three-dimensional phenylene-substituted calix[4]arene rigid segment. In contrast to the classical one-dimensional 4-cyano-4′-alkoxybiphenyl mesogens, which exhibit nematic and/or smectic A phases, cyano-phenyl-calix[4]arene and methyl-biphenyl calix[4]arene derivatives exhibit highly ordered smectic supramolecular organization as evidenced by the polarizing optical microscopy and X-ray diffraction studies. On the basis of the results of X-ray diffraction, an interdigitated antiparallel packing mode is proposed for the ordered smectic mesophase. The presence of the strong dipoles in the rigid calixarene segments not only facilitates and stabilizes the formation of the highly ordered
lamellar smectic structure but also enhances the optical properties of calix[4]arene derivatives. F. Yang et al. [198] have recently reported synthesis and mesomorphism of novel symmetrical triads of triphenylene-calix[4]arene-triphenylene (Figure 17).

Menon and co-workers [199, 200] Figure : 18 (A) studied a series of new LCs based on Schiff base calix[4]arene prepared by the reaction of tetraaminocalix[4]arene with aldehydes (4-hydroxy benzaldehyde, 2-vanillin, 4-vanillin and 2-hydroxy naphthaldehyde). All these compounds exhibit thermotropic liquid crystalline behaviours over wide range of temperatures and exhibit smectic and nematic phase. The behaviour of the dielectric constant studies shows that as the frequency increases the dielectric loss and decreases approaching near zero value. Micrographs show birefringence due to the mesomorphic nature of the compounds. The order of the phase transition is Smectic A texture-nematic texture-isotropic texture. The compounds showed mosaic and lancet texture like Smectic A phase and broken focal conic fan texture like Smectic C phase, grass like and fan like texture.

Menon et al. [201] (B) also synthesized a series of substituted calix[4]arene with two different linking groups, which exhibit rod-like, focal conic and mosaic texture of smectic C phase, schlieren texture with four brushes of nematic phase. Mesogenic property for all these compounds was confirmed by DSC and POM. Smectic C phases of representative compounds were confirmed by powder XRD at their transition temperature. These compounds were also studied for their dielectric property at various temperatures. These results indicated the linking groups in exterior point make great influence on mesogenic properties of calixarene LCs. they also (C) synthesized a series of lower rim azocalix[4]arene basket type mesogens. The proposed supramolecular organization displayed needle shaped, rod like, focal conic and schlieren textures [202].
Figure 18: Some of the previously reported mesogens based on calix[4]arene scaffold by our research group.

Figure 19: Some of the previously reported mesophases by our research group.
1.3.3 Biological activities

In 1955, Cornforth et al. [203] reported the first biological activity of calixarenes. Their studies concerned para-octyl-calix[8]arene having polyoxyethylene units on the lower rim and have shown that they were active as an anti-tuberculosis agent. The mechanism of action is completely different from the other drugs currently used against tuberculosis and, since the resistance toward conventional chemotherapeutic agents is increasing, these compounds are very promising. During more than 40 years, no more studies were carried out on the biological activities of calixarenes, until the patent of Hwang [204] concerning the anti-viral activity of some calixarene derivatives.

1.3.3.1 Anti-cancer activity

Shear induced carboplatin binding within the cavity of a phospholipid mimic for increased anticancer efficacy [205]. Research on the therapeutic applications of calixarene derivatives is an emerging area of interest. Due to their superior geometric shape, calixarenes can accommodate drug molecules by forming inclusion complexes. Controlled release of anticancer drugs by calixarenes might help in targeted chemotherapy [206].

