4. RESULTS AND DISCUSSION

This chapter deals with synthesis of N-(5-Aminopentyl)-3-[[5-(N-hydroxyacetamido)-pentyl]carbamoyl]propionohydroxamic acid (L₁), Deferoxamine B mesylate (L₂), and characterized by elemental analysis, IR, NMR, Mass spectral analysis. Copper(II), nickel(II), iron(III), cobalt(III), vanadium(IV), manganese(III), magnesium(II) and zinc(II) complexes of ligand L₁ and L₂ was synthesized and characterized by elemental analysis and IR, Electronic, Mass spectral analysis. DNA binding properties of copper(II), nickel(II), iron(III), cobalt(III), vanadium(IV), manganese(III), magnesium(II) and zinc(II) complexes towards (genomic) calf thymus (CT) DNA were studied by UV-absorption and fluorescence spectral methods. The DNA cleavage activities of the copper(II), nickel(II) and zinc(II) complexes towards (plasmid) pBR322 DNA were studied by agarose gel electrophoresis.

Deferoxamine B mesylate is a linear trihydroxamate ligand which forms a very stable hexacoordinate, octahedral complex with trivalent metal ions such as chromium(III), aluminium(III) and iron(III). It is white to off-white powder and freely soluble in water, methanol and ethanol. Deferoxamine mesylate USP (Desferal) is an iron-chelating agent to remove excess iron from the body¹ and available in vials containing 500 mg and 2 g of deferoxamine mesylate USP in sterile, lyophilized form for intramuscular, subcutaneous and intravenous administration.

Deferoxamine B mesylate is N-[5-[[5-([5-aminopentyl]hydroxycarbamoyl]propionamido]-pentyl]-3[[5-(N-hydroxyacetamido)pentyl]carbamoyl]propionohydroxamic acid methane sulfonate salt and its structural formula is,
Desferrioxamine B was first synthesized in 1962 by Prelog et al from 1-amino-5-nitropentane. However, because of the number of steps in the synthesis and the low yield of the sequence, the method does not enable the production of large quantities of the chelator or its analogs. Further, Bergeron et al. has also prepared Deferoxamine B from 4-cyanobutanal as well as 1,5-diaminopentane with number of steps involves series of condensations and catalytic hydrogenation to obtain Deferoxamine B. It was observed that catalytic hydrogenation cleaves benzyl protective group of N-OH simultaneously while saturating nitrile group and further reduces N-OH group to form about 6-12 % of unwanted acetamide impurities which are very difficult to remove by purification or other conventional crystallization techniques. Even though several different approaches were performed to overcome from the formation of this impurity, they could not succeed and hence the process was not scalable commercially. This is encouraged us to investigate and develop a simple and commercially viable process for the preparation of Deferoxamine B without the formation of these impurities. During the course of investigation, we could develop a novel process for the preparation of key intermediate N-Benzyloxy carbonylamino-5-hydroxylaminopentane by protecting 5-aminopentanol with Benzyloxy carbonyl chloride and followed by mesylation with methanesulfonyl chloride and displacement reaction with hydroxyl amine. The synthesis scheme is as given below,
In the beginning of our research, we have also observed the formation of acetamide impurity about 6 to 12% during the deprotection of Benzyloxy carbonyl group of Deferoxamine and its intermediate by catalytic hydrogenation. Surprisingly, these impurities formation was not observed by converting the N-OH group into its sodium salt using sodium hydroxide prior to catalytic deprotection of Benzyloxy carbonyl group from amine. The generalized scheme is as given below,
This novel method gave Deferoxamine B in high pure form with good yield. This novel process is commercially scalable, cost effective and high pure than Deferoxamine B prepared by our procedure have higher purity than sample available in market.
4.1 Synthesis and characterization of L\textsuperscript{1} precursor compounds 1-8.

4.1.1. Synthesis and characterization of N-Benzylxocarbonylamino-1-pentanol (1)

Synthesis of N-(5-Aminopentyl)-3-[[5-(N-hydroxyacetamido)pentyl]carbamoyl]-propionohydroxamic acid involves following steps

**Synthesis of N-Benzylxocarbonylamino-1-pentanol (1)**

**Characterization of N-Benzylxocarbonylamino-1-pentanol (1)**

**IR spectral analysis**

IR spectrum of compound 1 is shown in Fig. 1. A peak at 3400 cm\textsuperscript{-1} corresponds to O-H stretching vibration. The band at 3335 cm\textsuperscript{-1} due to the N-H stretching vibration and a peak at 1685 cm\textsuperscript{-1} was observed due to the carbonyl group (C=O).

**NMR spectral analysis**

$^1$H NMR spectrum compound 1 as shown in Fig. 2 $\delta$ 7.3-7.4 due to aromatic protons. A singlet at $\delta$ 5.1 due to methylene protons attached to phenyl ring. A singlet observed at $\delta$ 4.8 due to O-H proton. Methylene protons observed in the region $\delta$ 3.2 to 1.4.

**Mass spectral analysis**

ESI-MS spectrum of compound 1 is shown in Fig. 3. The compound 1 shows a molecular ion peak [M$^+$] at m/z 237 is equivalent to its molecular weight.
Fig. 1. IR spectrum of 1.
Fig. 2. $^1$H NMR spectrum of 1.
Fig. 3. ESI-MS spectrum of 1.
4.1.2. Synthesis and Characterization of N-Benzoxycarbonylamino-5-hydroxylamino-pentane (3).

Synthesis of N-Benzoxycarbonylamino-5-hydroxylaminopentane (3).

Characterization of N-Benzoxycarbonylamino-5-hydroxylaminopentane (3).

IR spectral analysis

IR spectrum of 3 exhibit a broad peak at 3350 cm\(^{-1}\) due to O-H stretching vibration and a strong band in the region around 3300 cm\(^{-1}\) due to the N-H stretching vibration. The band observed at 1690 cm\(^{-1}\) is due to the carbonyl group (C=O) as shown in Fig. 4.

NMR spectral analysis

\(^1\)H NMR spectrum compound 3 exhibits a peak in the region \(\delta\) 7.3-7.4 due to aromatic protons. A singlet at \(\delta\) 5.1 due to methylene protons attached to phenyl ring. A singlet observed at \(\delta\) 4.85 due to O-H proton. Methylene protons observed in the region \(\delta\) 3.6 to 1.3 as shown in Fig. 5.

Mass spectral analysis

The ESI-MS spectrum of compound 3 shows a molecular ion peak \([M+H]^+\) at \(m/z\) 253 is equivalent to its molecular weight as shown in Fig. 6.
Fig. 4. IR spectrum of 3.
Fig. 5. $^1$H NMR spectrum of 3.
Fig. 6. ESI-MS spectrum of 3.
4.1.3 Synthesis and characterization of N-Benzyloxy carbonylamino-5-(N-succinylhydroxyl-amino)pentane (4)

Synthesis of N-Benzyloxy carbonylamino-5-(N-succinyl-hydroxylamino)pentane (4)

Characterization of N-Benzyloxy carbonylamino-5-(N-succinylhydroxylamino)pentane (4)

**IR spectral analysis**

IR spectrum of 4 exhibits a broad peak at 3345 cm\(^{-1}\) due to O-H stretching vibration and a strong band in the region around 3150 cm\(^{-1}\) due to the N-H stretching vibration as shown in Fig. 7. The band observed at 1702 and 1686 cm\(^{-1}\) are due to the carbonyl group (C=O) of carboxylic acid and amide respectively.

**NMR spectral analysis**

\(^1\)H NMR spectrum compound 4 exhibits a peak in the region δ 7.3-7.4 due to aromatic protons. A singlet observed at δ 6.3 due to oxime proton. A singlet observed at δ 5.1 due to methylene protons attached to phenyl ring. Methylene protons observed in the region δ 1.3 to 3.75. The formation of new peak at δ 6.3 due to oxime group which indicate the formation of compound 4 as shown in Fig. 8.

**Mass spectral analysis**

As shown in Fig. 9. The ESI-MS spectrum of compound 4 shows a molecular ion peak [M+H]\(^+\) at m/z 353 is equivalent to its molecular weight.
Fig. 7. IR spectrum of 4
Fig. 8. $^1$H NMR spectrum of 4.
Fig. 9. ESI-MS spectrum of 4.
4.1.4. Synthesis and characterization of N-(5'-Benzyloxy carbonylaminopentyl)tetrahydro-3, 6-dioxo-1, 2-oxazine (5)

Synthesis of N-(5'-Benzyloxy carbonylaminopentyl)tetrahydro-3, 6-dioxo-1, 2-oxazine (5)

Characterization of N-(5'-Benzyloxy carbonylaminopentyl)tetrahydro-3, 6-dioxo-1, 2-oxazine (5)

IR spectral analysis

IR spectrum of 5 exhibits a broad peak at 3345 cm$^{-1}$ is due to O-H stretching vibration and a strong band in the region around 3300 cm$^{-1}$ is due to the N-H stretching vibration. The band observed at 1770 and 1660 cm$^{-1}$ are due to the carbonyl group (C=O) of carboxylic acid and amide respectively as shown in Fig 10.

Mass spectral analysis

$^1$H NMR spectrum compound 5 exhibits a peak in the region $\delta$ 7.3-7.4 is due to aromatic protons. A singlet observed at $\delta$ 5.1 is due to methylene protons attached to phenyl ring. Methylene protons observed in the region $\delta$ 1.3 to 3.75 as shown in Fig. 11.

Mass spectral analysis

The ESI-MS spectrum of compound 5 shows a molecular ion peak [M]$^+$ at m/z 334 is equivalent to its molecular weight as shown in Fig. 12.
Fig. 10. IR spectrum of 5
Fig. 11. $^1$H NMR spectrum of 5.
Fig. 12. ESI-MS spectrum of 5.
4.1.5. Synthesis and characterization of N-(5-Benzyloxy carbonylaminopentyl)-N-(hydroxyacetamide) (6)

Synthesis and of N-(5-Benzyloxy carbonylaminopentyl)-N-(hydroxy)acetamide (6)

Characterization of N-(5-Benzyloxy carbonylaminopentyl)-N-(hydroxy)acetamide (6)

IR spectral analysis

As depicted in Fig. 13, IR spectrum of 6 exhibit a broad peak at 3347 cm\(^{-1}\) due to O-H stretching vibration and a strong band in the region around 3125 cm\(^{-1}\) due to the N-H stretching vibration. The band observed at 1686 cm\(^{-1}\) due to the carbonyl group (C=O) of amide moiety.

