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Synthesis and photophysical, electrochemical, antibacterial, DNA binding studies of Triazinocalix[2]arenes

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Abstract

Oxygen bridged highly functionalized novel Calix[2]arene[2]triazine derivatives were synthesized from 2,6-pyridinedimethanol and cyanuric chloride. All the synthesized oxacalixarenes showed absorbance band between 315-339 nm and two emission bands at 269-272 nm and 289-339 nm. All the oxacalixarenes exhibited quasireversible oxidation reduction signals in cyclic voltametry. The binding property of the oxacalixarenes towards calf thymus DNA (CT-DNA) was investigated by Viscosity measurements, UV-VIS and Circular Dichorism studies and the oxacalixarenes possesses comparable antibacterial activity against *B. cereus, S. aureus* and *E. coli* as supported by docking studies.

Introduction

The design and synthesis of crown ether[1] type macrocycles, chiral macrocycles and their complexation ability with electron deficient guest molecules is of interest in supramolecular chemistry. Crown ethers such as spherands,[2] cryptands,[3] have promoted tremendous advance in supramolecular chemistry and one of the recent examples is calixarene.[4] Calixarene constitute an important and indispensable part in supramolecular chemistry. Calixarenes are characterized by a wide upper rim and a narrow lower rim and a central annulus. Calix[n]arene are [1_n] metacyclophane which are relatively easy to prepare and functionalize due to their high level preorganization and conformational preferences. calix[n]arenes have been widely used as molecular platforms and hosts in supramolecular chemistry. Calixarene finds

potential applications in the field of ion selective electrode, sensors,[5] optical sensors,[6] self assembly,[7] catalysis,[8] drug discovery [9] and as molecular recognition devices for solid phase extraction and as stationary phase and modifiers.[10] An important application of calixarenes is their use as sodium selective electrodes for the measurement of sodium levels in blood.[11] Calixarenes have hydrophobic cavity that can hold small molecules and ions and belongs to the category of cavitands which are known in host guest chemistry.[12] Calixarenes are macrocycles synthesized by the condensation reaction between a para substituted phenol and formaldehyde.[13] Synthesize of calixarenes are quiet challenging for synthetic community because the system always ends up with complex mixtures of linear and cyclic oligomers with different number of repeating units.

Heterocalixarene[14] has attracted growing interest in calixarene chemistry because of their easy availability, fine tunable cavity structure and powerful recognition properties. Macrocyclic host molecules recognizing various guest species including cations,[15] anions[16] and neutral molecules[17] including fullerenes.[18] Oxacalixarenes[19] are an important class of heterocalixarenes and could be efficiently synthesized by nucleophilic aromatic substitution of resorcinol with appropriate activated electrophilic reagents. Although more sophisticated oxacalixarenes have been synthesized and documented in the recent literature, the application of such macrocycle in supramolecular chemistry remains largely unexplored. Katz and co-workers[20] first reported the synthesize of oxacalix[2]arene[2]hetarenes in a single step cyclooligomerization.

Oxygen atom bridged calixaromatics exhibit fine tuned supramolecular properties. Efficient and convenient synthesis of oxacalix[2]arene[2]triazine is based on the fragment coupling approach. Heteroatom particularly nitrogen can adopt Sp^3/Sp^2 hybridization and can have different degree of conjugation with neighboring aromatic units which could result in the alteration of bond length and bond angle.[21] Melamine is one of the triazine derivative which can act both as hydrogen bond donor and acceptor to bind the guest species like cyanuric acid and

uric acid derivatives through multiple hydrogen bonding interaction.[22] Homo[2]heterocalix[2]arene[2]triazine exist in thermodynamically favored 1,3 alternate or saddle conformation. However, the conformational flexibility of such macrocyclic system limits their broad application in supramolecular chemistry. Oxacalix[2]arene[2]triazine can be synthesized by stepwise coupling approach between the highly reactive cyanuric chloride and aromatic diol through aromatic nucleophilc substitution pathway. The present investigation focus on the synthesis and structural characterization of oxacalixarenes **2**, **3**, **4**, **5**, **6**, **7**, **8** and **9** and investigation of their photophysical and electrochemical properties. Further, as the calixarenes has both oxygen and nitrogen heteroatom, it would be of interest to carry out DNA binding studies and antibacterial activity of the synthesized oxacalixarenes **2-9** against both gram positive and gram negative bacteria. The results of the antibacterial activity are also supported by molecular docking studies.Molecular structure of oxacalixarenes **2**, **3**, **4**, **5**, **6**, **7**, **8** and **9** are shown in **Fig 1**.