Baggetto et al. [207] synthesized and studied the effect of calix[4]arene derivatives on various tumor cells (MU2, MU2F, HT1080, SP6.5, 1PC227, Jurkat, MEWO, H1-60, Huh7, Hep-G2, MEWO, DLM.1) and compared the activity of these compounds with standard anticancer drugs. They discovered that these functionalized calix[4]arene derivatives are potent anticancer agents, particularly in lymphoblastic leukemia and melanoma cell lines. Likewise, Nasuhi Pur and Dilmaughani [208] synthesized calix[4]arene functionalized with four platinum (II) centers and reported that it was a prospective anticancer agent. When compared with a chemotherapeutic agent, car-
boplatin, the newly synthesized compound showed better activity against non-small cell lung cancer, hepatocellular cancer, and breast cancer. In another study, the in vivo effect of glycoconjugates of calix[4]arene in a mouse melanoma model was reported to include a substantial reduction in tumor growth within 2 weeks [209]. Anthony et al. [210] patented a novel calixarene derivative as anticancer agent and demonstrated the anticancer effect of calix[4]arene dihydroxyphosphonic acid on different tumor cells in culture, in particular fibrosarcoma, melanoma and leukemic cells. Moreover, they compared the anticancer effect of calix[4]arene dihydroxyphosphonic acid, p-octanoyl-calix[4]arene dihydroxyphosphonic acid and p-tert-butylcalix[4]arene dihydroxyphosphonic acid on cell lines (Figure 20). Calixarene derivatives are known to form water-soluble host-guest capsules with anti-cancer agents such as carboplatin, doxorubicin, topotecan, paclitaxel, etc. These complexes were found to have more solubility than the standard drug and the release of the drugs was also in a controlled and target-specific manner [211-214]. Neagu et al. [215] studied the antitumoral effect of p-sulfonato-calixarenes human K562 myelogenous leukemia cell line in experimental photodynamic therapy.

**Figure 20**: Chemical structures of four calix[4]arene dihydrophosphonic derivatives.
Bezouska et al. [216] investigated the attractive role of thiacalix[4]arene and carboxylated calixarenes for protection of leukocyte killer cells in combined animal tumor therapies. They assessed three calixarene scaffold and revealed that thiacalix[4]arene had the highest affinity for CD69 leukocyte membrane receptor. They proved that carboxylated calixarenes were effective at protection of CD69 lymphocytes from apoptosis triggered by a multivalent ligand or antibody.

1.3.3.2 Anti-mycobacterial

Tuberculosis is the leading cause of death among infectious diseases, accounting for more than two million deaths annually and its incidence is increasing owing to the resurgence of drug-resistant strains of mycobacterium tuberculosis. Calixarenes are able to modify its growth and is effective in controlling its infections. Colston et al. [217] revealed that macrocyclons (Figure 21) were effective in athymic mice and they synthesized a number of structurally related calixarenes expressing significant anti-mycobacterial activity. They showed that macrocyclon significantly affected mycobacterial growth in murine macrophages by a mechanism involving L-arginine metabolism and inducible nitric oxide synthase (iNOS) activity. They also described the antimycobacterial activity of calixarenes bearing polymeric polyethylene glycol (PEG) chain lengths at the lower rim or t-octyl group at the upper rim. In another report, sodium p-sulfonatocalix[n]arenes was used to improve the physicochemical and biopharmaceutical properties of isoniazid, a first line antituberculosis drug [218]. Cationic and anionic calixarenes are also known which have anti-mycobacterial activity comparable to frontline drugs like isoniazide [219]. Para-octyl-calix[8]arene having polyoxyethylene units at the lower rim of the calixarene was shown to be active as an anti-tuberculosis agent. Two derivatives were studied; Macrocyclon having one chain containing from 12 to 13 ethyleneglycol units and HOC-60 having
60 ethyleneglycol units. The growth of mycobacterium tuberculosis in infected macrophages is inhibited by macrocyclon and stimulated by HOC-60. The mechanism of action is completely different from that of other drugs currently used against tuberculosis and these compounds are promising since the resistance toward conventional chemio-therapeutic agents is increasing [220]. Goodworth et al. [221] synthesized a series of large-ringed calix[6,7,8]arene analogues and their affect against mycobacterium tuberculosis in vivo.

![Figure 21: The chemical structures of macrocyclons acting antimycobacterial agents](image)