NMR spectral analysis

\(^1\)H NMR spectrum compound 6 exhibits a singlet at \(\delta\) 8.5 is due to amide N-H proton. The peak observed in the region \(\delta\) 7.3-7.4 due to aromatic protons. A singlet observed at \(\delta\) 5.0 is due to methylene protons attached to phenyl ring. A singlet observed at \(\delta\) 4.9 is due to oxime proton. Methylene protons observed in the region \(\delta\) 1.5 to 3.6. \(^1\)H NMR spectra of 6 exhibits a singlet at \(\delta\) 2.1 confirms the acylation of 3 as shown in Fig. 14 respectively.

Mass spectral analysis

As represented in Fig. 15, the ESI-MS spectrum of compound 6 shows a molecular ion peak \([\text{M+H}]^+\) at m/z 295 is equivalent to its molecular weight.
Fig. 13. IR spectrum of 6.
Fig. 14. $^1$H NMR spectrum of 6.
Fig. 15. ESI-MS spectrum of 6.
4.1.6. Synthesis and characterization of N-(5-Aminopentyl)-N-(hydroxy)acetamide (7)

Synthesis of N-(5-Aminopentyl)-N-(hydroxy)acetamide (7)

Characterization of N-(5-Aminopentyl)-N-(hydroxy)acetamide (7)

NMR spectral analysis

$^1$H NMR spectrum compound 7 exhibits a peaks at $\delta$ 3.4, $\delta$ 2.8, $\delta$ 1.8, $\delta$ 1.4, $\delta$ 1.1 are due to methylene protons. The disappearance of peaks at $\delta$ 8.5 is due to amide N-H proton, $\delta$ 7.3-7.4 is due to aromatic protons and a single peak at $\delta$ 5.0 is due to methylene protons attached to phenyl ring which indicates the formation of 7. A singlet at $\delta$ 1.8 is due to methyl protons of acetyl group as represented in Fig. 16.

Mass spectral analysis

As depicted in Fig. 17, the ESI-MS spectrum of compound 7 shows a molecular ion peak $[M+H]^+$ at m/z 161 is equivalent to its molecular weight.
Fig. 16. $^1$H NMR spectrum of 7.
Fig. 17. ESI-MS spectrum of 7.
4.1.7. Synthesis and characterization of N-(5-Benzoxycarbonylaminopentyl)-3-[[5-(N-hydroxyacetamido)pentyl]carbamoyl]propionohydroxamic acid (8)

Synthesis of N-(5-Benzoxycarbonylaminopentyl)-3-[[5-(N-hydroxyacetamido)pentyl]carbamoyl]propionohydroxamic acid (8)

Characterization of N-(5-Benzoxycarbonylaminopentyl)-3-[[5-(N-hydroxyacetamido)pentyl]carbamoyl]propionohydroxamic acid (8)

IR spectral analysis

IR spectrum of 8 exhibits a broad peak at 3325 cm\(^{-1}\) due to O-H stretching vibration as shown in Fig. 18. A strong band observed around 3125 cm\(^{-1}\) is due to the N-H stretching vibration. The band observed at 1685 due to the carbonyl group (C=O) of carboxylic acid.

NMR spectral analysis

\(^1\)H NMR spectrum of compound 8 exhibits a singlet at δ 8.1 is due to N-H proton. Aromatic protons observed in the range δ 7.3-7.4. A singlet observed at δ 5.0 is due to methylene protons attached to phenyl ring. Methylene protons observed in the region δ 1.3 to 3.6 as shown in Fig. 19.

Mass spectral analysis

The ESI-MS spectrum of compound 8 as shown in Fig. 20 shows a molecular ion peak [M]\(^+\) at m/z 494 is equivalent to its molecular weight.
Fig. 18. IR spectrum of 8.
Fig. 19. $^1$H NMR spectrum of 8.
Fig. 20. ESI-MS spectrum of 8.
4.2. Synthesis and characterization of \( L^1(9) \).

**Synthesis of N-(5-Aminopentyl)-3-[[5-(N-hydroxyacetamido)pentyl]carbamoyl]propionohydroxamic acid (9) (\( L^1 \)).**

**Characterization of N-(5-Aminopentyl)-3-[[5-(N-hydroxyacetamido)pentyl]carbamoyl]propionohydroxamic acid (9) (\( L^1 \)).**

**IR spectral analysis**

IR spectrum of 9 exhibits a broad peak at 3310 cm\(^{-1}\) due to O-H stretching vibration. A strong band observed around 3100 cm\(^{-1}\) due to the N-H stretching vibration. The band observed at 1630 cm\(^{-1}\) is due to the carbonyl group (C=O) as represented in Fig. 21.

**NMR spectral analysis**

As shown in Fig. 22, \(^1\)H NMR spectrum of 9 exhibits a singlet at \( \delta \) 8.1 is due to N-H proton. The absence of peak in the range \( \delta \) 5-7.5 indicates that the formation of compound 9. A singlet observed at \( \delta \) 1.8 is due to methyl proton. The peaks observed at triplet at \( \delta \) 3.3, \( \delta \) 2.87, \( \delta \) 2.6, \( \delta \) 2.1, and \( \delta \) 0.9 – 1.08 are due to methylene protons. \(^{13}\)C NMR spectrum of 9 exhibits carbonyl carbons at \( \delta \) 173.7, 172.9, and 170. The peaks observed at \( \delta \) 50.5, 48.2, 47.1, 29.786, 26.152, 24.336, 23.723 and 23.556 are due to methylene carbons. Methyl carbon was observed at \( \delta \) 19 as shown in Fig 23.

**Mass spectral analysis**

The ESI-MS spectrum of compound 9 shows a molecular ion peak \([\text{M+H}]^+\) at m/z 361 is equivalent to its molecular weight as presented in Fig 24.
Fig. 21. IR spectrum of 9
Fig. 22. $^1$H NMR spectrum of 9.
Fig. 23. $^{13}$C NMR spectrum of 9.
Fig. 24. ESI-MS spectrum of 9.
4.3. Synthesis and characterization of $L^2$ precursor compound (10)

Characterization of N-[5-[3-[(5-benzyloxycarbonylamino)pentyl]hydroxylcarbamoyl]-propionamido)pentyl]-3[[5-(N-hydroxyacetamido)pentyl]carbamoyl]propiono-hydroxamic acid (10)

NMR spectral analysis

As represented in Fig. 26, $^1$H NMR spectrum of 10 in DMSO-d$_6$ showed a singlet at $\delta$ 7.78 is due to three NH (amide) protons. Aromatic protons observed in the range $\delta$ 7.36-7.5. A peak observed at $\delta$ 4.99 is due to methylene protons attached to phenyl ring. A singlet observed at $\delta$ 1.93 is corresponds to methyl protons. Methylene protons observed as triplet at $\delta$ 3.4 ($J = 3.45$ Hz), $\delta$ 3.02 ($J = 4.35$ Hz), $\delta$ 2.28 ($J = 5.4$ Hz), multiplet at $\delta$ 2.5, $\delta$ 1.49, $\delta$ 1.34, $\delta$ 1.2.

Mass spectral analysis

The ESI-MS spectrum of 10 shows a molecular ion peak [M$^+$] at m/z 694 are equivalent to its molecular weight as shown in Fig. 27.
Fig. 25. IR spectrum of 10.
Fig. 26. $^1$H NMR spectrum of 10.
Fig. 27. ESI-MS spectrum of 10.
4.4 Synthesis and characterization of Deferoxamine B mesylate ($L^2$).

Synthesis of Deferoxamine B mesylate ($L^2$).
Characterization of Deferoxamine B mesylate (L²).

IR spectral analysis

IR spectrum of deferoxamine B mesylate (L²) showed a peak at 3330 cm⁻¹ due to O-H stretching vibration. A peak observed in the range 3130 cm⁻¹ is due to N-H stretching vibration. A sharp peak at 1650 cm⁻¹ is due to carbonyl group as represented in Fig. 28.

NMR spectral analysis

¹H NMR spectrum deferoxamine B mesylate (L²) showed a singlet peak at δ 8.0 is due to two NH protons. Methyl protons observed as singlet at δ 1.94. Methylene protons observed as triplet at δ 3.41 (J = 8.7 Hz), δ 2.97 (J = 4.8 Hz), δ 2.8 (J = 5.5 Hz) and δ 2.3 (J = 5.25 Hz), and multiplet as δ 2.6, δ 1.41 - 1.54, δ 1.30 - 1.37 and δ 1.16 – 1.2. The absence of peaks in the range δ 4.8 to 7.5 which is also indicates that conversion of amide to amine as represented in Fig. 29.

Mass spectral analysis

As depicted in Fig. 30, Deferoxamine B mesylate (L²) shows a peak at m/z 561 corresponds to its [M+1]⁺ ion.
Fig. 28. IR spectrum of $L^2$. 
Fig. 29. $^1$H NMR spectrum of $\mathbf{L}^2$. 
Fig. 30. ESI-MS spectrum of L².

4.5.1. Synthesis and characterization of Cu(II) complex.

Synthesis of Cu(II) complex (9a).

Characterization of Cu(II) complex (9a).

**IR spectral analysis**

IR spectrum of 9a exhibits a broad band around 3433 cm$^{-1}$ due to $\nu$ O-H stretching vibration of water molecule. N-H str vibration observed around 3100 cm$^{-1}$. A peak observed around 1640 cm$^{-1}$ due to carbonyl (C=O) group.

**Absorption spectral analysis**

The electronic spectrum of aqueous solution of complex 9a exhibited absorption maxima at 614 nm d-d transition of copper ion in visible region as shown in Fig 31.

**Mass spectral analysis**

As represented in Fig. 33, the complex 9a shows a peak at m/z 422 corresponds to its $[\text{CuL}^1+1]^+$ ions.
Electrochemical studies

The electrochemical behavior of complex 9a has been studied by cyclic voltammetry in water containing 0.1 M TBAP. The electrochemical data are summarized in table 1. The mononuclear complex 9a exhibits a quasireversible redox wave for a one-electron transfer process corresponding to the Cu(II)/Cu(I) redox couple with an anodic peak potential $E_{p_a}$ of -0.40 V and cathodic peak potential of -0.48 V vs Ag/AgCl. The spectrum is shown in Fig. 32.