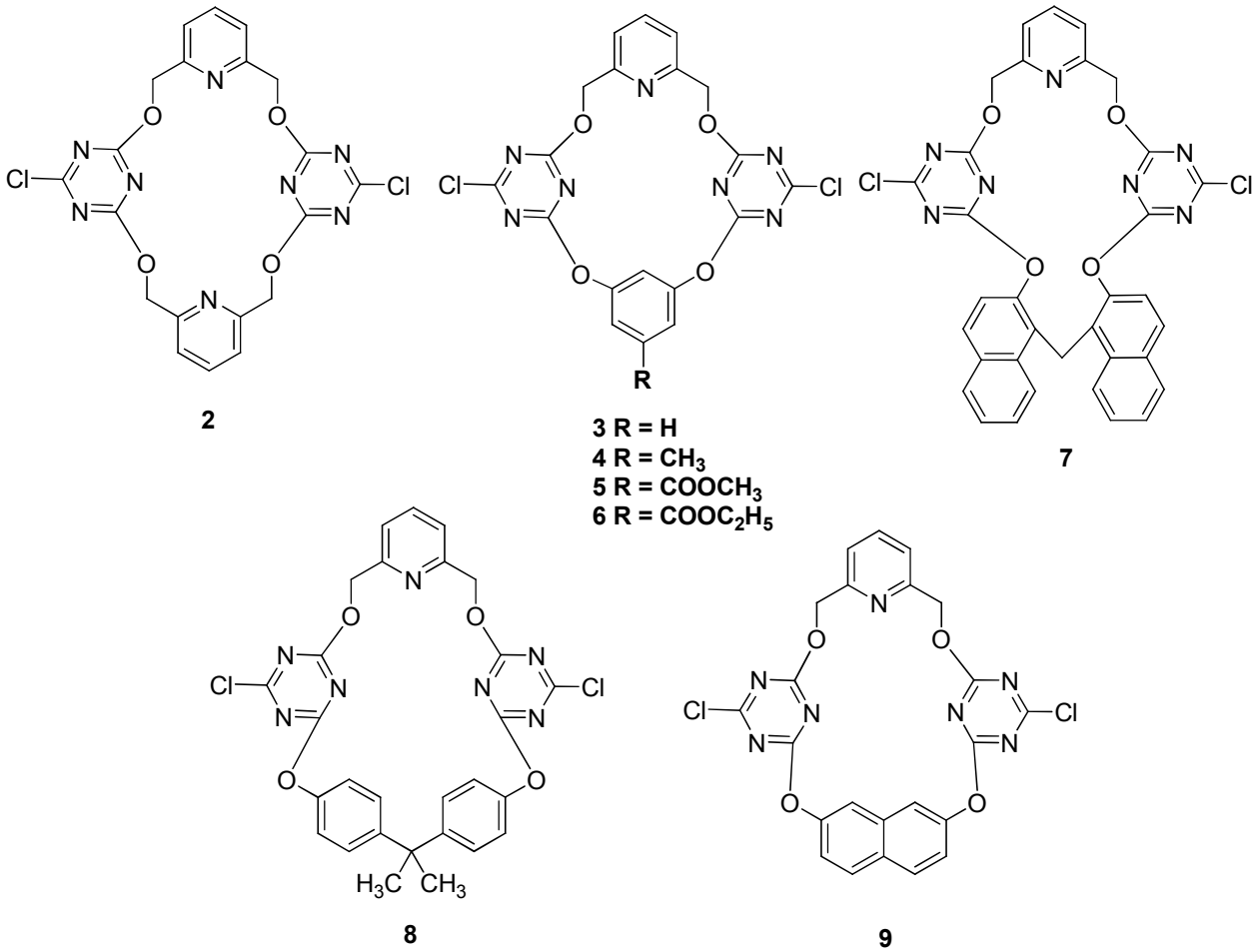
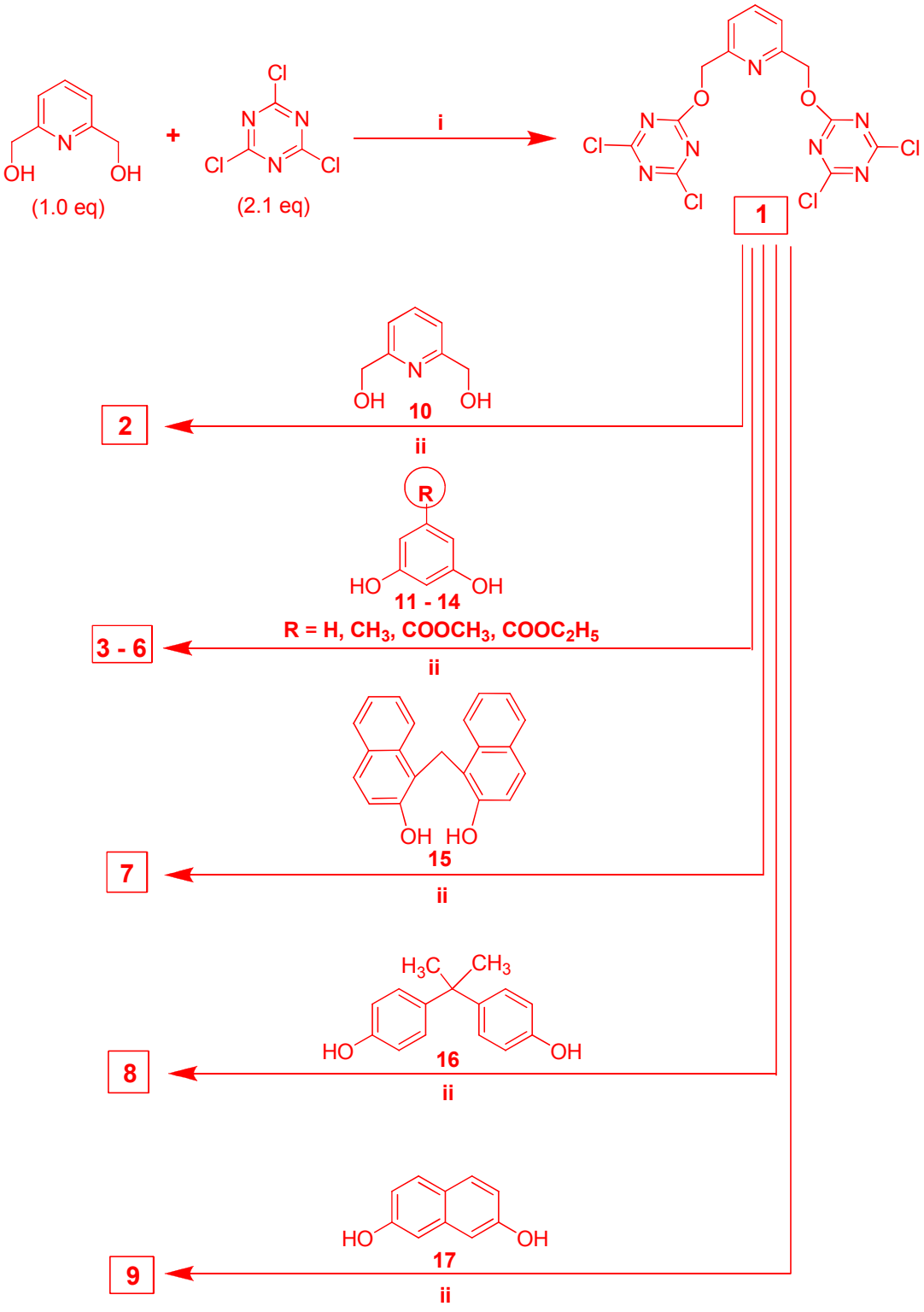


Fig. 1 Molecular structure of Oxacalix[2]arene[2]triazine **2**, **3**, **4**, **5**, **6**, **7**, **8** and **9**

Results and discussion

Tetraoxacalix[2]arene[2]triazines **2-9** were synthesized from the precyclophane **1** by aromatic nucleophilic substitution reaction through addition elimination process. 2,6-pyridinedimethanol function as the nucleophilic reagent and reacts with 2.2 equiv. of cyanuric chloride in the presence of diisopropylethylamine (DIPEA) in dry THF to give 2,6-bis((4,6-dichloro-1,3,5-triazin-2-yloxy)methyl)pyridine **1** in 61% yield.[23] The temperature and molar ratio of the reactants can be controlled to get higher yield of **1**. The reaction has to be carried out at 0-5 ^{0}C and with slightly higher equivalent of cyanuric chloride in order to achieve better yields of **1**. The ^{1}H NMR spectrum of the precyclophane **1** displayed a four proton singlet at δ 5.63 for *O*-methylene protons

and one proton triplet at δ 7.81 and a two proton doublet at δ 7.46 for the pyridine ring. The ^{13}C NMR spectrum of **1** showed *O*-methylene carbon at δ 71.2 in addition to the signals for the aromatic carbons. Macrocyclic coupling between one equivalent of 2,6-pyridinedimethanol **10** with one equivalent of the precyclophane **1** in the presence of DIPEA in dry acetone under high dilution conditions at room temperature for 48 h afforded the desired oxacalixarene **2** in 20% yield. In the ^{1}H NMR spectrum, the compound **2** showed an eight proton singlet at δ 5.43 for *O*-methylene protons in addition to the signals for the aromatic protons. In ^{13}C NMR spectrum, the compound **2** displayed the *O*-methylene carbons at δ 68.9, in addition to the signals for the aromatic carbons. The mass spectrum of oxacalixarene **2** showed molecular ion peak at *m/z* 501 $[M + H]^{+}$ Similarly, reaction of resorcinol **11** with precyclophane **1** afforded **3** in 23% yields. The yield of **3** could be increased to 30% by using acetonitrile instead of acetone as the solvent for the coupling reaction. However, the yields of oxacalixarenes **2** and **3** cannot be improved by increasing the molar ratio of DIPEA in the coupling reaction. Similarly, the reaction of the substituted resorcinol **12**, **13** and **14** with the precyclophane **1** gave oxacalixarenes **4**, **5** and **6** in 22%, 21% and 20% yields respectively. Macrocyclization of the precyclophane **1** under similar conditions in acetonitrile with methylene binaphthol **15** at room temperature for 48 h gave tetraoxacalix[2]arene[2]triazine **7** in 27% yield.