**1.3.3.3 Antibacterial activity**

Lamartine et al. [222] have studied the anti-microbial activity of a series of water-soluble calixarenes. Preliminary screening of 57 calixarenes was conducted to assay their potential as anti-microbially active compounds against corynebacterium. Of these compounds, seven calixarenes, amongst those SC4, SC6 and SC8 were found to exhibit suitable anti-microbial activity. These seven samples were then further tested to elucidate any anti-microbial activity they might have versus additional species. After examining the growth and inhibition values of these selected compounds, calixarenes SC4, SC6 and SC8 were shown to also display antimicrobial activity.
against Fusarium solani f. sp. Mori [F.s.-26] with an inhibition range of approximately 60–70%. Additionally, SC4, SC6, SC8 and others exhibited excellent and selective anti-microbial activity against the fungal strains, Rosellinia necatrix [R-8], and colletotrichum dematium [C.d.8901]. Loftsson et al. [223] reports the characterization of three cationic amphiphillic aminocalix[4]arenes as potential antimicrobial agents in vitro. Their minimum inhibitory concentrations (MIC) against Escherichia coli (E. coli) and S.aureus were in the 16–32 µg/ml concentration range, while minimum lethal concentrations (MLC) varied from 16 to 256 µg/ml depending on the bacteria and aminocalix[4]arene considered. Jean-Bernard Regnouf-de-Vains et al. [224] synthesized tetra-para-guanidinoethylcalix[4]arene and have been evaluated on Gram-positive and Gram-negative bacteria. Yilmaz et al. [225] synthesized two new Cu(II) complexes of p-tert-butylcalix[4]arene amide derivatives and investigated their antimicrobial activities. Dibama et al. [226] synthesized a water-soluble calixarene bearing the nalidixic acid, a quinolone antibiotic, and examined its prodrug behavior in vitro by high performance liquid chromatography. Figure 22 presents the molecular structure of the synthetic prodrug. Langmuir–Blodgett films of resorcinarene bis-crown silver complexes and tetra-p-guanidinoethylcalix[4]arene trifluoroacetate salt also have been reported recently as antibacterial agents [227,228].

![Figure 22](image-url): Calix[4]arene derivative used in antibacterial agents
1.3.3.4 Antifungal activity

p-Sulfonato-calix[n]arenes SC₄, SC₆ and SC₈, and the phenyl diazo derivative have been evaluated by Lamartine et al. [222] against fungi that are pathogenic for Fusarium solani f. sp. Mori [F.s.-26] with an inhibition range of approximately 60–70%. These compounds also inhibited completely the C. dematium growth and thus seem to be promising as agents for the minimization of economic loss in agriculture caused by fungus-related crop diseases. Fungi are some of the most neglected pathogens in terms of new drug discovery [229]. The biological profile of calix[n]arenes shows that they have significant antifungal properties. Oliveira et al. [230] have reported the synthesis of six calix[n]arenes and their antiparacoccidioides activity. The activity of amphotericin β calix[4]arene conjugates against Saccharomyces cerevisiae was studied by Paquet and coworkers [231] (Figure 23). Memon et al. [232] have studied the anti-fungal activity of a series of water-soluble calixarenes. Bagatin et al. [233] have studied the anti-fungal activity against C. albicans of a 8-oxyquinolinepropoxycalix[4]arene and 5-Cl-8- oxyquinolinepropoxy calix[4]arene ligands, showing fairly good results.

Figure 23: calix[n]arenes derivative antifungal agents
1.3.3.5 Anti-proliferative

Rouge et al. [234] used calix[4]arenes bearing alkyl ester and alkyl acid moieties at the lower rim, Pires et al. [235] used calix[4]arenes bearing two hydrazide function or ornithine, glutamic/aspartic acid groups at the lower rim, and Latxague et al. [236] used calix[4]arenes bearing diamino-tetraesters, diamino-tetraalcohols, diamino-tetraacid and tetraaryloxypentoxy groups at the lower rim (Figure 24). They compared their calixarene derivatives with 4-3,5-bis-(2-hydroxyphenyl)-1,2,4-triazol-1-yl-benzoic acid as a new oral chelator. The antiproliferative effect of these compounds, which was inhibited by intracellular iron level, were studied and the results revealed that the antiproliferative effect was due to their cytotoxicity. They show that novel substituted calix[4]arenes open the way to novel valuable medicinal chemistry scaffolding.

Figure 24: Anti proliferativity of Calix[4]arene derivative

Krenek et al. [237] linked the calixarenes substituted with 2-acetamido-2-deoxy-beta-D-glucopyranose by a thiourea spacer and tested it as activation receptors for the human macrophages and the rat natural killer cells. They showed that 5,11,17,23-tetrakis N-(2-acetamido-2-deoxy-beta-D-glucopyranosyl)- thioureido-25,26,27,28-tetrapropoxycalix[4]arene has the best ligand abilities towards the human Macrophages. Liu et al. [238] have studied Inclusion complex between water-soluble
sulphonatocalix[4]arene (SC4A) and irinotecan (CPT-11) was investigated by using UV spectrophotometry, $^1$H NMR and DSC analysis, and the complex was showed antiproliferative activity.