Fig. 31. Electronic spectrum of complex 9a
Fig. 32. Cyclic voltagram of complex 9a.
Fig. 33. ESI-MS spectrum of 9a.
4.5.2. Synthesis and characterization of Ni(II) complex.

Synthesis of Ni(II) complex (9b).

Characterization of Ni(II) complex (9b).

IR spectral analysis

A peak observed at 1650 cm\(^{-1}\) is due to carbonyl (C=O) group. The peak observed at 3290 cm\(^{-1}\) is due to N-H stretching vibration.

Electronic spectral analysis

Complex 9b exhibits absorption maxima at 655, 749 and 1073 nm due to d-d transition of nickel ion in visible region as shown in Fig. 34. This suggests that the coordination geometry around the metal ion may be distorted octahedral.

Mass spectral analysis

As shown in Fig. 36, Complex 9b shows a peak at m/z 417 corresponds to its [NiL\(^{1+1}\)]\(^{+}\) ion.

Electrochemical studies

The electrochemical behavior of complex 9b has been studied by cyclic voltammetry in water containing 0.1 M TBAP. The electrochemical data are summarized in table 1. The cyclic voltammogram of complex 9b reveals a one-electron quasireversible reduction wave.
corresponding Ni(II)/Ni(I) redox couple with \( E_{pa} = -0.52 \) V and \( E_{pc} = -0.61 \) V Vs Ag/AgCl. As shown in Fig. 35, The complex exhibits a quasireversible redox wave for a one-electron transfer process corresponding to the Ni(II)/Ni(III) redox couple with an anodic peak potential \( E_{pa} = 0.46 \) V and \( E_{pc} = 0.37 \) V Vs Ag/AgCl.

Table 1.
Electrochemical data for the mononuclear Cu(II) and Ni(II) complexes

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Cathodic region</th>
<th>Anodic region</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 9a )</td>
<td>(-0.48)</td>
<td>(-0.40)</td>
</tr>
<tr>
<td>( 9b )</td>
<td>(-0.61)</td>
<td>(-0.52)</td>
</tr>
</tbody>
</table>

Measured by Cyclic Voltammograms at 100 mV/s. E Vs Ag/AgCl. Conditions: GC working and Ag/AgCl reference electrodes; supporting electrolyte, TBAP; \([\text{complex}]= 1 \times 10^{-3} \) M; \([\text{TBAP}]= 1 \times 10^{-1} \) M.
Fig. 34. Electronic spectrum of 9b.

Fig. 35. Cyclic Voltagram 9b.
Fig. 36. ESI-MS spectrum of 9b.
4.5.3. Synthesis and characterization of Zn(II) complex

Synthesis of Zn(II) complex (9c).

Characterization of Zn(II) complex (9c).

**IR spectral analysis**

The peak observed at 3290 cm\(^{-1}\) is due to N-H stretching vibration. Complex 9c exhibited a peak at 1651 cm\(^{-1}\) is due to carbonyl group (C=O).

**Mass spectral analysis**

As shown in Fig. 37, Complex 9c shows a peak at m/z 423 corresponds to its [ZnL\(^1\) + 1]\(^+\) ion.
Fig. 37. ESI-MS spectrum of 9c.
4.5.4. Synthesis and characterization of Mg(II) complex

Synthesis of Mg(II) complex (9d).

Characterization of Mg(II) complex (9d).

**IR spectral analysis**

Complex 9d exhibits a peak at 1625 cm$^{-1}$ due to carbonyl group (C=O). The peak 3100 cm$^{-1}$ was observed corresponds to N-H stretching vibration.

**Mass spectral analysis**

ESI-MS mass spectrum of complex 9d shows a peak at m/z 383 corresponds to its $[\text{MgL}^1+1]^+$ ion as represented in Fig. 38.
Fig. 38. ESI-MS spectrum of 9d.
4.5.5. Synthesis and characterization of Fe(III) complex

Synthesis of Fe(III) complex (9e).

Characterization of Fe(III) complex (9e).

**IR spectral analysis**

A strong band in the region around 1090 cm\(^{-1}\) and a sharp peak in the region around 630 cm\(^{-1}\) was observed which was corresponds to the antisymmetric stretch and antisymmetric bending vibration of perchlorate ions, respectively. A peak around 3120 cm\(^{-1}\) corresponds to N-H stretching vibration. A peak observed at 1690 cm\(^{-1}\) was due to the carbonyl group (C=O).

**Electronic spectral analysis**

A weak band observed in the range 430 nm was due to the d-d transition of Fe(III) ion. A strong band in the range 220 nm was due to an intraligand charge transfer transition as shown in Fig. 39.

**Mass spectral analysis**

As depicted in Fig. 40, The ESI-MS spectrum of complex 9e shows a molecular ion peak [M\(^+\) + 1] at m/z 414 that is equivalent to its molecular weight.
Fig. 39. Electronic spectrum 9e.
Fig. 40. ESI-MS Spectrum 9e.
4.5.6. Synthesis and characterization of Co(III) complex

Synthesis of Co(III) complex (9f).

Characterization of Co(III) complex (9f).

**IR spectral analysis**

A strong band in the region around 1080 cm\(^{-1}\) and a sharp peak in the region around 626 cm\(^{-1}\) was observed which was corresponds to the antisymmetric stretch and antisymmetric bending vibration of perchlorate ions, respectively. A peak around 3200 cm\(^{-1}\) corresponds to N-H stretching vibration. A peak observed at 1710 cm\(^{-1}\) was due to the carbonyl group (C=O).

**Electronic spectral analysis**

A weak band observed in the range 614 nm was due to the d-d transition of Co(III) ion. A strong band in the range 210 nm was due to an intraligand charge transfer transition as shown in Fig. 41.

**Mass spectral analysis**

As represented in Fig. 42, ESI-MS spectrum of complex 9f shows a peak at m/z 417 is corresponds to its [Co(III)L\(^{1}+1\)]\(^+\) ion.
Fig. 41. Electronic spectrum of \textbf{9f}. 
Fig. 42. ESI-MS spectrum of 9f.
4.5.7. Synthesis and characterization of Mn(III) complex

Synthesis of Mn(III) complex (9g).

Characterization of Mn(III) complex (9g).

**IR spectral analysis**

A strong band in the region around 1085 cm$^{-1}$ and a sharp peak in the region around 625 cm$^{-1}$ was observed which was corresponds to the antisymmetric stretch and antisymmetric bending vibration of perchlorate ions, respectively. A peak around 3180 cm$^{-1}$ corresponds to N-H stretching vibration. A peak observed at 1680 cm$^{-1}$ was due to the carbonyl group (C=O).

**Electronic spectral analysis**

A weak band observed in the range 710 nm was due to the d-d transition of Mn(III) ion as shown in Fig. 43. A strong band in the range 240 nm was due to an intraligand charge transfer transition.

**Mass spectral analysis**

ESI-MS spectrum of complex 9g shows a peak at m/z 413 is corresponds to its [Mn(III)L$^1$ + 1]$^+$ ion as represented in Fig. 44.
Fig. 43. Electronic spectrum of 9g.
Fig. 44. ESI-MS spectrum of 9g.
4.5.8. Synthesis and characterization of VO(IV) complex

Synthesis of VO(IV) complex (9h).

Characterization of VO(IV) complex (9h).

**IR spectral analysis**

A peak around 3150 cm\(^{-1}\) corresponds to \(\nu\)(N-H) stretching vibration. A peak observed at 1675 cm\(^{-1}\) was due to the carbonyl group \(\nu\) (C=O). A band at around 980 cm\(^{-1}\), which is assigned to \(\nu\)(V=O) vibration.

**Electronic spectral analysis**

A weak band observed in the range 625 nm was due to the d-d transition of VO(IV) ion as represented in Fig. 45. A strong band in the range 210 nm was due to an intraligand charge transfer transition.

**Mass spectral analysis**

As depicted in Fig. 46, ESI-MS spectrum of complex 9h shows a peak at m/z 425 is corresponds to its [VO(IV)L\(^{1}\)]\(^{+}\) ion.
Fig. 45. Electronic spectrum of 9h.
Fig. 46. ESI-MS spectrum of 9h.

4.6.1. Synthesis and characterization of Cu(II) complex

Synthesis of Cu(II) complex (11a).

Characterization of Cu(II) complex (11a).

IR spectral analysis

IR Spectrum of 11a exhibit a broad band around 3090 cm⁻¹ due to ν(N-H) stretching vibration. A peak observed around 1650 cm⁻¹ is due to carbonyl (C=O) group.

Absorption spectral analysis

Fig. 47 shows the electronic spectra of aqueous solution of complex 11a exhibited absorption maxima at 634 nm d-d transition of copper ion in visible region. A strong band in the range 214 nm was due to an intraligand charge transfer transition.

Mass spectral analysis

Complex 11a shows a peak at m/z 622 corresponds to its [CuL² + 1]⁺ ion as represented in Fig. 48.
Fig. 47. Electronic spectrum of 11a.
Fig. 48. ESI-MS spectrum of 11a.
4.6.2. Synthesis and characterization of Ni(II) complex

Synthesis of Ni(II) complex (11b).

Characterization of Ni(II) complex (11b).

IR spectral analysis

A peak observed at 1650 cm\(^{-1}\) is due to carbonyl (C=O) group. The peak observed at 3100 cm\(^{-1}\) due to N-H stretching vibration.

Electronic spectral analysis

Complex 11b exhibits absorption maxima at 667, 765 and 1068 nm due to d-d transition of nickel ion in visible region as shown in Fig. 49, suggesting that the coordination geometry around the metal ion may be distorted octahedral.

Mass spectral analysis

Complex 11b shows a peak at m/z 617 corresponds to its [NiL\(^2\)+1]\(^+\) ion as shown in Fig. 50.
Fig. 49. Electronic spectrum of 11b.
Fig. 50. ESI-MS spectrum of 11b.
4.6.3. Synthesis and characterization of Zn(II) complex

Synthesis of Zn(II) complex (11c).

Characterization of Zn(II) complex (11c).

**IR spectral analysis**

The peak observed at 3290 cm\(^{-1}\) is due to N-H stretching vibration. Complex 11c exhibit a peak at 1650 cm\(^{-1}\) is due to carbonyl group (C=O).