Scheme 1 (i) DIPEA, dry THF, 0 °C to rt, 4 h, **1** (61%). (ii) DIPEA, Dry acetone/acetonitrile, rt, 48 h, **2** (20%), **3** (27%), **4** (22%), **5** (21 %), **6** (20%), **7** (28.6%), **8** (37%) and **9** (28%).

The ^{1}H NMR spectrum of oxacalixarene **7** displayed a two proton singlet at δ 4.34 for methylene protons located in between the two naphthyl rings and a four proton singlet at δ 5.43 for *O*-methylene protons in addition to the signals for the aromatic protons. The ^{13}C NMR spectrum of **7** showed methylene carbon between the two naphthyl rings and *O*-methylene carbon at δ 24.5, 70.7 respectively, in addition to the signals for the aromatic carbons. The mass spectrum of **7** showed the molecular ion peak at *m/z* 661 $[M + H]^{+}$ Reaction of the bisphenol A **16** with the precyclophane **1** in the presence of DIPEA in dry acetone under high dilution conditions at room temperature for 48 h gave the oxacalixarene **8** in 37% yield. In ^{1}H NMR spectrum, the oxacalixarene **8** showed a singlet at δ 1.76 for two methyl protons and a sharp four proton singlet at δ 5.38 for *O*-methylene protons in addition to the signals for the aromatic protons. In ^{13}C NMR spectrum, the oxacalixarene **8** displayed the methyl carbon and *O*-methylene carbon at δ 30.6, 70.2 respectively, in addition to the signals for the aromatic carbons. The mass spectrum of **8** showed a peak at *m/z* 590 $[M + H]^{+}$ Usually substituted tetraoxacalix[2]arene[2]triazine are sterically hindered and hence can exhibit fluxional behavior in dissolved state and could undergo rapid conformational transformation by structural inter conversion. Finally, in order to create structural rigidity the precyclophane **1** was reacted with dihydroxy naphthalene **17** to give the oxacalixarene **9** in 28% yield. The structure of the oxacalixarenes **2**, **3**, **4**, **5**, **6**, **7**, **8** and **9** was confirmed from spectral and analytical data **(Scheme 1)**.

Photophysical studies

The UV-vis absorption and emission spectra of oxacalixarenes **2-9** were recorded in DMF and the λmax values are summarized in **Table 1**. The absorption spectrum of oxacalixarenes **2-9** showed strong absorption band between 315-339 nm. The absorption maxima observed for all the oxacalixarenes could be due to π-π* transition. The absorption spectrum of all the oxacalixarenes was recorded at the same concentration of 1 X 10^{-3} mol L^{-1}.

The intensity of the absorption band for oxacalixarene **2** and **3** is found to be high, whereas the intensity of the absorption band for oxacalixarene **5** is found to be low due to the presence of electron withdrawing carbethoxy group. In the fluorescence spectroscopy, the emission spectrum was obtained by exciting all the oxacalixarenes **2** to **9** at the corresponding absorption maximum. The oxacalixarenes **2-9** exhibited strong florescence emission in the region of 269-339 nm. Structural resembles of the oxacalixarenes **2-6** could be responsible for the observed weak emission band at 269-272 nm and a strong emission band at 289-339 nm. The emission spectrum of oxacalixarene **7** and **9** exhibited broad emission band at 269 - 315 nm due to the unique molecular frame.

Table 1 Photophysical properties of oxacalixarenes **2-9** in DMF (1 X 10^{-3} mol L^{-1})

Oxacalixarenes	λ_{max} (nm)	ε (mol/L)	λ_{em} (nm)
2	331	819	271, 337
3	334	1018	272, 339
4	332	289	272, 333
5	318	136	272, 334
6	315	495	271, 289
7	338	764	315
8	326	304	273,284, 293, 306, 313, 321, 330
9	339	537	269

Electrochemical studies

The electrochemical property of the oxacalixarenes **2-9** was studied by recording cyclic voltammetry in DMF at room temperature in the scan rate at 50mV/s with *n*-Bu_4NPF_6 as supporting electrolyte, Glassy carbon electrode as working electrode, Ag/AgCl as reference electrode and Pt electrode as counter electrode. All the synthesized oxacalixarenes exhibited electrochemical response in cyclic voltammogrametry and the electrochemical parameters are shown in **Table 2**. Cyclic voltametry of oxacalixarenes **2** to **5** and **6** to **9** are shown in **Fig 2** and the cyclic voltametry oxacalixarene **3** and **4** with different scan rate are shown in **Fig 3**. Oxidation and reduction potential for all the oxacalixarenes appeared in the potential range -2.0 to +1.8 V. In the cyclic voltammetry three oxidation peaks appeared between -1.10 to -0.51 V and two reduction peaks appeared between -0.85 to +1.32 V for oxacalixarenes **4**, **5** and **6**. Oxidation and reduction peaks for the oxacalixarenes **4, 5** and **6** may be attributed to the presence of electron withdrawing or donating group. The oxacalixarenes **2**, **3**, **7**, **8** and **9** exhibited two oxidation peaks between -1.08 to -0.55 V and two reduction peaks between -0.93 to +1.47 V. The Calix[2]arene[2]triazine derivatives **7**, **8** and **9** with extensive π- conjugate system of triazine moiety requires more energy in the reduction process[24] and hence in the cyclic voltametry, potential range was shifted from lower to higher potential value. The oxacalixarene **9** has showed a high positive potential range at +0.93 to +1.50 V due to extended conjugation of aromatic moiety. All the oxacalixarenes show quasireversible behavior in cyclic voltametry. Hence, quasi reversible nature of the compounds satisfies the following conditions. (i) The E_{pc} and E_{pa} current change with increase in scan rate (ii) ΔE_p values increases with increasing the scan rate and are greater than 50 mV (iii) Cathodic peak currents (I_{pc}) are lesser than the anodic peak currents (I_{pa}).