## 1.3.3.6 Antiviral activity

One of the interesting biological activities of the p-sulfonato-calix[n]-arenes is their potential use in the treatment of viral diseases, such as HIV and Herpes. Hwang et al. [204] patented a method for inhibiting cell infection by an enveloped virus, by administering to an infection site, a therapeutically effective amount of a calix[n]arene-derived compound, with polar substituents having terminal sulfonate (SO$_3$H[n]), carboxylate (COOH[n]), and phosphate (POOH[n]) groups, including esters and amides which are cleavable in vivo. The mechanism of this action is proposed to be by interaction of the molecule with the viral envelope via electrostatic interactions, thus masking the recognition site for cells. Another potent compound based on a tetrabutoxy-calix[4]-arene scaffold that possesses dual inhibition for both HIV and HCV has been described very recently by Hamilton et al. [239]. They have demonstrated remarkable anti-HIV and anti-HCV activities for a series of compounds based on the tetrabutylcalix[4]arene scaffold, and carboxylic derivatives on the upper rim. They have shown that maintaining the cone conformation of the scaffold is important for antiviral activity. In addition, aromatic isophthalate spacers at the upper rim are essential for anti-HIV activities and the diacid groups are also necessary for the observed anti-HCV effects. Furthermore, they have identified a potent compound that possesses dual inhibition for both HIV and HCV in vitro.

Regnouf-de-Vains and coworkers [240] studied the anti-HIV evaluation of other nine anionic calix[n]arenes, functionalized with sulfonate, carboxylate or phosphonate groups on the upper rim and with bithiazolyl podands on the lower rim (Figure 25).
These derivatives have been evaluated as anti-HIV agents on PBMC (Peripheral Blood Mononuclear Cell), MT4 (membrane type-4) and CEM-SS (Controlled Ecological mouse support System) cells infected, respectively, with Bal, LAI and III-b HIV strains. No cellular toxicity has been observed at 100 μM for all the compounds. For the nonsubstituted sulfonate and carboxylate calix[4]arene derivatives, which have modest activity, the substitution of the bithiazole units resulted in a gain of activity, the major gains being obtained with the sulfonated family.

![Figure 25](image)

**Figure 25**: Anionic calix[4]arene derivatives studied for their anti-HIV activity

Menon et al. [241] synthesized novel calixarene based heterocyclic compounds in which 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives have been coupled with 5,11,17,23-tetra-tertbutyl-25,27-bis(chlorocarbonyl-methoxy)-26,28-dihydroxy calix[4]arene. All the newly synthesized calixarene based heterocyclic compounds
have been characterized by elemental analysis and various spectroscopic methods like FTIR, $^1$H NMR, $^{13}$C NMR, and FAB-MS. (Figure: 26) All the final scaffolds have been subjected to antioxidant activity, in vitro antimicrobial screening against two gram (+ve) bacteria (S. aureus, S. pyogenes), two gram (−ve) bacteria (E. coli, P. aeruginosa) and two fungal strains (C. albicans, A. clavatus) and also have been screened for their antitubercular activity against Mycobacterium tuberculosis H$_{37}$Rv.

Figure 26: Some of the previously reported calix[4]arene based heterocyclic moiety by our research group.
1.3.3.7 Antimalarial Activity

Malaria is a parasitic infection of the red blood cells, transmitted by the female Anopheles mosquito through biting and transfer of plasmodium parasites by saliva. Malaria is the third most infectious disease after tuberculosis and HIV/AIDS, and affects over 100 countries in Africa, Asia and South America [242]. Despite intensive efforts towards its eradication in the early 1960s, malaria remains a major public health problem to date. According to the World Health Organization (WHO) nearly 300–500 million people throughout the world become infected with malaria every year. The mortality rate is estimated to be around 1.1 million deaths per year, mostly children under the age of five. Eighty percent of malaria cases worldwide occur in Africa; two thirds of the remaining cases are found in six countries, of which India is one. Malarial infections in humans are caused by four species of the genus *plasmodium*, and the majority are caused by *plasmodium falciparum* (*P. falciparum*) or *plasmodium vivax* (*P. vivax*) [243]. *p. falciparum*, the life-threatening parasite, is endemic in South and East Asia, South America, the Caribbean, the Middle East and Africa whereas *P. vivax*, which is typically not lethal, is endemic in Central America, India and parts of the Eastern Mediterranean. *plasmodium ovale* and *plasmodium malariae* are two rare, nonlethal parasites, most commonly found in Africa and Papau New Guinea [244]. Finally, in parts of Southeast Asia, *plasmodium knowlesi*, a type of monkey malaria, has been identified recently as a fifth human malaria parasite [245].