**Mass spectral analysis**

Complex 11c is shows a peak at m/z 623 corresponds to its [ZnL\(^{2+}\)]\(^{+}\) ion as shown in Fig. 51.
Fig. 51. ESI-MS spectrum of 11c.
4.6.4. Synthesis and characterization of Mg(II) complex

Synthesis of Mg(II) complex (11d).

![Synthesis of Mg(II) complex](image)

Characterization of Mg(II) complex (11d).

**IR spectral analysis**

Complex 9d exhibit a peak at 1630 cm\(^{-1}\) is due to carbonyl group (C=O). O-H str vibration of water molecule observed around 3300 cm\(^{-1}\).

**Mass spectral analysis**

As depicted in Fig. 52, complex 11d shows a peak at m/z 583 corresponds to its [MgL\(^2\) + 1]\(^+\) ion.
Fig. 52. ESI-MS spectrum of 11d.
4.6.5. Synthesis and characterization of Fe(III) complex

Synthesis of Fe(III) complex (11e).

![Synthesis Diagram]

Characterization of Fe(III) complex (11e).

**IR spectral analysis**

Complex 11e exhibit a peak at 1650 cm\(^{-1}\) due to carbonyl group (C=O). A peak observed around 3140 cm\(^{-1}\) is due to N-H stretching vibration.

**Electronic spectral analysis**

An aqueous solution of complexes 11e exhibits absorption maxima in the region 431 nm due to d-d transition of Fe(III) ion as shown in Fig. 53. The strong band observed in the range 210 nm due to intraligand charge transfer.

**Mass spectral analysis**

Complex 11e shows a peak at m/z 614 corresponds to its [FeL\(^2\)+1]\(^+\) ion as represented in Fig. 54.
Fig. 53. Electronic spectrum of 11e.
Fig. 54. ESI-MS spectrum of 11e.
4.6.6. Synthesis and characterization of Co(III) complex

Synthesis of Co(III) complex (11f).

![Synthesis of Co(III) complex](image)

Characterization of Co(III) complex (11f).

**IR spectral analysis**

Complex 11f exhibit a peak at 1650 cm$^{-1}$ due to carbonyl group (C=O). The peak observed at 3110 cm$^{-1}$ due to N-H stretching vibration. O-H str vibration of water molecule observed around 3420 cm$^{-1}$.

**Electronic spectral analysis**

An aqueous solution of complexes 11f exhibits absorption maxima in the region 614 nm due to d-d transition of Co(III) ion as given in Fig. 55. The strong band observed in the range 220 nm due to intraligand charge transfer.

**Mass spectral analysis**

Complex 11f shows a peak at m/z 617 corresponds to its $[\text{CoL}^2+1]^+$ ion as presented in Fig. 56.
Fig. 55. Electronic spectrum of 11f.
Fig. 56. ESI-MS spectrum of 11f.
4.6.7. Synthesis and characterization of Mn(III) complex

**Synthesis of Mn(III) complex (11g).**

![Chemical structure of Mn(III) complex]

**Characterization of Mn(III) complex (11g).**

**IR spectral analysis**

IR Spectrum of 11g exhibit a broad band around 3450 cm\(^{-1}\) due to ν O-H stretching vibration of water molecule. N-H str vibration observed around 3100 cm\(^{-1}\). A peak observed around 1650 cm\(^{-1}\) due to carbonyl (C=O) group.

**Electronic spectral analysis**

As depicted in Fig. 57, aqueous solution of complexes 11g exhibits absorption maxima in the region 580 nm due to d-d transition of Mn(III) ion. The strong band observed in the range 210 nm and 310 nm are due to intraligand charge transfer.

**Mass spectral analysis**

Complex 11g shows a peak at m/z 613 corresponds to its [MnL\(^2+1\)]\(^+\) ions as given in Fig. 58.
Fig. 57. Electronic spectrum of 11g.
Fig. 58. ESI-MS spectrum of 11g.
4.6.8. Synthesis and characterization of VO(IV) complex

Synthesis of VO(IV) complex (11h).

![Chemical structure of VO(IV) complex (11h)]

Characterization of VO(IV) complex (11h).

**IR spectral analysis**

IR spectrum of complex 11h is shown in Fig. 102. IR spectrum of complex 11h exhibits a peak at 1650 cm\(^{-1}\) due to carbonyl group. A peak at 3100 cm\(^{-1}\) due to N-H stretching vibrations. O-H stretching frequency was observed around 3422 cm\(^{-1}\). A band at around 961 cm\(^{-1}\), which is assigned to \(\nu(V=O)\) vibration.

**Electronic spectral analysis**

Electronic spectrum of aqueous solution of complex 11h in visible region exhibits a weak band around 570 nm due to d-d transition of VO(IV) metal ion as given in Fig. 59. A strong band in the range 210 nm was due to an intraligand charge transfer transition.

**Mass spectral analysis**

ESI-MS spectrum of complex 11 h shows a peak at m/z 625 due to [VO(IV)]\(L^2\)]\(^+\) ion as shown in Fig. 60.
Fig. 59. Electronic spectrum of 11h.
Fig. 60. ESI-MS spectrum of 11h.
4.7. DNA Binding and cleavage studies of complexes with L.¹

4.7.1. DNA Binding and cleavage studies of Cu(II), Ni(II), Zn(II) and Mg(II) complexes.

4.7.1.1. DNA Binding studies

Absorption spectral studies

Interaction of metal complexes with CT-DNA can be monitored by absorption spectrum titrations. The absorption spectra of the new synthesized complexes 9a – 9d in absence and presence of DNA is shown in Fig. 61-64. An aqueous solution of the 9a complex shows bands at 220 nm due to intraligand charge transfer transitions in Fig. 31. Addition of CT-DNA (0 to 250 µM) into complex, 49% hypochromism was observed for intraligand charge transfer transitions of complex 9a. Intraligand charge transfer transitions of complexes 9b, 9c and 9d exhibited 37%, 18% and 30% hypochromism upon addition of DNA into complex solution and are shown in Figures 61 - 64. The intrinsic binding constant $K_b$ has been obtained from the changes in intraligand charge transfer transition around 220 nm and the values are given in table 2. These values are ~100 times lower than reported for typical classical intercalators (eg. ethidium bromide, $-10^6 \text{M}^{-1}$).¹³⁵

Table 2. DNA binding parameters for 9a, 9b, 9c and 9d complexes

<table>
<thead>
<tr>
<th>Complexes</th>
<th>$K_b (\text{M}^{-1})$</th>
<th>$K_{sv}$</th>
<th>$K_{app} (\text{M}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>9a</td>
<td>$2.5 \times 10^4$</td>
<td>0.14</td>
<td>$1.8 \times 10^5$</td>
</tr>
<tr>
<td>9b</td>
<td>$2.8 \times 10^4$</td>
<td>0.14</td>
<td>$1.8 \times 10^5$</td>
</tr>
<tr>
<td>9c</td>
<td>$8.7 \times 10^4$</td>
<td>0.11</td>
<td>$2.5 \times 10^5$</td>
</tr>
<tr>
<td>9d</td>
<td>$1.8 \times 10^4$</td>
<td>0.12</td>
<td>$1.0 \times 10^5$</td>
</tr>
</tbody>
</table>
Fig. 61. Absorption spectra of \(9a\) complex (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Plot of \([\text{DNA}] / (\varepsilon_a - \varepsilon_f)\) vs [DNA] for absorption titration of CT-DNA and \(9a\) complex. The arrow shows the intensity changes on increasing the DNA concentration.

Fig. 62. Absorption spectra of \(9b\) complex (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Plot of \([\text{DNA}] / (\varepsilon_a - \varepsilon_f)\) vs [DNA] for absorption titration of CT-DNA and \(9b\) complex.

<table>
<thead>
<tr>
<th>Complex</th>
<th>(K_b) (M(^{-1}))</th>
<th>(K_{app}) (M(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(9a)</td>
<td>2.5 x 10(^4)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>1.8 x 10(^5)</td>
<td></td>
</tr>
<tr>
<td>(9b)</td>
<td>2.8 x 10(^4)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>1.8 x 10(^5)</td>
<td></td>
</tr>
<tr>
<td>(9c)</td>
<td>8.7 x 10(^4)</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>2.5 x 10(^5)</td>
<td></td>
</tr>
<tr>
<td>(9d)</td>
<td>1.8 x 10(^4)</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>1.0 x 10(^5)</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 63. Absorption spectra of 9c complex (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Plot of [DNA]/(ε_a - ε_f) vs [DNA] for absorption titration of CT-DNA and 9c complex.

Fig. 64. Absorption spectra of 9d complex (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Plot of [DNA]/(ε_a - ε_f) vs [DNA] for absorption titration of CT-DNA and 9d complex.
Fluorescence spectral studies

In competitive ethidium bromide (EB) binding studies, the complexes 9a, 9b, 9c and 9d were added to DNA pretreated with EB ([EB] = 2 µM, [DNA] = 280 µM) and then emission intensities of DNA-induced EB were measured [Fig. 65 – 68]. Addition of a complex would quench the EB emission by either replacing DNA-bound EB (if it binds to DNA more strongly than EB) and/or by accepting the excited state electron from EB\(^{136}\). The non replacement-based quenching has been suggested with DNA-mediated electron transfer from the excited ethidium bromide to acceptor metal complexes. As there is no complete quenching of the EthBr-induced emission intensity, an intercalative mode of DNA-binding of 9a, 9b, 9c and 9d observed values is ruled out. Further, the quenching extents “K\(_{sv}\)” for 9a, 9b, 9c and 9d have been estimated by using Stern–Volmer equation and K\(_{app}\) values are also calculated from fluorescence spectral titrations and are given in table 2. These values are lower than the classical intercalators which suggested that the complexes 9a, 9b, 9c and 9d may be bind to DNA grooves.

![Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex 9a.](image)

**Fig. 65.** Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex 9a. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM (λ\(_{ex}\) = 520 nm). The arrow shows the intensity changes on increasing the complex concentration. The plot of I\(_0\)/I vs. [Complex]/[DNA] for fluorescence quenching curves of DNA-EB by complex 9a.
Fig. 66. Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex 9b. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (ex = 520 nm). The arrow shows the intensity changes on increasing the complex concentration. The plot of I0/I vs. [Complex]/[DNA] for fluorescence quenching curves of DNA-EB by complex 9b.

Fig. 67. Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex 9c. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (ex = 520 nm). The arrow shows the intensity changes on increasing the complex concentration. The plot of I0/I vs. [Complex]/[DNA] for fluorescence quenching curves of DNA-EB by complex 9c.
Fig. 68. Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex 9d. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (ɛex = 520 nm).