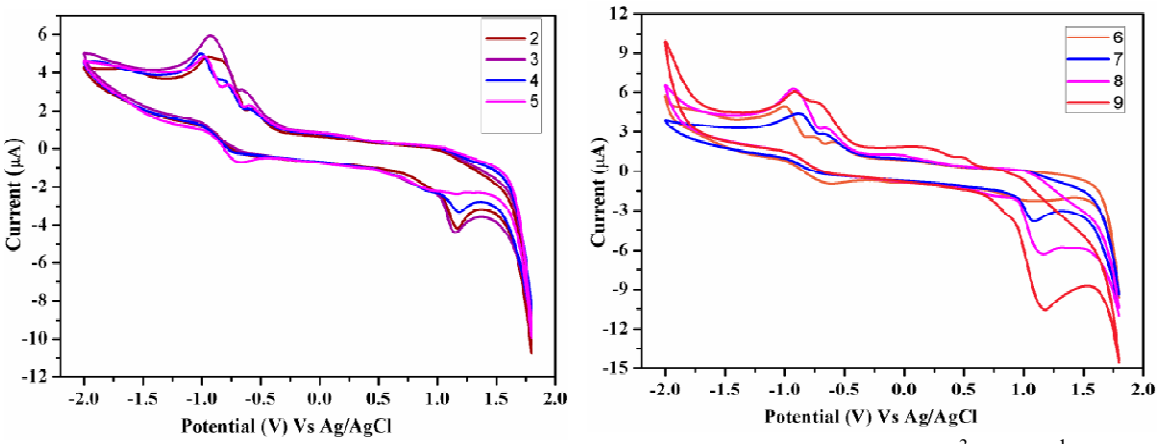


Fig. 2 Cyclic voltammograms of oxacalixarene **2-5** and **6-9** in DMF (1 X 10^{-3} mol L^{-1}), scanned at 50mV s^{-1}

Table 2 Electrochemical parameters of oxacalixarenes **2-9** in DMF (1 X 10^{-3} mol L^{-1})

Oxacalixarenes	$E_{pc}1$ (V)	$E_{pc}2$ (V)	$E_{pc}3$ (V)	$E_{pa}1$ (V)	$E_{pa}2$ (V)	ΔEp1(V)	ΔEp2 (V)	ΔEp3 (V)
2	-0.89	-0.57	-	1.17	-0.75	-2.06	0.18	-
3	-0.92	-0.65	-	1.15	-0.78	-2.07	0.13	-
4	-1.01	-0.80	-0.60	1.18	-0.75	-2.19	-0.05	-0.60
5	-0.97	-0.76	-0.58	0.89	-0.69	-1.86	-0.07	-0.58
6	-1.00	-0.75	-0.58	1.08	-0.62	-2.08	-0.13	-0.58
7	-0.87	-0.65	-	1.08	-0.75	-1.95	0.10	-
8	-0.92	-0.65	-	1.16	-0.66	-2.08	0.01	-
9	-0.92	-0.72	-	1.17	-0.68	-2.09	-0.04	-

The electrochemical behavior of oxacalixarenes **3** and **4** was studied at different scan rate Vs Ag/AgCl electrode. The scan rate range was increased from 50 to110 mVs^{-1}. The oxidation peak currents are proportional to the scan rate and the linear regression equation is y = mx + C with the increase of scan rate. The oxidation peak potential shifts positively and reduction peak potential shifts negatively. The linearity coefficient equation I (μA) = 0.0299 + 2.7722 is in the form y = mx + C with R^2 value as 0.9932 and I (μA) = 0.0272 + 2.7722 is also in the form

y = mx + C with R^2 value again as 0.9958. The oxacalixarenes **3** and **4** shows good stability with the increase in scan rate. **Fig. 4** shows linearity coefficient curve of oxacalixarene **3** and **4**.

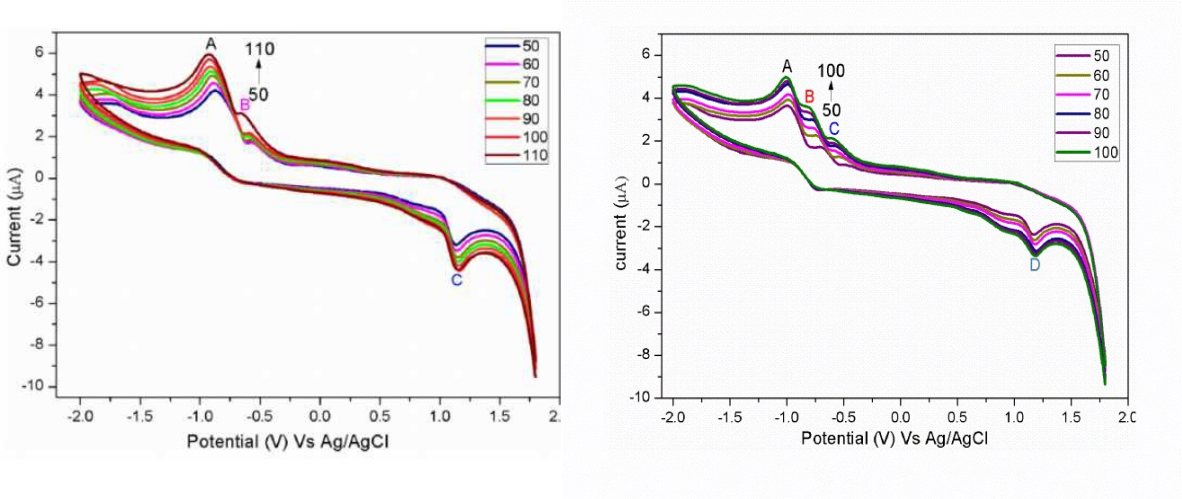


Fig. 3 Cyclic voltammograms of oxacalixarene **3** and **4** in DMF (1 X 10^{-3} mol L^{-1}) using different scan rate at 50mVs^{-1} to110 mVs^{-1}

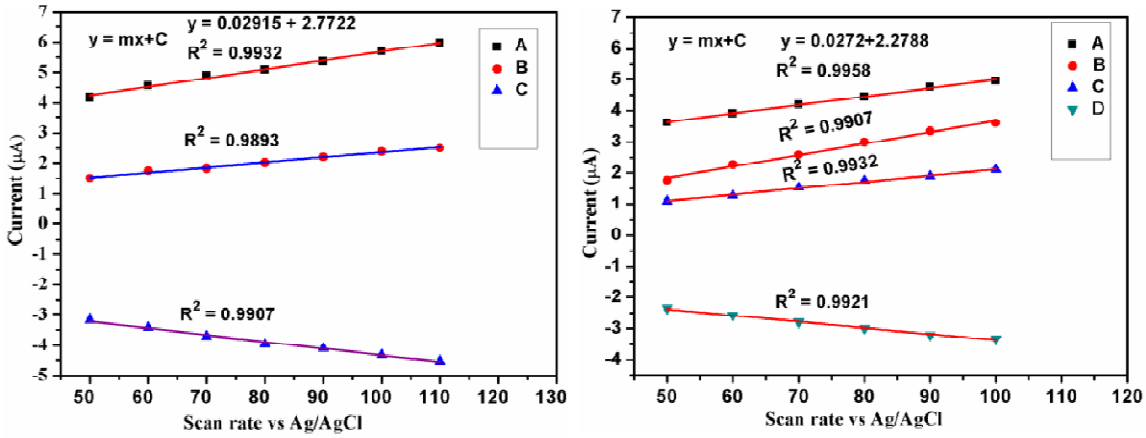


Fig. 4 linearity coefficient curve of oxacalixarenes **3** and **4** in DMF (1 X 10^{-3} mol L^{-1})

DNA binding studies

The DNA binding experiment was performed in Tris-HCl/ NaCl buffer (50Mm tris-HCl/1mM NaCl buffer, pH 7.2) using DMF solution of oxacalixarenes. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient 6600 M^{-1} cm^{-1} at 260 and 280 nm A260/A280 of ca. 1.8-1.9, indicating that the DNA

was sufficiently free of protein.[25] The stock solutions were stored at 4 ^{0}C and used within 4 days. Absorption titrations were carried out by varying the concentration of CT-DNA from 0 to 250 µL and with constant concentration of the oxacalixarene (60 µL). In order to eliminate the absorbance of DNA itself; reference cell containing DNA alone was prepared at the same concentration to which increments of the DNA stock solution were added. The binding of the oxacalixarenes to DNA led to decrease in the absorption in UV-vis spectrum, indicating that π* orbital of the oxacalixarenes can couple with π orbital of the DNA base pairs, resulting in the decrease in the π-π* transition energy and causing in bathochromic shift. The binding constants K_b of the oxacalixarenes to CT-DNA were determined by monitoring the changes in the absorbance with increasing the concentration of CT-DNA (**Fig. 5**). The absorption data were analyzed to evaluate the intrinsic binding constant (K_b) determined from the spectroscopic titration data using the following equation.