Due to the unavailability of effective vaccines, chemotherapy remains the only option for the treatment of malaria. After the discovery of quinine in the late 1600s, a huge number of potent antimalarial agents such as chloroquine, amodiaquine, primaquine,
pamaquine, mefloquine and related compounds were developed. Chloroquine (CQ) has been the mainstay of malaria therapy for decades because of its efficacy, safety and low cost, until the emergence and spread of CQ-resistance. Pyrimethamine-sulfadoxine (Fansidar) was one of the best therapeutic options after CQ, but was rendered ineffective in most malaria-endemic regions due to the spread of resistance. Currently, natural endoperoxide artemisinin and its semi-synthetic derivatives (artemether, arteether and artesunate) are the most potent and fast-acting antimalarials effective against resistant strains of \( P. falciparum \). In order to combat the resistance problem, combination therapy has been introduced by the WHO, in which artemisinin and its analogue in combination with 4-aminoquinoline antimalarials are used to treat malaria. Although artemisinin combination therapy (ACT) is well-tolerated and is nearly 95% effective in treating malaria, its use is limited in some regions due to some serious issues such as the higher cost of treatment and safety during pregnancy [246–251]. In addition, resistance to artemisinin derivatives has also been reported in Southeast Asian countries and may continue to increase, subsequently making malaria chemotherapy more complicated [249–252].

Malaria has been teasing human populations from a long time. Presently, several classes of antimalarial drugs are available in market, but the issues of toxicity, lower efficacy and the resistance by malarial parasites have decreased their overall therapeutic indices. Thus, the search for new promising antimalarials continues, however, the battle against malaria is far from over. Ferroquine is a derivative of chloroquine with antimalarial properties. It is the most successful of the chloroquine derivatives. Not only ferroquine, but also its derivatives have shown promising potential as antimalarials of clinical interest. Presently, much research is dedicated to the development of ferroquine derivatives as safe alternatives to antimalarial
CHAPTER - 1

INTRODUCTION

Several classes of ferroquine derivatives including hydroxyferroquines, trioxaferroquines, chloroquine-bridged ferrocenophanes, thiosemicarbazone derivatives, ferrocene dual conjugates, 4-N-substituted derivatives, and others have been discussed [253].

Quinoline-containing compounds, such as quinine and chloroquine, have a long-standing history as potent antimalarial agents. However, the increasing resistance of the *plasmodium* parasite against these drugs and the lack of licensed malaria vaccines have forced chemists to develop synthetic strategies toward novel biologically active molecules. A strategy that has attracted considerable attention in current medicinal chemistry is based on the conjugation of two biologically active molecules into one hybrid compound. Since quinolines are considered to be privileged antimalarial building blocks, the synthesis of quinoline-containing antimalarial hybrids has been elaborated extensively in recent years [254].

Figure 27: Basis for the synthesis of tetrazole derivatives of 4-aminoquinoline

A class of hybrid molecules consisting of 4-aminoquinoline and pyrimidine were synthesized and tested for antimalarial activity against both chloroquine (CQ)-sensitive (D6) and chloroquine (CQ)-resistant (W2) strains of *plasmodium falciparum* through an in vitro assay [255]. Singh et al. [256] have discussed the life cycle of
malaria parasite followed by quinoline based antimalarial drugs. Rawat et al. [257] synthesized a series of highly active 4-aminoquinoline-pyrimidine hybrids and evaluated for their antimalarial activity against CQ-sensitive (NF54) and CQ-resistant (Dd2) strains of *P. falciparum* in an in-vitro assay. A series of novel tetrazole derivatives of 4-aminoquinoline were synthesized and screened for their antimalarial activities against both chloroquine-sensitive (3D7) and chloroquine-resistant (K1) strains of *plasmodium falciparum* as well as for cytotoxicity against VERO cell lines. Most of the synthesized compounds exhibited potent antimalarial activity as compared to chloroquine against K1-strain [258] (*Figure 27*).