Viscosity measurements

Mode of binding of complexes to CT-DNA was investigated by using the viscosity measurements also. Complex that interact in the DNA grooves by partial and or non classical intercalation leads no change in relative viscosity of CT-DNA solution when an increasing the concentration of complex. A classical intercalative mode causes a significant increase in viscosity of DNA solution due to increase in separation of base pairs at intercalation sites and hence an increase in overall DNA length. To explore the interaction between the complexes to CT-DNA, viscosity measurements were carried out by keeping the DNA concentration as constant and varying the concentration of complex. An increasing amount of complex into CT-DNA no change in the relative viscosity of DNA solution and are shown in
Fig. 69. The data suggest that the new synthesized complexes interact to grooves of DNA base pairs.

4.7.1.2. DNA cleavage studies of complexes Cu(II), Ni(II), Zn(II) and Mg(II).

The nuclease activity of the complexes has been studied, supercoiled pBR322 DNA (150 μg/μL) was incubated with complex in aqueous buffer solution (5 mM Tris–HCl/40 mM NaCl, pH 7.2). The DNA-cleaving ability of complexes was demonstrated initially by a plasmid relaxation assay, in which the conversion of supercoiled form (Form I) to nicked circular (Form II) and linear open circular (Form III) DNA were monitored.

Mechanistic investigations were done using various quenchers to understand the nature of the reactive species involved in the DNA cleavage reactions. The active chemical species that was responsible for the DNA cleavage, we investigated in the presence of hydroxyl radical scavengers (DMSO, KI), singlet oxygen quenchers (NaN₃, L-histidine, and ethanol), superoxide scavenger (SOD) and chelating agent (EDTA) under our experimental conditions. From the Fig. 70, there was no apparent inhibition in the DNA cleavage activity of the complex 9a in the presence of singlet oxygen quenchers such as NaN₃ and L-histidine and superoxide scavenger (SOD). Complete inhibition in DNA cleavage was observed in the presence of hydroxyl radical
(•OH) quenchers such as KI, DMSO and in presence of chelating agent EDTA (0.5 mM) (Fig.
71, Lane 2). The mechanistic results indicate the formation of •OH as the DNA cleaving agent.
The EDTA, a Cu(II) specific chelating agent that strongly binds to Cu(II) forming a stable
complex, can efficiently inhibit DNA cleavage, indicating Cu(II) complexes play the key role in
the cleavage.

Fig. 70. Cleavage of supercoiled pBR322 DNA incubated with 9a (50 µM) in the presence of
hydrogen peroxide in Tris – HCl buffer (pH = 7.2) at 37 ºC for 1 h. Lane 1, DNA control,
Lane 2, DNA + 9a (50 µM) + H₂O₂ (0.08 %), Lane 3, DNA + 9a (50 µM) + H₂O₂ (0.08 %) +
KI (0.5 mM), Lane 4, DNA + 9a (50 µM) + H₂O₂ (0.08 %) + NaN₃ (0.5 mM), Lane 5, DNA +
9a (50 µM) + H₂O₂ (0.08 %) + DMSO (4 µl), Lane 6, DNA + 9a (50 µM) + H₂O₂ (0.08 %) +
L-histidine (50 µM), Lane 7, DNA + 9a (50 µM) + H₂O₂ (0.08 %) + SOD (4 units), Lane 8,
DNA + 9a (50 µM) + H₂O₂ (0.08 %) + ethanol (1 µL, 10 %).

Fig. 71. Cleavage of supercoiled pBR322 DNA incubated with 9b (75 µM) in Tris – HCl (pH
= 7.2) at 37 ºC for 3 h. Lane 1, DNA control, Lane 2, DNA + 9b (75 µM), Lane 3, DNA +
9b (75 µM) + NaN₃ (0.5 mM), Lane 4, DNA + 9b (75 µM) + DMSO (4 µl), Lane 5, DNA +
9b (75 µM) + L-histidine (50 µM), Lane 6, DNA + 9b (75 µM) + SOD (4 units), Lane 7,
DNA + 9b (75 µM) + KI (0.5 mM).
The cleavage of supercoiled pBR323 DNA by complexes 9b and 9c and their mechanistic study of DNA cleavage has shown in Fig. 71 and Fig. 72 respectively. Complexes 9b and 9c cleave the pBR322 DNA and no apparent inhibition was observed in presence of hydroxyl radical scavenger (DMSO, KI), singlet oxygen quencher (NaN₃, L-histidine, ethanol) and superoxide scavenger (SOD). These results rules out the possibility of cleavage by hydroxyl radical, singlet oxygen and superoxide anion, respectively. Experimental results suggested that the DNA cleavage reaction by complex 9b and 9c should be due to hydrolytic mechanism. The EDTA efficiently inhibits the DNA cleavage activity of the 9b and 9c complexes (Fig. 71, Lane 3 and Lane 4).

![Fig. 72. Cleavage of supercoiled pBR322 DNA incubated with 9c (75 μM) in Tris – HCl (pH = 7.2) at 37 °C for 3 h. Lane 1, DNA control, Lane 2, DNA + 9c (75 μM), Lane 3, DNA + 9c (75 μM) + NaN₃ (0.5 mM), Lane 4, DNA + 9c (75 μM) + DMSO (4 μl), Lane 5, DNA + 9c (75 μM) + L-histidine (50 μM), Lane 6, DNA + 9c (75 μM) + SOD (4 units), Lane 7, DNA + 9c (75 μM) + KI (0.5 mM).](image)

![Fig. 73. Cleavage of supercoiled pBR 322 DNA incubated with 9a - 9c in Tris-Hcl buffer. Lane 1, DNA control, Lane 2, DNA + H₂O₂ (0.08 %) + 9a (50 μM) + EDTA (0.5 mM), Lane 3, DNA + 9b (75 μM) + EDTA (0.5 mM), Lane 4, DNA + 9c (75 μM) + EDTA (0.5 mM).](image)
Minor groove binding agent DAPI and major groove binding agent methyl green were used to explore the potential interacting site of the complexes with plasmid pBRR322 DNA. Fig. 74, there was no apparent inhibition in the DNA cleavage activity of complexes, when methyl green (major groove binding agent) treated with pBR322 DNA prior to addition of complexes and external additives (Lane 2, 4, 7). Addition of DAPI (minor groove binding agent) to pBR322 DNA prior to addition of complexes inhibit the DNA cleavage activity of complexes 9a, 9b and 9c are shown in Fig. 74, Lane 3, 5, 6. This result clearly suggests that the complexes prefer to bind to pBR322 DNA minor groove.

![Image](image.png)

Fig. 74. Lane 1, DNA control, Lane 2, DNA + H₂O₂ (0.08 %) + 9a (50 µM) + methyl green (0.5 mM), Lane 3, DNA + H₂O₂ (0.08 %) + 9a (50 µM) + DAPI (2 µL), Lane 4, DNA + 9b (75 µM) + methyl green (0.5 mM), Lane 5, DNA + 9b (75 µM) + DAPI (2 µl), Lane 6, DNA + 9c (75 µM) + DAPI (2 µl), Lane 7, DNA + 9c (75 µM) + methyl green (0.5 mM).

The nuclease activity of complex 9d has been studied, supercoiled pBR322 DNA (150 µg/µL) was incubated with complex in aqueous buffer solution (5 mM Tris–HCl, pH 7.2) for an one hour. Cleavage activity of 9d is shown in Fig. 75. Complex 9d does not cleave the supercoiled pBR322 DNA in presence and absence of hydrogen peroxide.
Fig. 75. Cleavage of supercoiled pBR322 DNA incubated with 9d (75 µM) in Tris – HCl (pH = 7.2) at 37 ºC for 3 h. Lane 1, DNA control, Lane 2, DNA + 9d (50 µM), Lane 3, DNA + 9d (50 µM) + (0.08 %) and Lane 4, DNA + 9d (100 µM) + (0.08 %).

4.7.2. DNA Binding and cleavage studies of Fe(III), Co(III), Mn(III) and VO(IV) complexes.

4.7.2.1. DNA binding studies

Absorption spectral studies

Interaction of metal complexes with CT-DNA can be monitored by absorption spectral titrations. The absorption spectra of the new synthesized 9e-9h complexes in absence and presence of DNA is shown in Fig.76 - 79. An aqueous solution of 9e-9h complexes showed a band at 220 nm due to the intraligand charge transitions and exhibited 51%, 51%, 45%, 68%, 40% and 41% hypochromism respectively with an increase in the concentration of CT-DNA upto 250 µM and are shown in Fig. 76 - 79. The intrinsic binding constant $K_b$ has been obtained from the changes in intraligand charge transfer transition around 220 nm and the values are given in table Fig. 76-79. These values are ~100 times lower than reported for typical classical intercalators (eg. ethidium bromide, $10^6$ M$^{-1}$).
Fig. 76. Absorption spectra of 9e complex (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Plot of [DNA]/(ε_a - ε_f) vs [DNA] for absorption titration of CT-DNA and 9e complex.

Fig. 77. Absorption spectra of 9f complex (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Plot of [DNA]/(ε_a - ε_f) vs [DNA] for absorption titration of CT-DNA and 9f complex.
Fig. 78. Absorption spectra of 9g complex (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Plot of [DNA] / (ε_a - ε_f) vs [DNA] for absorption titration of CT-DNA and 9g complex.

Fig. 79. Absorption spectra of 9h complex (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Plot of [DNA] / (ε_a - ε_f) vs [DNA] for absorption titration of CT-DNA and 9h complex.
**Fluorescence spectral studies**

In competitive ethidium bromide (EB) binding studies, the complexes 9e - 9f were added to DNA pretreated with EB ([EB] = 2 µM, [DNA] = 280 µM) and then emission intensities of DNA-induced EB were measured [Fig. 80 - 83]. Addition of a complex would quench the EB emission by either replacing DNA-bound EB (if it binds to DNA more strongly than EB) and/or by accepting the excited state electron from EB. The non replacement-based quenching has been suggested with DNA-mediated electron transfer from the excited ethidium bromide to acceptor metal complexes. As there is no complete quenching of the EB-induced emission intensity, an intercalative mode of DNA-binding of 9e - 9f observed values is ruled out. Further, the quenching extents “Ksv” for have been estimated by 9e - 9f using Stern–Volmer equation and Kapp values are also calculated from fluorescence spectral titrations and are given in table 5. These values are lower than the classical intercalators which suggested that the complexes 9e-9h may be bind to DNA grooves.