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

The 'apparent' extinction coefficient (ε_a) was obtained by calculating Absorbance/[oxacalixarenes]. The terms ε_f and ε_b correspond to the extinction coefficients of free (unbound) and the fully bound oxacalixarenes. The binding constant (K_b) was calculated using a plot (**Fig. 6**) of [DNA]/ ($\varepsilon_a - \varepsilon_f$) Vs [DNA] from the ratio of slope to intercept.[26]

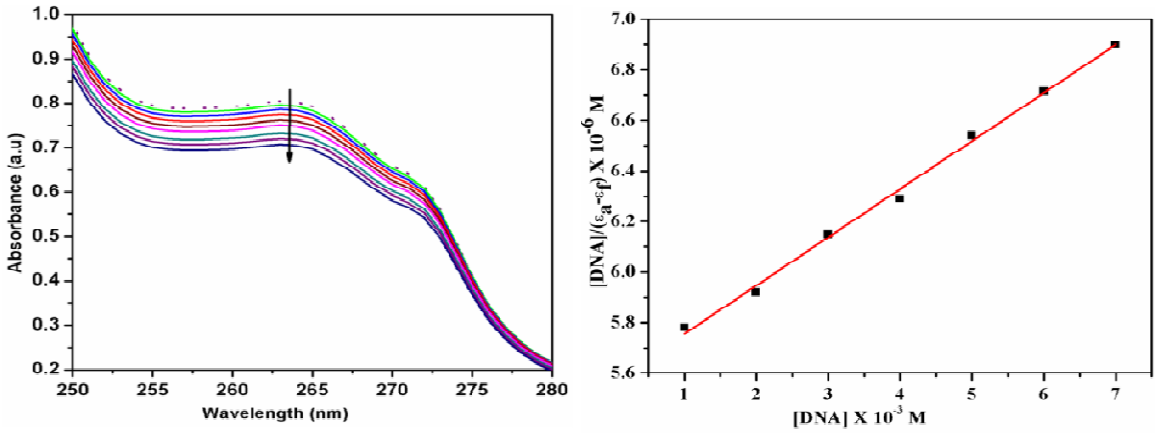


Fig. 5 Absorption spectra of oxacalixarene **3** in the absence (dotted line) and presence of (thick line) increasing amounts of CT-DNA (0 - 250 µM) at 25 ºC in 50 mM Tris-HCl (pH = 7.2).

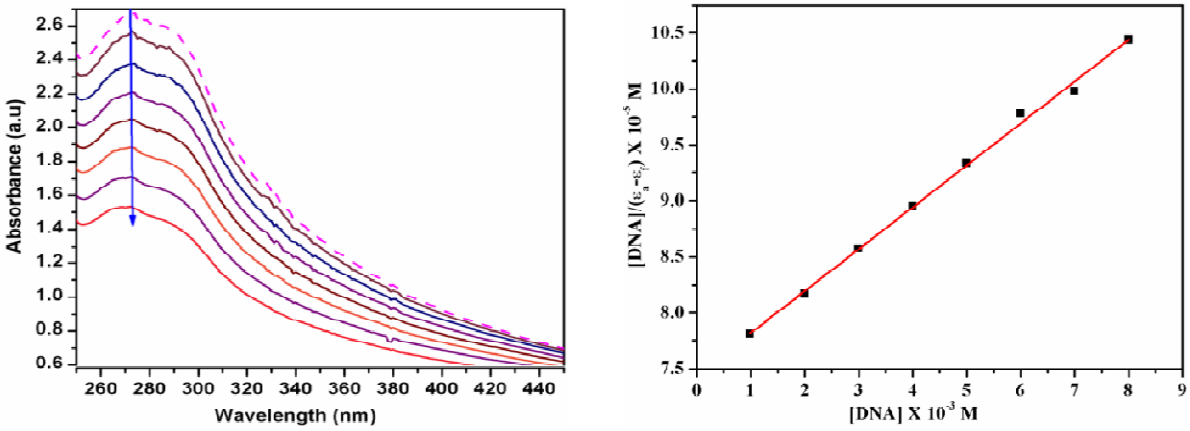


Fig. 6 Absorption spectra of oxacalixarene **9** in the absence (dotted line) and presence of (thick line) increasing amounts of CT-DNA (0 - 250 µM) at 25 ºC in 50 mM Tris-HCl (pH = 7.2).

In the present study, heteroatom present in oxacalixarene aromatic moiety can easily bind DNA base pairs[27]. Drugs with phenoxy functional groups are known for their major groove in DNA binding. The interaction of the oxacalixarene with increase in calf thymus DNA in (1%) DMF solution is shown in **Fig. 5** and **Fig. 6** shows similar such interaction of oxacalixarene **9** with CT-DNA. In the absorption spectrum, oxacalixarene exhibit more intense absorption bands, which are attributed to strong stacking interaction between the planar extended π-system of the oxacalixarene, facilitating a non covalent interaction of the oxacalixarene with base pair of DNA molecule[28]. While increasing the concentration of the calf thymus DNA, the absorption band of the oxacalixarene is affected, this results in hypochromism and bathochromic red shift. Here, the oxacalixarenes **3**, **7** and **9** exhibited 12%, 13% and 43% of hypochromism respectively, while they exhibited 10 nm, 15 nm and 40 nm of bathochromic shift respectively upon addition of DNA into the oxacalixarenes. The extent of hypochromism gives a measure of the strength of the intercalative binding. The presence of the naphthalene ring in the oxacalixarene **9** is

responsible for more hypochromism, indicating the extent of interaction of oxacalixane **9** as stronger than other oxacalixarenes. The oxacalixarene containing easily displaceable chloride ions bind via covalent interaction with DNA like cis platin to form novel DNA adduct species[29]. The oxacalixarene **9** exhibited strong hydrophobic CT-DNA interaction due to the presence of extended π-system of aromatic naphthalene ring and planarity of molecule. The binding constant (K_b) of oxacalixarene **9** with CT-DNA was found to be 3.4×10^5 M^{-1}. Addition of calf thymus DNA to oxacalixarene **3** and **5** shows a decrease in absorbance of the π-π* band which indicates that the binding of oxacalixarene with DNA is due to a strong interaction. The oxacalixarenes **3** and **5** showed greater binding strength to CT-DNA and the binding constants are 5.05 X 10^4 and 6.77 X 10^4 M^{-1} respectively due to the presence of electron withdrawing group. The binding constant (K_b) values of the oxacalixarenes **3**, **5** and **9** occur in the following order **9** > **5** > **3**. **Table 3** shows the static binding constant of oxacalixarenes **2-9** with CT-DNA.