In the search of novel chemotherapeutic agents for emerging drug resistant parasites, the hybridization approaches have successfully emerged as an efficient tool in malarial chemotherapy. Recently there is a reported work on a rational design and synthesis of novel 8-aminoquinoline and pyrazolopyrimidine hybrids and their antimalarial activity against wild type *plasmodium falciparum* (Pf_NF54) and resistant strain (Pf_K1). The medicinal chemistry approach to expand the scope of this series resulted in an identification of potent compounds with nanomolar potency (best IC$_{50}$ 5–10 nM). Systematic structure activity relationship (SAR) studies revealed that pyrazolopyrimidine and 8-aminoquinoline ring are essential for achieving good *p. falciparum* potency [259].

![Fig. 28 : Structures of some 4-aminoquinoline derivatives with antimalarial activity.](Image)
Some quinoline-based compounds bearing a ferrocenyl unit in the 2-position of the heterocyclic system were synthesized from ferrocenyl-o-nitrochalcones through a simple hydrogenation/intramolecular cyclization sequence and fully characterized. The obtained ferrocenyl derivatives were evaluated in vitro as antimalarial agents against chloroquine-susceptible D10 and chloroquine-resistant W2 strains of *Plasmodium falciparum* and a beneficial effect of the organometallic moiety was evidenced in comparison with the phenyl-substituted analogues. All the ferrocenyl heterocycles inhibited the parasite growth in µM range and the lowest values of IC₅₀ were determined for derivatives containing a dimethylamino group as additional substituent [260].

A survey of literature on the biological activities of calixarenes reveals that though functionalized calixarenes have been designed to investigate anti-cancer, anti-mycobacterial, anti-bacterial, anti-fungal and anti-viral activities, no work has been reported so far on the use of calixarene scaffolds as anti-malarial agents. And hence, in the present investigation we designed quinoline and pyrimidine functionalized calixarene scaffolds to initiate the studies on anti malarial activity.
1.3.4 Staining

1.3.4.1 Introduction

Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image. Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing. Bacteria are microscopic organisms that cannot be seen with unaided eye. They can be seen even in unstained preparations such as a wet mount or hanging drop preparation but the morphology is not clear. Bacteria are colorless and when suspended in saline they don’t offer any contrast. Besides, bacterial motility makes it difficult to observe the morphology clearly. Hence, bacteria have to be stained to observe them. There are several dyes that can be used to stain the bacteria. During microscopy the cells must be dark enough to see, that is they must have contrast to the light. To create contrast a simple stain can be used. Simple staining technique utilizes single basic dye such as crystal violet, methylene blue, basic fuchsin etc, most bacterial cells using bright field. All bacteria take up the basic dye uniformly and appear in the same colour. Only the morphology of the bacteria can be appreciated upon staining.

The use of stains that react chemically with cell material will enhance the contrast between the cell and the background. A stain is a dye consisting of a colored ion (a chromophore) and a counter ion to balance the charge. Attachment of the chromophore part of the dye complex to a cellular component represents the staining reaction. Depending upon the dye, the chromophore can be either positively charged (cationic) and have an affinity for negative ions or negatively charged (anionic) with an affinity for positive ions. Bacteria carry a net negative charge at pH 7. Therefore, cationic dyes such as methylene blue, basic fuchsin, or crystal violet are useful for the direct staining of cells, whereas anionic stains, such as eosin and nigrosin, will not
directly stain bacterial cells. However, negatively charged stains, are useful for revealing the outlines of bacterial cells; anionic dyes stain the background, leaving the bacterial cells clear and bright against a dark background. Simple staining implies the use of only a single stain, which is usually sufficient to reveal the morphological features of most microbial cells, including relative size, shape, and characteristic arrangements for groups of cells [261-264].