![Fluorescence spectra of EB bound to DNA](image-url)

**Fig. 80.** Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex 9e [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (ëex = 520 nm).
Fig. 81. Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex \( \mathbf{9f} \) \([\text{EB}] = 2 \mu\text{M}, [\text{DNA}] = 280 \mu\text{M}, [\text{Complex}] = 0 \text{ to } 280 \mu\text{M}. (\epsilon_{\text{ex}} = 520 \text{ nm}).

Fig. 82. Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex \( \mathbf{9g} \) \([\text{EB}] = 2 \mu\text{M}, [\text{DNA}] = 280 \mu\text{M}, [\text{Complex}] = 0 \text{ to } 280 \mu\text{M}. (\epsilon_{\text{ex}} = 520 \text{ nm}).
Fig. 83. Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex 9h. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (ex = 520 nm).

Table 5. DNA binding parameters for 9e - 9h complexes

<table>
<thead>
<tr>
<th>Complexes</th>
<th>K_b</th>
<th>K_av</th>
<th>K_app</th>
</tr>
</thead>
<tbody>
<tr>
<td>9e</td>
<td>1.2 x 10^4</td>
<td>0.1</td>
<td>1.3 x 10^5</td>
</tr>
<tr>
<td>9f</td>
<td>1.2 x 10^4</td>
<td>0.1</td>
<td>1.6 x 10^5</td>
</tr>
<tr>
<td>9g</td>
<td>1.0 x 10^4</td>
<td>0.09</td>
<td>1.2 x 10^5</td>
</tr>
<tr>
<td>9h</td>
<td>1.1 x 10^4</td>
<td>0.09</td>
<td>1.5 x 10^5</td>
</tr>
</tbody>
</table>

**Viscosity measurements**

Complex that interact in the DNA grooves by partial and or non classical intercalation leads no change in relative viscosity of CT-DNA solution when an increasing the concentration of complex. A classical intercalative mode causes a significant increase in viscosity of DNA solution due to increase in separation of base pairs at intercalation sites and hence an increase in
overall DNA length. To explore the interaction between the 9e-9h complexes to CT-DNA, viscosity measurements were carried out by keeping the DNA concentration as constant and varying the concentration of complex. An increasing amount of complex 9e into CT-DNA no change in the relative viscosity of DNA solution and are shown in Fig. 84. The data suggest that the new synthesized complexes interact to grooves of DNA base pairs.

![Graph showing changes in relative viscosity of CT-DNA on increasing amounts of complex 9e.](image)

**Fig. 84. Changes in relative viscosity of CT-DNA on increasing amounts of complex 9e.**

### 4.7.2.2. DNA cleavage studies

The DNA-cleaving ability of complexes was demonstrated initially by a plasmid relaxation assay, in which the conversion of supercoiled form (Form I) to nicked circular (Form II) and linear open circular (Form III) DNA were monitored. Mechanistic investigations were done using various additives to understand the nature of the reactive species involved in the DNA cleavage reactions. The active chemical species that was responsible for the DNA cleavage, we investigated in the presence of hydroxyl radical scavengers (DMSO, KI), singlet oxygen quenchers (NaN₃, L-histidine, and ethanol), superoxide scavenger (SOD) and chelating agent (EDTA) under our experimental conditions.
DNA cleavage activities of 9e complex

The plasmid DNA cleavage activity of complex 9e was investigated by agarose gel electrophoresis. The cleavage activity of the complex 9e is shown in Fig. 85. Complex 9e does not cleave the plasmid DNA in absence and presence of hydrogenperoxide.

Fig. 85. cleavage of supercoiled pBR322 DNA incubated with 9e in Tris-Hcl at 37 °C for 3 hrs. Lane 1: DNA control, Lane 2: DNA + 9e (50 μM), Lane 3: DNA + 9e (50 μM) + H₂O₂ (0.08%), Lane 4, DNA + 9e (100 μM) + H₂O₂ (0.08%).

DNA cleavage studies of complex 9f

The plasmid DNA cleavage activity of complex 9f is shown in Fig. 86. From Fig. 86 complex 9f cleave the pBR322 DNA in absence of additives under physiological condition (Lane 2). No inhibition was observed in presence of hydroxyl radical scavengers (DMSO), singlet oxygen quenchers (NaN₃), superoxide scavenger (SOD) (lane 3-5). These results rules out the possibility of cleavage by hydroxyl radical, singlet oxygen and superoxide anion, respectively. This fact implies that the DNA cleavage reaction by complex 9f may be due to hydrolytic mechanism. The EDTA efficiently inhibits the DNA cleavage activity of the 9f complex under our experimental conditions (Lane 6).
Fig. 86. Cleavage of supercoiled PBR322 DNA included with 9f (50 μM) in Tris-Hcl (Ph=7.2) at 37°C for 3 hrs. Lane 1: DNA Control, Lane 2: DNA + 9f (50 μM), Lane 3: DNA + 9f (50 μM) + NaN₃(0.5mM), Lane 4: DNA + 9f (50 μM) + DMSO(4 μl), Lane 5: DNA + 9f (50 μM) + SOD (4 units), Lane 6: DNA + 9f (50 μM) + EDTA(50 μM).

4.7.2.3. DNA cleavage studies of complex 9g

Complex 9g cleaves the pBR 322 DNA in presence of H₂O₂ and from Fig. 87. Lane 2 and Lane 3, higher concentration of the complex 9g exhibit higher cleavage activity than lower concentration of the complexes under same experimental conditions. The DNA cleavage activity of complex 9g was inhibited in presence of singlet oxygen quenchers such as NaN₃ and L-histidine. No apparent inhibition in the DNA cleavage activity of the complex 9g was observed in the presence of hydroxyl radical (•OH) quenchers such as KI, DMSO and superoxide scavenger (SOD). The mechanistic results indicate the formation of singlet oxygen as the DNA cleaving agent.

Fig. 87. Cleavage of supercoiled PBR322 DNA incubated with complex 9g in Tris-Hcl(pH=7.2) at 37 °C for 3 hrs. Lane 1: DNA control, Lane 2: DNA + 9g (50 μM) + H₂O₂ (0.08%), Lane 3: DNA + 9g (100 μM) + H₂O₂ (0.08%), Lane 4: DNA + 9g (100 μM) + H₂O₂ (0.08%) + KI (0.5mM), Lane 5: DNA + 9g (100 μM) + H₂O₂ (0.08%) + L-Histidine(50 μM), Lane 6: DNA + 9g (100 μM) + H₂O₂ (0.08%) + NaN₃ (0.5mM), Lane 7: DNA + 9g (100 μM) + H₂O₂ (0.08%) + SOD (4 units), Lane 8: DNA + 9g (100 μM) + H₂O₂ (0.08%) + DMSO(4 μl).
4.7.2.4. DNA cleavage activity of complex 9h

Complex 9h cleaves the pBR 322 DNA in presence of H₂O₂ and from Fig. 68. Lane 2, Lane 3 and Lane 4, higher concentration of the complex 9h exhibit higher cleavage activity than lower concentration of the complexes under same experimental conditions. The DNA cleavage activity of complex 9h dependent on the concentration of the complex. The DNA cleavage activity of complex 9h was inhibited in presence of singlet oxygen quenchers singlet oxygen quenchers such as NaN₃ and L-histidine (Lane 5, Lane 6). No apparent inhibition in the DNA cleavage activity of the complex 9h was observed in the presence of hydroxyl radical (•OH) quenchers such as DMSO. The mechanistic results indicate the formation of singlet oxygen as the DNA cleaving agent. The EDTA efficiently inhibits the DNA cleavage activity of the 9h complex under our experimental conditions. The EDTA, a chelating agent that strongly binds to V(IV) forming a complex, can inhibit DNA cleavage, indicating complex 9h play the key role in the cleavage.

Fig. 88. Cleavage of supercoiled PBR322 DNA incubated with complex 9h in Tris-Hcl(pH=7.2) at 37 °C for 3 hrs. Lane 1, DNA control, Lane 2: DNA + 9h (50 μM) + H₂O₂ (0.08%), Lane 3: DNA + 9h (75 μM) + H₂O₂ (0.08%), Lane 4: DNA + 9h (100 μM) + H₂O₂ (0.08%), Lane 5, DNA + 9h (75 μM) + H₂O₂ (0.08%) + L-Histidine(50 μM), Lane 6, DNA + 9h (75 μM) + H₂O₂ (0.08%) + NaN₃ (0.5mM), Lane 7, DNA + 9h (75 μM) + H₂O₂ (0.08%) + DMSO (4 μl), Lane 8: DNA + 9h (75 μM) + H₂O₂ (0.08%) + EDTA (0.5mM).
4.8. DNA Binding and cleavage studies of complexes with L$_2$

4.8.1. DNA binding and cleavage studies of Cu(II), Ni(II), Zn(II) and Mg(II) complexes.

4.8.1.1. DNA Binding studies

Absorption spectral studies

Interaction of metal complexes 11a to 11d with CT-DNA can be monitored by absorption spectral titrations. The absorption spectra of 11a in the absence and presence of calf thymus (CT-DNA) at different concentrations are given in Fig. 89. In the UV region, the complex exhibits absorption band around 210 nm, which are attributed to the л-л* transition, as increasing the calf thymus (CT-DNA) concentration, 74 % hypochromism and a slight red shift were observed, which suggested that interaction between the 11a and calf thymus (CT-DNA). The intrinsic binding constant K$_b$ of 3.14 × 10$^4$ M$^{-1}$ is determined from a plot of [DNA]/($\varepsilon_a-\varepsilon_f$) versus [DNA] (Figure 1) using the absorption at 210 nm. The K$_b$ value is 100 times lower than that of classical intercalators EB$^{139, 140}$. The observed hypochromism of intraligand charge transfer spectra of complexes 11b – 11d (Fig 90-92) and their binding constant values on increasing the CT-DNA concentration upto 250 µM are given in Table 8. These results suggest that the reported complexes have a weaker binding of DNA than the classical intercalators, and it is also likely that the complex binds to CT-DNA via groove mode$^{141, 142}$. 