Table 3 Static binding constants (K_b in L mol^{-1}) for the interaction of oxacalixarenes **2-9** with CT- DNA at 298 K.

Oxacalixarenes	Binding constant K_b (L mol^{-1})
2	(1.68 ± 0.40) X 10^3
3	(5.02 ± 0.25) X 10^4
4	(2.58 ± 0.47) X 10^4
5	(6.77 ± 0.20) X 10^4
6	(1.16 ± 0.6) X 10^4
7	(2.68 ± 0.35) X 10^4
8	(1.89 ± 0.45) X 10^4
9	(3.4 ± 0.15) X 10^5

Viscosity studies

Another evidence for the interaction of oxacalixarenes with CT-DNA is obtained from viscosity measurements, carried out on CT-DNA by varying the concentration of the oxacalixarenes. The interaction of oxacalixarenes with CT-DNA was also evaluated by viscosity measurements in solution and it gives further evidence for an intercalative binding mode. In general, a classical intercalation model lengthens the DNA helix, the base pairs of DNA are separated and binding, leads to increased DNA viscosity[30]. The viscosity of DNA solution increases due to partial and/or complete intercalation of oxacalixarene in between the DNA base stacks, which reduce electrostatic/covalent interactions with in DNA. The relative specific viscosities of DNA were investigated after addition of the oxacalixarenes. Upon increasing the concentration of oxacalixarenes, the relative viscosity of DNA simultaneously increases due to the intercalation of aromatic chromophobic unit in oxacalixarenes with CT-DNA base pairs and increases the length of CT-DNA. **Fig. 7** clearly show that the order of increasing viscosity of the CT-DNA solution **7** < **3** < **5** < **9**. The relative viscosity of CT-DNA increases due to presence of oxacalixarene, indicating that the oxacalixarenes may bind to DNA through groove binding mode[31]. Furthermore, the binding of the oxacalixarene **9** with CT-DNA was more significant than that of the oxacalixarenes **3**, **5** and **7** indicating that the affinity of oxacalixarene **9** to DNA was the strongest among the four oxacalixarenes. The results suggest that the oxacalixarene **9** can bind to DNA through intercalation, due to presence of naphthalene ring system in one compartment of the oxacalixarene.

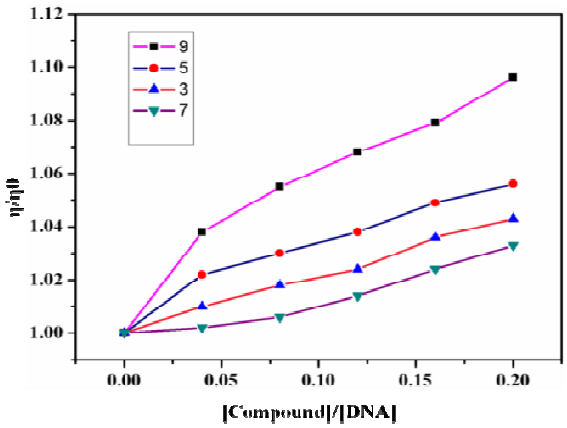


Fig.7 Relative viscosity shows the effect of increasing the concentration of oxacalixarenes **7**, **3,** **5** and **9** to CT-DNA at 25 ºC. [DNA] = 0.5 mM and [Compound] / [DNA] = 0.00, 0.04, 0.08, 0.12, 0.16, 0.2 respectively.

CD spectral studies

Circular dichorism (CD) is yet another useful method to decide whether nucleic acids undergo conformational changes of CT-DNA induced by oxacalixarenes. The CD spectrum of oxacalixarenes **3**, **5** and **9** were determined in Tris-HCl buffer medium at 25 ºC and shown in **Fig. 8**. The UV CD spectrum of CT DNA consists of a positive band at 276 nm and negative band at 245 nm corresponding to the base stacking and the helicity of normal B-DNA conformation. The change in ellipticity and shifting to higher energy of the positive CD signals are due to intercalative mode of binding[32]. Incubation with oxacalixarenes **3**, **5** and **9** induced considerable changes in CD spectrum[33]. At a concentration ratio of oxacalixarene and CT DNA at 2: 1, oxacalixarenes **3**, **5** and **9** produced shift to higher energy for the positive CD signal as well as an enhancement of CD ellipticity at 276 nm. The increasing ellipticity was observed in the following order **3 < 5 < 9** which reveals that the conformational changes induced by oxcalixarene **9** have higher affinity with CT DNA due to presence of aromatic naphthalene ring, which enhances the maximum interaction, while the oxacalixarene **3** and **5** induced only slight conformational changes in DNA.

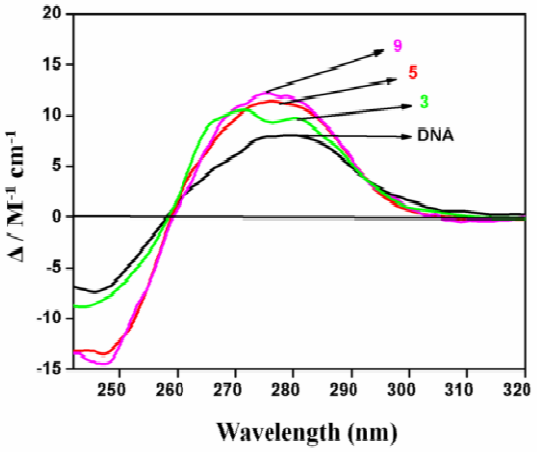


Fig. 8 CD spectra of CT-DNA (200 μM) in the absence and the presence of oxacalixarenes **3**, **5** and **9** (100 μM).

Antibacterial activity

Minimum inhibitory concentration (MIC)

The MIC for the oxacalixarenes was measured by agar well diffusion assay[34] using Mueller Hinton agar medium. The MIC is the least concentration of the test oxacalixarenes which inhibit the growth of bacteria. The activities of oxacalixarenes were observed by the zone of inhibition and were expressed in microgram per milliliter for the MIC. Three bacterial pathogens Viz *B. cereus, S. aureus* and *E. coli* were used for the antibacterial activity. Among all the eight oxacalixarenes tested, the oxacalixarenes **4** exhibited comparable activity against all the three human pathogenic bacteria than other oxacalixarenes. Similar studies are reported earlier by S. C. Ozkan et al[35]. The MIC values for the oxacalixarenes vary in a range of 50μg to 300μg/mL. The oxacalixarenes **7** and **8** showed good antibacterial activity against *E. coli* but not against the bacteria *S. aureus* (**Table 4**). The oxacalixarenes **5**, **6**, **7**, **8** and **9** showed moderate activities against the tested bacterial pathogens. Ampilicin 50μg/mL was used as the standard drug in the analysis and 9% DMSO was used as a control.

Table 4 The range of inhibitory concentration (MIC) values of the oxacalixarenes **2-9**.