1.3.4.2 Mechanisms of staining (principle)

A stain has a ability to bind a cellular component. These abilities depend upon the charges present on cellular component and charges present on chromophore group of stain. Bacteria has large number of carboxyl group on its surface and these carboxyl group has negative charge. When these carboxyl group carry out ionization reaction it shows \( \text{COO}^- \) and \( \text{H}^+ \). In nature these \( \text{H}^+ \) ions are present on cell surface and further replaced by other positively charged ions like \( \text{Na}^+ \) or \( \text{K}^+ \). Now when these simple stains are used it has chloride group, further these stain carry out dissociation for example if the stain is Malachite green it will carry out dissociation and give free radicals like \( \text{Mg}^+ \) and \( \text{Cl}^- \). Now these free \( \text{Mg}^+ \) ions give positive charge on chromophore group. When these stain is applied to a cell these positively charged \( \text{Mg}^+ \) ions replace the \( \text{K}^+ \) or \( \text{Na}^+ \) present on cell surface. Thus a ionic bond is formed in between positively charged \( \text{Mg}^+ \) ions and cell surface. Thus it result in staining of cell [265-267].

In our study we have developed series of different dyes based on calix[4]resorcinarene and made an attempt to check their staining ability for different groups of cell membrane of microorganisms. Here we have used simple staining
solutions, namely crystal violet as standard and azocalix[4]resorcinarenes as staining agent. We used these dye to stain cocci and bacilli.

1.4 Aim and scope of the work

Calix[n]arenes are the third generation of supramolecules, after crown ethers and cyclodextrins. They are phenol-formaldehyde based macrocycles, which are able to form stable and selective complexes with cations, anions or neutral molecules. Calix[4]resorcinarene is a macrocycle, or a cyclic oligomer, based on the condensation of resorcinol and an aldehyde. Resorcinarenes can be characterized by a wide upper rim and a narrow lower rim. They have been found to be outstanding platforms for creating attractive host molecules. Literature survey reveals that calix[n]arenes and calix[4]resorcinarenes have been used for a multitude of applications, e.g., in liquid crystal display, polymer synthesis, electrochemical sensors, optical sensors, chiral recognition devices, HPLC as stationary phases, electroanalytical techniques such as voltammetry and potentiometry, chromatography, enzyme mimics, ion channels, self assembling monolayers, catalysis, ion selective electrodes, phase transfer catalysts, drug delivery, fluorescent sensors, metal ion sensors, DNA chip technology/biosensing technology, biotechnology, biology, drug discovery, liquid liquid extraction, liquid membrane transport and solid lipid nanoparticles. Some of the promising fields of application of calix and calixresorcinarene which are pursued exhaustively are in the field of cation sensing, especially for the monitoring of toxic metal ions, for the designing of new liquid crystalline material and for the synthesis of novel medicinal agents and hence the present investigation focuses on these applications.
Elemental arsenic and arsenic compounds are classified as "toxic" and "dangerous for the environment". The International Agency for Research on Cancer (IARC) recognizes arsenic and arsenic compounds as group 1 carcinogens. In accordance with the World Health Organization and the U.S. Environmental Protection Agency the acceptable level for maximum As contamination in drinking water is 10 ppb. Drinking water with a higher level of As contamination will cause adverse health effects, including hyperkeratosis on the palm or feet, fatigue, cancer of the bladder, arsenicosis and genotoxic and mutagenic effects. Analytical techniques capable of determining ppb or sub-ppb levels of As are therefore very important for this critical public health threat. Motivated by necessity, we proposed development of anthraquinone functionalized calix[4]arene fluorescent probe for selective and sensitive determination of arsenic from waste water samples.

Functionalized calix[4]arenes with various linking groups were found to give an ideal platform to supramolecular chemists to design novel mesogens based on supramolecules and explore new LC phases for their utility in various fields and hence, it was proposed to synthesize novel lower rim ester linked p-hydroxy benzoic acid derivatives linked with rigid scaffold calix[4]arene with various flexible aliphatic chains to study their mesogenic properties.

Malaria is a parasitic infection of the red blood cells, transmitted by the female anopheles mosquito through biting and transfer of plasmodium parasites by saliva. Malaria causes symptoms that typically include fever, fatigue, vomiting and headaches. In severe cases it can cause yellow
skin, seizures, coma or death. Malaria has been teasing human populations from a long time. Presently, several classes of antimalarial drugs are available in market, but the issues of toxicity, lower efficacy and the resistance by malarial parasites have decreased their overall therapeutic indices. Thus, the search for new promising antimalarials continues, however, the battle against malaria is far from over. Here we proposed development of novel calix[4]arenes linked to substituted quinoline and pyrimidine derivatives to evaluate their antimalarial activity against *p. falciparum* strain.