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Table 8 DNA binding parameters

<table>
<thead>
<tr>
<th>Complexes</th>
<th>$K_b$ (M$^{-1}$)</th>
<th>% of hypochromism</th>
<th>Stern-Volmer quenching constant $K_{sv}$</th>
<th>$K_{app}$ (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11a</td>
<td>$3.14 \times 10^4$</td>
<td>74 %</td>
<td>0.22</td>
<td>$3.5 \times 10^4$</td>
</tr>
<tr>
<td>11b</td>
<td>$3.1 \times 10^4$</td>
<td>54 %</td>
<td>0.15</td>
<td>$2.8 \times 10^4$</td>
</tr>
<tr>
<td>11c</td>
<td>$3.05 \times 10^5$</td>
<td>58 %</td>
<td>0.3</td>
<td>$4.6 \times 10^4$</td>
</tr>
<tr>
<td>11d</td>
<td>$2.24 \times 10^4$</td>
<td>26 %</td>
<td>0.15</td>
<td>$2.2 \times 10^5$</td>
</tr>
</tbody>
</table>

Fig. 89. Absorption spectra of complex 11a (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Inset shows plot of $[\text{DNA}]/(\varepsilon_a - \varepsilon_d)$ vs $[\text{DNA}]$ for absorption titration of CT-DNA and complex 11a. The arrow shows the intensity changes on increasing the DNA concentration.
Fig. 90. Absorption spectra of complex 11b (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Inset shows plot of [DNA]/(ε_a − ε_f) vs [DNA] for absorption titration of CT-DNA and complex 11b. The arrow shows the intensity changes on increasing the DNA concentration.

Fig. 91. Absorption spectra of complex 11c (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Inset shows plot of [DNA]/(ε_a − ε_f) vs [DNA] for absorption titration of CT-DNA and complex 11c. The arrow shows the intensity changes on increasing the DNA concentration.
Fig. 92. Absorption spectra of complex 11d (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Inset shows plot of [DNA]/(ε_a – ε_f) vs [DNA] for absorption titration of CT-DNA and complex 11d. The arrow shows the intensity changes on increasing the DNA concentration.

**Fluorescence spectral studies**

To further clarify the interaction of the complexes with DNA, a competitive binding experiment was carried out. EB emits intense fluorescence at about 600 nm in the presence of DNA due to its strong intercalation between adjacent DNA base pairs. It was previously reported that the enhanced fluorescence could be quenched by the addition of metal complexes. Two mechanisms have been proposed to account for this reduction in the emission intensity: the replacement of molecular fluorophores and/or electron transfer. The relative binding of complex 11a to CT-DNA was studied with an EB-bound CT-DNA solution in Tris – HCl buffer (pH 7.2) (Fig. 93). Fluorescence intensities at 610 nm (510 nm excitation) were measured at different complex concentrations. The fluorescence quenching curves of DNA-bound EB by complexes illustrates that the quenching of EB bound to DNA by the complexes are in good agreement with the linear Stern-Volmer equation. In the linear fit plot of I_0/I versus
[complex]/[DNA], K is given by the ratio of the slope to intercept. The Ksv values for the complex 11a are 0.22. The apparent binding constants (Kapp) at room temperature were calculated to be 3.5 x 10^4 M⁻¹ for 11a. The stern-volmer quenching constant and apparent binding constants of the complexes 11b – 11d are given in table 8 and Fig. (94 - 96). These values are lower than the classical intercalators which suggested that the complexes may be bind to DNA grooves.

Fig. 93. Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of complex 11a. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (ëex = 520 nm). The plot of I₀/I vs [Complex]/[DNA] for fluorescence quenching curves of DNA-EB by complex 11a.
Fig. 94. Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of complex 11b. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (ëex = 520 nm). The plot of $I_0/I$ vs [Complex]/[DNA] for fluorescence quenching curves of DNA-EB by complex 11b.

Fig. 95. Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of complex 11c. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (ëex = 520 nm). The plot of $I_0/I$ vs [Complex]/[DNA] for fluorescence quenching curves of DNA-EB by complex 11c.
Viscosity measurements

Mode of binding of complexes to CT-DNA was investigated by using the viscosity measurements also. Complex that interact in the DNA grooves by partial and or non classical intercalation leads no change in relative viscosity of CT-DNA solution when an increasing the concentration of complex. A classical intercalative mode causes a significant increase in viscosity of DNA solution due to increase in separation of base pairs at intercalation sites and hence an increase in overall DNA length\textsuperscript{146,147}. To explore the interaction between the complexes to CT-DNA, viscosity measurements were carried out by keeping the DNA concentration as constant and varying the concentration of complex. An increasing amount of complex into CT-DNA no change in the relative viscosity of DNA solution and are shown in Fig. 97. The data suggest that the new synthesized complexes interact to grooves of DNA base pairs.
Fig. 97. Changes in relative viscosity of CT-DNA on increasing amounts of complex 11a.

4.8.1.2. DNA cleavage studies

The cleavage of plasmid pBR322 DNA was monitored by gel electrophoresis to investigate the ability of the present complexes to serve as metallonucleases. The naturally occurring supercoiled form (Form I), when nicked, gives rise to an open circular relaxed form (Form II) and further cleaves to a linear form (Form III). When subjected to gel electrophoresis, Form I shows the fastest migration compared to Forms II and III. Form II migrates very slowly prior to its relaxed structure whereas Form III migrates somewhere between the positions of Form I and Form II\textsuperscript{148}. Mechanistic investigations were done using various quenchers to understand the nature of the reactive species involved in the DNA cleavage reactions. The active chemical species that was responsible for the DNA cleavage, we investigated in the presence of hydroxyl radical scavengers (DMSO, KI), singlet oxygen quenchers (NaN\textsubscript{3}, L-histidine, and ethanol), superoxide scavenger (SOD) and chelating agent (EDTA) under our experimental conditions.
From the Fig. 98, 11a cleave the plasmid DNA in presence of hydrogen peroxide. Complexes 11b and 11c cleave the pBR 322 DNA without any additives. Complex 11d does not cleave the plasmid DNA in presence of hydrogen peroxide. 11c shows higher nuclease activity compare to other complexes.

Complex 11a cleaves the plasmid DNA by oxidative path involving •OH as the reactive species responsible for the nuclease activity. The cleavage of supercoiled pBR323 DNA by complexes 11b and 11c and their mechanistic study of DNA cleavage has shown in Figs. 99a and 99b. Complexes 11b and 11c cleave the pBR322 DNA and no apparent inhibition was observed in presence of hydroxyl radical scavenger (KI), singlet oxygen quencher (NaN₃) and superoxide scavenger (SOD). These results rules out the possibility of cleavage by hydroxyl radical, singlet oxygen and superoxide anion, respectively. This fact implies that the DNA cleavage reaction by complexes 11b and 11c should be due to hydrolytic mechanism. The EDTA efficiently inhibits the DNA cleavage activity of the 11b and 11c.
Fig. 99a. Cleavage of supercoiled pBR322 DNA incubated with complexes 11b and 11c (50 μM) in Tris – HCl (pH = 7.2) at 37 ºC for 1 h. Lane 1, DNA control, Lane 2, DNA + 11b (50 μM) + SOD (4 units), Lane 3, DNA + 11b (50 μM) + NaN₃ (0.5 mM), Lane 4, DNA + 11b (50 μM) + KI (0.5 mM), Lane 5, DNA + 11c (50 μM) + NaN₃ (0.5 mM), Lane 6, DNA + 11c (50 μM) + KI (0.5 mM), Lane 7, DNA + 11c (50 μM) + KI (0.5 mM).

99b. Lane 1, DNA control, Lane 2, DNA + 11c (50 μM) + EDTA (0.5 mM), Lane 5, DNA + 11c (50 μM) + EDTA (0.5 mM).

Minor groove binding agent DAPI and major groove binding agent methyl green (Fig. 100) were used to examine the potential interacting site of complexes with supercoiled plasmid DNA. The cleavage patterns (Fig. 86, Lanes 2-4), demonstrated that in presence of methyl green DNA cleavage activity is not affected significantly, and in presence of DAPI (Fig. 100, Lanes 5-7) inhibit the DNA cleavage activity. This result clearly suggests that the complexes 11a to 11f prefer to bind to pBR322 DNA minor groove.
4.8.2 DNA binding and cleavage studies of Fe(III), Co(III), Mn(III) and VO(IV) complexes.

4.8.2.1 DNA binding studies

Absorption spectral analysis

The absorption spectra of complexes 11e - 11f are shown in Fig. 101 - 103. Upon addition of increasing amount of CT-DNA from 0 to 250 µM, a significant “hypochromic’’ effect and red shift observed in intraligand charge transfer bands around 215 nm. These spectral characteristic suggest that the complex interacts with CT-DNA. To study quantitatively, the binding ability of complexes with CT-DNA, the intrinsic binding constant $K_b$ values determined as given in Table 12. From this data, DNA binding affinity of complexes 11e - 11f is 100 times lower than classical intercalators- like ethidium bromide. The weaker DNA binding of
complexes 11e - 11f proposes that the cationic complexes interacts DNA probably through groove binding.

Fig. 101. Absorption spectra of 11e complex (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Plot of [DNA]/(ε_a − ε_f) vs [DNA] for absorption titration of CT-DNA and 11e complex.

Fig. 102. Absorption spectra of 11f complex (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Plot of [DNA]/(ε_a − ε_f) vs [DNA] for absorption titration of CT-DNA and 11f complex.
Fig. 103. Absorption spectra of \textbf{11g} complex (10 \(\mu\)M) in the absence and presence of increasing amounts of CT-DNA (0 - 250 \(\mu\)M) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Plot of \([\text{DNA}] / (\varepsilon_a - \varepsilon_i)\) vs [DNA] for absorption titration of CT-DNA and \textbf{11g} complex.

\textbf{Flourescence spectral analysis}

Since the complexes are non-emissive both in the presence and absence of CT-DNA, competitive ethidium bromide (EB) binding studies were carried out as represented in Fig. 104 - 106. They involve the addition of the present complexes to DNA pretreated with EB and measurement of emission intensities of DNA-bound EB were carried out. The observed enhancement in emission intensity of EB in the presence of DNA is expected of its strong stacking interaction between the DNA. \textbf{11e-11g} binds to DNA probably through grooves, it cannot displace the strongly DNA bound EB. Addition of complexes \textbf{11e-11g} quenches the DNA-induced EB emission intensity but only to a smaller extent. The reduction of emission intensity occurred probably through photoinduced electron transfer.
Fig. 104. Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex 11e. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (ëex = 520 nm). The plot of I₀/I vs. [Complex]/[DNA] for fluorescence quenching curves of DNA-EB by complex 11e.