Minimum Inhibitory Concentration (μg mL^{-1})			
Oxacalixarenes	E. coli	S. aureus	B. cereus
2	150	200	-
3	100	-	300
4	100	300	300
5	150	-	250
6	-	200	-
7	50	-	200
8	50	-	-
9	-	-	200

Amphicillin	50	50	50
Control	NI	NI	NI

NI - no inhibition, "-" means not active

Docking studies

In addition to the wet lab antibacterial activity, we have also performed the computational work (molecular docking) for the oxacalixarenes. The Schrödinger software version 9.0 was used for the molecular docking. The bacterial D-alanyl-D-alanine transpeptitase are most important targets for the bata-lactums antibiotics[36]. The oxacalixarenes were docked against the cocalent Aceyl enzyme (PDB: 1PW8). Among all the eight oxacalixarenes tested, the three oxacalixarene derivatives **2**, **4** and **8** showed good docking in the active pocket of the target protein[37]. All the other oxacalixarenes doesn't show docking with the target protein. The oxacalixarene **8** exhibited good docking score, glide energy and strong hydrogen bond and hydrophobic interactions with the amino acids in the active pocket. The oxacalixarenes **2** and **4** showed good interactions with target protein. The oxacalixarene **8** showed strong hydrogen bond interactions with Ser62, Asn161, Thr301 and Tyr306 and hydrophobic interactions with Thr116, Phe120, Thr123, Tyr159, Trp233, Arg285, Gln303 and Asn327. The Glide energy (binding free energy) was found to be -59.07 kcal mol^{-1} and the glide score (docking score) is -5.06 **(Table 5** and **Fig. 9)**. The oxacalixarene **4** also showed hydrogen bonding interactions with Tyr306, Asn327 and hydrophobic interactions with Leu332, Val329, Thr306, Ser326, Leu214, Gln303, Thr301, Trp233 and Phe120. The Glide energy (binding free energy) is -45.0 kcal mol^{-1} and the glide score (docking score) -4.92 (**Table 5**). The oxacalixarene **2** showed hydrogen bond interactions with Tyr306 and hydrophobic interactions with Phe120, Asn328, Asn327, Val329, Thr301 and Thr116 with a Glide energy (binding free energy) of -39.99 kcal/mol with the Glide score (docking score) of -3.65. The oxacalixarene **8** has crucial binding interaction with Covalent Acyl enzyme when compared with other oxacalixarenes **2** and **4**. The oxacalixarene **8** is considered as good lead based on its crucial interaction with the receptor.

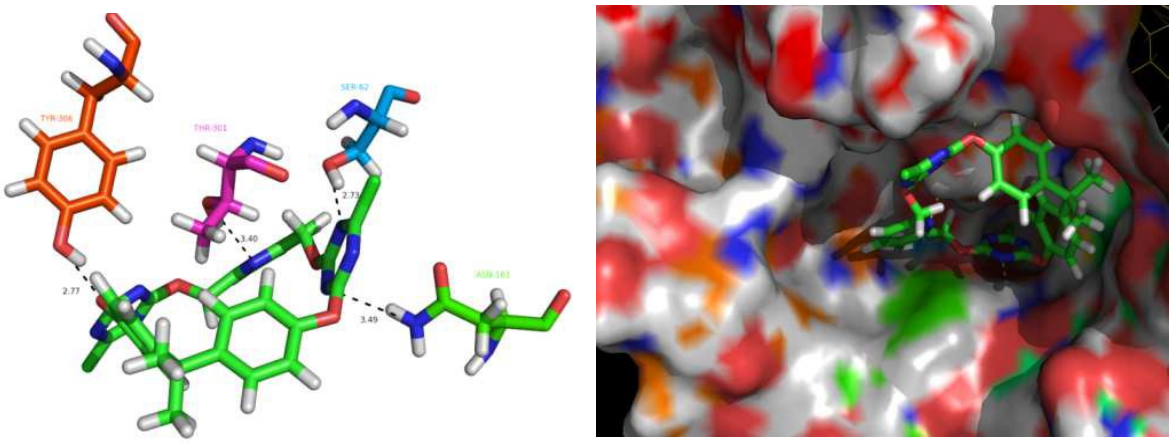


Fig. 9 (a) binding of oxacalixarene **8** with the Covalent Acyl enzyme (PDB ID: 1PW8). **(b)** The oxacalixarene substrate **8** bound to the binding site of quadruple mutant, showing the fitting of the test oxacalixarene on the substrate surface.

Table 5 Interaction of oxacalixarenes with the target Covalent Acyl enzyme.

Oxacalixarenes	Glide Energy Kcal mol^{-1}	Docking Score	Hydrogen Bonds Interaction [D-H…A]	Distance [Å]
2	-39.99	-3.65	Tyr306 (O-H...O)	2.88
4	-44.99	-4.92	Tyr306 (O-H...O)	3.02
8	-59.07	-5.05	Asn327 (N-H...O)	3.16
			Ser62 (O-H...N)	2.73
			Asn161 (N-H...N)	3.49
			Thr301 (O-H...N)	3.40
			Tyr306 (O-H...O)	2.77

D: donor; A: acceptor

Conclusions

A series of novel oxacalixarenes **2-9** were synthesized and characterized from spectral and analytical data. The photophysical properties revealed that the presence of electron rich groups in oxacalixarene show better absorption and emission values than the oxacalixarene with electron deficient carbethoxy group. All the synthesized oxacalixarenes show good electrochemical response in cyclic voltametry. The binding constant (K_b) of the oxacalixarenes **9** and **5** with DNA are 3.4 X 10^5 and 6.77 X 10^4 M^{-1} respectively and also carried out viscosity measurements and circular dichroism studies also prove used the binding interaction of oxacalixarenes with DNA. The antibacterial activity reveals that all the synthesized oxacalixarene exhibit comparable activity against the gram positive and gram negative human pathogenic bacteria namely *B. cereus, S. aureus* and *E. coli*. Molecular docking studies also supported this observation. Synthesis of other bioactive oxacalixarenes and their application towards DNA binding and antimicrobial activity is underway.

Experimental Section

All the reagents and solvents were obtained from Alfa Aesar, Merck or Avra chemical companies. The known products were identified by the comparison of their melting points and spectral data is already reported in the literature. Melting points were uncorrected and were determined using Toshniwal melting point apparatus by the open capillary tube method. The UV-Vis spectra were recorded on a Hitachi U-3210 spectrophotometer. The emission spectra were recorded on a HORIBA JOBIN YVON Fluoromax-4 spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz NMR spectrometer. The chemical shifts are reported in ppm (δ) with TMS as an internal standard and the coupling constants (*J*) are expressed in Hz. Elemental analyses were performed on a Perkin-Elmer 240B elemental analyzer. Electrochemical studies were carried out on a CH Instrument electrochemical analyzer.

CT- DNA was purchased from biochemical (India). Tris(hydroxymethyl)aminomethane-HCl (Tris-HCl) buffer was prepared using deionized water. Viscosity measurements were carried out Brookfield Viscometer at 25.0 ± 0.1 °C. CD measurements were carried out on a Jasco J-715 spectropolarimeter at 25 ^{0}C.