A new field of application of calix[4]resorcinarene explored in the present investigation is in microbiology, as a staining agent. It was proposed to synthesize azo dyes derived from calix[4]resorcinarene for this study.

Thus the proposed research work converges into the design, synthesis and characterization of four categories of functionalized calix[4]arene for their use in four promising areas of applications namely

I. toxic metal sensing

II. liquid crystal studies

III. antimalarial activity and

IV. in monochrome staining
1.5 Present Investigation

Novel lower and upper rim modified calix[4]arene derivatives and calix[4]resorcinarene derivatives were synthesized and the studies were carried out thoroughly and were discussed exhaustively in the thesis, which comprises of subsequently mentioned five chapters with relevant list of references. The detailed literature of the last fifteen years on the synthesis and applications of various functionalized calix[4]arenes and calix[4]resorcinarene in various fields have been summarized in chapter 1.

Chapter 2 represents a highly sensitive and selective method, having low detection limit and fast response time PET based switch on-off fluorescence sensor 1-aminoanthraquinonephenylazo calix[4]arene dye (AQAC), which has been synthesised for the recognition of As$^{3+}$. Recognition event was supported by emission titration, UV-visible, ESI-MASS and colorimetric detection under fluorescent light. Proposed fluorescence probe has lower sensing limit as well as high selectivity towards As$^{3+}$ (0.94 nM). This highly sensitive, selective, easy, rapid detection and cost-effective fluorometric method will provide great interest for routine analysis of As$^{3+}$. Moreover, this probe has been applied for As$^{3+}$ recognition from waste water samples.

Chapter 3 describes the synthesis of novel basket shaped molecules having alkoxy benzoic acid linked with calix[4]arene as central rigid core possessing alkoxy material in outer space unit in the lengthening arm. The synthesized compounds have been purified and characterized by elemental analysis and different spectroscopic methods such as fourier transform infrared spectroscopy (FT-IR), electron spin ionization mass
spectroscopy (ESI-MS) and proton nuclear magnetic resonance spectroscopy ($^1$H NMR). Mesomorphism behavior of novel materials was studied using polarizing optical microscopy (POM), differential scanning calorimetry (DSC) and higher temperature powder x-ray diffraction (PXRD) method to confirm smectic C phase. The compounds exhibited rod like, needle shaped and schlieren textures. Nematic phases were also quite often found in materials during cooling and heating stage from isotropic phases. These compounds were studied for their dielectric properties in the frequency range 100 Hz–2 MHz at variable temperatures (50–110$^0$C). These studies will give adequate prospects in the field of supramolecular system especially in calixarene organization to explore new epoch of calixarene based liquid crystals with alkoxy groups of different chain lengths.

Chapter 4 represents the synthesis of novel calix[4]arenes linked to substituted quinoline and pyrimidine derivatives and evaluation of their antimalarial activity. The synthesized compounds were purified and characterized by elemental analysis, FT-IR, $^1$H NMR and ESI-MS and screened for their anti-malarial activity against *plasmodium falciparum* strains. IC50 values of many synthesized compounds exhibited significant activity as compared to standard drugs of chloroquine and quinine.

Chapter 5 deals with the synthesis of a series of novel azocalix[4]resorcinarene derivatives and their application for bacterial staining in the field of microbiology. All the synthesized compounds were purified and characterized by elemental analysis, FT-IR, $^1$H NMR, $^{13}$C NMR, ESI-MS and FAB-MS. The new molecules designed exhibited excellent binding ability to stain the gram +ve cocci and bacilli. All the results are compared with the staining of gram +ve cocci and bacilli using crystal
violet standard by standard monochrome staining protocol and the mechanism of staining is discussed.
1.6 References


5. O. Manasse, Ber., 1894, 27(2), 2409-2413.


CHAPTER - 1

INTRODUCTION

105. B. Valeur, J. Bourson, J. Pouget, A. W. Czarnik, Fluorescent Chemosensors
      for Ion and Molecule Recognition; ACS Symposium Series 538; American
      597.
      67, 2348.
      607.