Fig. 105. Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex 11f. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (ëex = 520 nm). The plot of I₀/I vs. [Complex]/[DNA] for fluorescence quenching curves of DNA-EB by complex 11f.
Fig. 106. Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex 11g. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (λex = 520 nm). The plot of I₀/I vs. [Complex]/[DNA] for fluorescence quenching curves of DNA-EB by complex 11g.

Table 12 DNA binding parameters

<table>
<thead>
<tr>
<th>Complexes</th>
<th>K_b (M⁻¹)</th>
<th>% of hypochromism</th>
<th>Stern-Volmer quenching constant Ksv</th>
<th>K_app (M⁻¹)</th>
</tr>
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<tr>
<td>11e</td>
<td>2.8 x 10⁴</td>
<td>62 %</td>
<td>0.15</td>
<td>1.6 x 10⁴</td>
</tr>
<tr>
<td>11f</td>
<td>2.7 x 10⁴</td>
<td>40 %</td>
<td>0.16</td>
<td>3.3 x 10⁴</td>
</tr>
<tr>
<td>11g</td>
<td>1.1 x 10⁴</td>
<td>66 %</td>
<td>0.1</td>
<td>1.6 x 10⁵</td>
</tr>
</tbody>
</table>

4.8.2.2. DNA cleavage studies of complexes 11e-11g.

The nuclease activity of complex 11e has been studied, supercoiled pBR322 DNA (150 µg/µL) was incubated with complex in aqueous buffer solution (5 mM Tris–HCl/40 mM NaCl, ...
pH 7.2). Complex 11e does not cleave in absence and presence of hydrogen peroxide as shown in Fig. 107.

Fig. 107. Cleavage of supercoiled pBR322 DNA incubated with complex 11e in Tris – HCl (pH = 7.2) at 37 °C for 1 h. Lane 1, DNA control, Lane 2, DNA + 11e (50 μM), Lane 3, DNA + 11e (100 μM), Lane 4, DNA + 11e (150 μM) + H2O2 (0.08 %).

Complexes 11f cleave the pBR322 DNA without any additives. No apparent inhibition was observed in presence of hydroxyl radical scavenger (DMSO), singlet oxygen quencher (NaN3) and superoxide scavenger (SOD) Fig. 108, (Lane 4 - 6). These results rules out the possibility of cleavage by hydroxyl radical, singlet oxygen and superoxide anion, respectively. This fact implies that the DNA cleavage reaction by complexes 11f should be due to hydrolytic mechanism. The EDTA efficiently inhibits the DNA cleavage activity of the 11f in lane 3.

Fig. 108. Cleavage of supercoiled pBR322 DNA incubated with complex 11f (50 μM) in Tris – HCl (pH = 7.2) at 37 °C for 1 h. Lane 1, DNA control, Lane 2, DNA + 11f (50 μM), Lane 3, DNA + 11f (50 μM) + EDTA (0.5 mM), Lane 4, DNA + 11f (50 μM) + DMSO (4 μl), Lane 5, DNA + 11f (50 μM) + NaN3 (50 μM), Lane 6, DNA + 11f (50 μM) + SOD (4 units).
Complex \textit{11g} cleaves the pBR 322 DNA in presence of H$_2$O$_2$ and from Fig. 108, Lane 2 and Lane 3, higher concentration of the complex \textit{11g} exhibit higher cleavage activity than lower concentration of the complexes under same experimental conditions. The DNA cleavage activity of complex \textit{11g} was inhibited in presence of singlet oxygen quenchers such as NaN$_3$ and L-histidine. No apparent inhibition in the DNA cleavage activity of the complex \textit{11g} was observed in the presence superoxide scavenger (SOD) as depicted in Fig. 109, The mechanistic results indicate the formation of singlet oxygen as the DNA cleaving agent. To probe the groove binding preferences of complexes \textit{11g}, the minor-groove binder DAPI and major-groove binder methylgreen was used. Prior to the addition of complex \textit{11g}, DNA was incubated with DAPI and methylgreen. The electrophoresis result (Fig. 100) shows an inhibition in the case of complex \textit{11g} in presence of DAPI (lane 10) while complex \textit{11g} in presence of methylgreen exhibits significant DNA cleavage (lane 11). This suggests minor groove preference for the complex \textit{11g}. Hence, it is clear that complex \textit{11g} binds to minor groove of DNA.

![Fig. 109. Lane 1, DNA control, Lane 2, DNA + \textit{11g} (25 μM) + H$_2$O$_2$ (0.08 %), Lane 3, DNA + \textit{11g} (50 μM) + H$_2$O$_2$ (0.08 %), Lane 4, DNA + \textit{11g} (75 μM) + H$_2$O$_2$ (0.08 %), Lane 5, DNA + \textit{11g} (100 μM) + H$_2$O$_2$ (0.08 %), Lane 6, DNA + \textit{11g} (100 μM) + H$_2$O$_2$ (0.08 %) + EDTA (0.5mM), Lane 7, DNA + \textit{11g} (100 μM) + H$_2$O$_2$ (0.08 %) + SOD (4 units), Lane 8, DNA + \textit{11g} (100 μM) + H$_2$O$_2$ (0.08 %) + L-histidine (50 μM), Lane 9, DNA + \textit{11g} (100 μM) + H$_2$O$_2$ (0.08 %) + NaN$_3$ (0.5mM), Lane 10, DNA + \textit{11f} (100 μM) + H$_2$O$_2$ (0.08 %) + DAPI (2 μl), Lane 11, DNA + \textit{11f} (100 μM) + H$_2$O$_2$ (0.08 %) + Methylgreen (0.5 mM).]
4.8.2.3. DNA binding and cleavage studies of 11h

4.8.2.3.1. DNA binding studies

Absorption spectral studies

An aqueous solution of complex 11h interacts with CT-DNA in Tris.HCl buffer and it was monitored by absorption spectra. The absorption spectra of complex 11h in presence and absence of CT-DNA is shown in Fig. 110. Upon addition of complex 11h from 0 to 240 µM, 70% hypochromism and slight red shift was observed. The binding constant K_b is found to be 2.2 \times 10^4 \text{ M}^{-1}. These spectral results suggest that the complex 11h may be interacts with grooves of DNA. Because, the binding constant value is 100 times lower than that of classical intercalators EB.

![Absorption Spectra](image)

Fig.110. Absorption spectra of complex 11h (10 µM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 µM) at room temperature in 50 mM Tris-HCl buffer pH=7.2). Plot of [DNA]/(\varepsilon_a - \varepsilon_d) vs [DNA] for absorption titration of CT-DNA and complex 11h.
Fluorescence spectral studies

Competitive binding studies were undertaken to monitor the interaction between the complex 11h and CT-DNA. The emission spectra EB bound CT-DNA in presence and absence of complex 11h is shown in Fig. 111. The binding constants $K_{sv}$ and $K_{app}$ values are calculated from spectral data. $K_{sv}$ is found to be 0.1 and $K_{app}$ is $1.8 \times 10^5$. $K_{sv}$ values indicate that less interaction between the complex and DNA. From the absorption and fluorescence spectral data complex, 11h may interact with CT-DNA through grooves.

![Emission spectra of EB bound to DNA in Tris – HCl buffer (pH 7.2) in the absence and presence of the complex 11h. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (λex = 520 nm). The arrow shows the intensity changes on increasing the complex concentration. The plot of $I_0/I$ vs. [Complex]/[DNA] for fluorescence quenching curves of DNA-EB by complex 11h.

Viscosity measurements.

Viscosity measurements are useful in determining whether a molecule interacts with DNA through intercalations or groove binding. Intercalators cause the unwinding and lengthening of DNA, increasing ratios of intercalator causes a significance increase in viscosity of DNA solutions. No change in relative viscosity of DNA solution upon increasing amount of
complex 11h. These results suggest that complex 11h interacts with DNA may be through grooves.

4.8.2.3.2. DNA cleavage studies of 11h

Complex 11h does not bring about DNA cleavage in the absence of any coreagent as can be seen from Fig. 112. This complex, however, brings about DNA cleavage in the presence of H₂O₂. As can be seen from lane 3, 50 μM of complex 11h was able to bring about DNA cleavage in the presence of H₂O₂. DNA cleaving experiments have been performed in the presence of hydroxyl radical quencher, such as DMSO and superoxide quencher, such as SOD. The results show that DMSO does not inhibit the cleavage of DNA (Fig. 107). This clearly shows that formation of hydroxyl radical or superoxide anion is not the reactive oxygen species for the observed cleavage in the presence of complex 11h and H₂O₂. DNA cleaving experiments have also been performed in the presence of singlet oxygen quencher, such as L-histidine and NaN₃. The results show that singlet oxygen quenchers inhibit the cleavage of DNA (Fig. 107). This clearly shows that formation of singlet oxygen is the reactive oxygen species for the observed cleavage in the presence of complex 11h and H₂O₂. To probe the groove binding preferences of complexes 11h, the minor-groove binder DAPI was used and major-groove binder methyl green. Prior to the addition of complex 11h, DNA was incubated with DAPI and methyl green. The electrophoresis result (Fig. 107) shows an inhibition in the case of complex 11h in presence of DAPI (lane 8) while complex 11h in presence of methyl green exhibits significant DNA cleavage (lane 9). This suggests minor groove preference for the complex 11h. Hence, it is clear that complex 11h binds to minor groove of DNA.
Fig. 112. Lane 1, DNA control, Lane 2, DNA + 11h (50 μM), Lane 3, DNA + 11h (50 μM) + H₂O₂ (0.08 %), Lane 4, DNA + 11h (50 μM) + H₂O₂ (0.08 %) + L-histidine (50 μM), Lane 5, DNA + 11h (50 μM) + H₂O₂ (0.08 %) + NaN₃ (0.5mM), Lane 6, DNA + 11h (50 μM) + H₂O₂ (0.08 %) + SOD (4 units), Lane 7, DNA + 11h (50 μM) + H₂O₂ (0.08 %) + DMSO (4 μl), Lane 8, DNA + 11h (50 μM) + H₂O₂ (0.08 %) + DAPI (2 μl), Lane 9, DNA + 11h (50 μM) + H₂O₂ (0.08 %) + methylgreen (0.5mM).