Synthesis of Precyclophane 1

To an ice cooled solution of cyanuric chloride (6.10 g, 33 mmol) in dry tetrahydrofuran (100 mL) was added dropwise a mixture of 2,6-pyridinedimethanol (2 g, 14 mmol) and diisopropylethylamine (DIPEA) (4.64 g, 36 mmol) in dry tetrahydrofuran (100 mL) during 1 h. The reaction mixture was stirred for another 4 h. After the reaction was complete, the diisopropylethylamine hydrochloride salt was filtered and the filtrate was evaporated under rotavapor vacuum. The residue obtained was extracted with chloroform (3 x 100 mL) and the combined organic layer was washed with water (100 mL), brine (50 mL) and dried over anhydrous sodium sulphate. The solvent was evaporated and the crude product obtained was purified by silica gel column chromatography with chloroform as the eluent to afford the pure 2,6-bis[(4,6-dichloro-1,3,5-triazin-2-yloxy)methyl]pyridine **1** as a yellow solid (3.8 g, 61%); mp: 190-192 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 5.63 (s, 4H); 7.46 (d, J = 7.8 Hz, 2H); 7.82 (t, J = 7.8 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δc 71.2, 121.3, 138.0, 154.0, 171.0, 173.0. ESI-MS m/z 435 $[M + H]^+$. Anal. Calc. for $C_{13}H_7Cl_4N_7O_2$: C, 35.89; H, 1.62; N, 22.54. Found: C, 33.57; H, 1.68; N, 22.37%.

General procedure for synthesis of oxacalixarene

To a solution of DIPEA (46 mmol) in dry acetone (200 mL) at room temperature were added dropwise both solutions of corresponding diol (18 mmol) in dry acetone (100 mL) and precyclophane **1** (18 mmol) in dry acetone (100 mL) at nearly the same rate during 1 h. The resulting mixture was allowed to stir for another 48 h at room temperature. After removal of the

solvent under rotavapor vacuum, the residue obtained was extracted with chloroform (3 x 100 mL), washed with water (3 x 100 mL) and dried over sodium sulphate. Evaporation of the organic layer gave a residue, which on purification using silica gel column chromatography with chloroform as eluent gave the corresponding oxacalixarene.

Oxacalixarene 2

Yield: 20%; mp: 196-198 ^{o}C; ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 5.43 (s, 8H); 7.02 (d, *J* = 7.8 Hz, 4H); 7.53 (t, *J* = 7.5 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δc 68.9, 118.8, 137.4, 154.1, 171.4, 171.7. ESI-MS m/z 501 $[M + H]^+$. Anal. Calcl. for $C_{20}H_{14}Cl_2N_8O_4$: C, 47.92; H, 2.82; N, 22.35. Found: C, 48.70; H, 2.87; N, 22.15%.

Oxacalixarene 3

Yield: 23%; mp: 214-218 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.25 (s, 4H); 6.52 (s, 1H); 6.88 (d, *J* = 7.8 Hz, 2H); 6.99 (d, *J* = 8.4 Hz, 2H); 7.44 (q, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δc 70.2, 114.0, 117.4, 120.3, 130.0, 137.6, 152.0, 155.1, 172.0, 172.1, 174.1. ESI-MS m/z 471 $[M + H]^+$. Anal. Calcl. for $C_{19}H_{11}Cl_2N_7O_4$: C, 48.32; H, 2.35; N, 20.76. Found: C, 48.05; H, 2.42; N, 20.51%.

Oxacalixarene 4

Yield: 22%; mp: 154-156 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 2.46 (s, 3H); 5.33 (s, 4H); 6.40 (s, 1H); 6.88 (s, 2H); 6.92 (d, *J* = 7.8 Hz, 2H); 7.50 (t, *J* = 7.8 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δc 21.4, 70.2, 111.0, 117.4, 121.0, 137.5, 141.0, 151.4, 155.0, 172.0, 172.1, 174.0. ESI-MS m/z 486 $[M + H]^+$. Anal. Calcl. for $C_{20}H_{13}Cl_2N_7O_4$: C, 49.40; H, 2.69; N, 20.16. Found: C, 49.18; H, 2.76; N, 19.85%.

Oxacalixarene 5

Yield: 21%; mp: 174-176 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.99 (s, 3H); 5.30 (s, 4H); 6.80 (s, 1H); 6.95 (d, J = 7.8 Hz, 2H); 7.49 (t, J = 7.8 Hz, 1H); 7.75 (s, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δc 52.9, 70.4, 117.4, 118.2, 121.7, 132.5, 137.6, 151.6, 155.0, 165.0, 172.0, 172.0, 174.2. ESI-MS m/z 530 $[M + H]^+$. Anal. Calcl. for $C_{21}H_{13}Cl_2N_7O_6$: C, 47.56; H, 2.47; N, 18.49. Found: C, 47.36; H, 2.56; N, 18.24%.

Oxacalixarene 6

Yield: 20%; mp: 112-114 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.35 (t, J = 7.2 Hz, 3H); 4.37 (m, 2H); 5.23 (s, 4H); 6.73 (s, 1H); 6.87 (d, J = 7.8 Hz, 2H); 7.42 (t, J = 7.8 Hz, 1H); 7.68 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δc 14.2, 62.0, 70.4, 117.5, 118.0, 121.6, 133.0, 137.6, 151.5, 155.0, 164.2, 171.7, 172.0, 174.2. ESI-MS m/z 543 $[M + H]^+$. Anal. Calcl. for $C_{22}H_{15}Cl_2N_7O_6$: C, 48.55; H, 2.78; N, 18.01. Found: C, 48.32, H, 2.91; N, 17.76%.

Oxacalixarene 7

Yield: 28.6%; mp: 252-256 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 4.34 (s, 2H); 5.42 (s, 4H); 6.97 (d, J = 7.5 Hz, 2H); 7.18 (d, J = 8.7 Hz, 2H); 7.30-7.44 (m, 5H); 7.78-7.89 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δc 24.4, 70.6, 121.0, 121.4, 124.2, 126.0, 126.3, 127.0, 128.8, 129.0, 132.3, 132.7, 137.1, 147.2, 154.8, 172.0, 172.4, 173.1. ESI-MS m/z 661 $[M + H]^+$. Anal. Calcl. for $C_{34}H_{21}Cl_2N_7O_4$: C, 61.64; H, 3.20; N, 14.80. Found: C, 61.38; H, 3.28; N, 14.54%.

Oxacalixarene 8

Yield: 37%; mp: 250-252 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.76 (s, 6H); 5.38 (s, 4H); 7.18 (d, J = 8.4 Hz, 4H); 7.29 (d, J = 8.4 Hz, 4H); 7.44 (d, J = 7.8 Hz, 2H); 7.77 (t, J = 7.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δc 30.6, 42.5, 70.2, 120.7, 120.8, 128.0, 138.1, 148.6, 149.5, 154.5, 171.6, 172.0, 173.7. ESI-MS m/z 590 $[M + H]^+$. Anal. Calcl. for $C_{28}H_{21}Cl_2N_7O_4$: C, 56.96; H, 3.59; N, 16.61. Found: C, 56.78; H, 3.66; N, 16.38%.

Oxacalixarene 9

Yield: 28%; mp: 246-248 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 5.13 (s, 4H); 7.04 (d, J = 7.8 Hz, 2H); 7.14 (d, J = 8.7 Hz, 4H); 7.54 (s, 2H); 7.60 (t, J = 8.4 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δc 70.7, 118.5, 122.6, 128.0, 137.4, 138.2, 152.0, 154.2, 172.0, 173.6, 174.1. ESI-MS m/z 521 $[M + H]^+$. Anal. Calcl. for $C_{23}H_{13}Cl_2N_7O_4$: C, 52.89; H, 2.51; N, 18.77. Found: C, 52.65; H, 2.59; N, 18.54%.

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