Influence of chirality using Mn(III) salen complexes on DNA binding and antioxidant activity
3.1. Introduction

Chirality plays a significant role in medicinal chemistry because only one enantiomer of a drug molecule is likely to show desirable therapeutic effects while other enantiomer can have a different or adverse biological response. A classical example is chloramphenicol drug where $R$ enantiomer showed antibacterial activity however $S$ enantiomer was inactive. Recently, the interaction of transition metal complexes with DNA evoked great interest, due to their importance in designing of new and promising drugs, probes for nucleic acids, $^1$-$^3$ DNA-dependent electron transfer reactions, DNA footprinting, sequence-specific cleaving agents and antitumor drugs. $^4$-$^8$ As DNA is chiral, its interaction with a molecule having a chiral center(s) is expected to be influenced by diastereomeric ion-pair formation between the two. Thus, different enantiomers of chiral metal complexes are expected to show different metallo-intercalation capacity with essentially chiral DNA and protein molecules. $^9$-$^{15}$ As a consequence of this interaction, different enantiomers of a metal complex may have variable effects on various biological reactions, especially those involving free-radicals like hydrogen peroxide decomposition, superoxide anion ($O_2^{-}$) dismutase, catalase, water oxidation and ribonuclease reduction. These reactions are crucial for scavenging superoxide ($O_2^{-}$) and hydroxyl radicals ('OH), which are largely responsible for DNA mutation and membrane protein damage that can lead to diseases like cancer, liver malfunction and cardiovascular problems. $^{16}$-$^{18}$ Among various transition metal complexes, Mn(II)$^{19}$-$^{21}$ and Mn(III)$^{22}$ salen complexes have shown promising results for the DNA binding and cleavage activity. Gravert and Griffin (1996)$^{23}$, in their exhaustive study on Mn(III) salen complexes, have demonstrated that the presence of substituents with different steric and electronic features on the salen moiety had a significant impact on their DNA binding/cleavage activity. They have also shown that in the case of chiral complexes (having no substituents on the salen moiety), different enantiomers have different DNA cleaving activity, the $R$ enantiomer being more potent. $^{23}$ Recently, Bin et al. (2009) have reported chiral Mn(III) salen complexes with a naphthaldehyde moiety for their potential DNA binding and cleavage activities. $^{19}$ However, in this study the difference in DNA binding/cleavage activity between the two enantiomers was found to be less pronounced. These results clearly demonstrated that the DNA binding and cleavage activity of the Mn(III) salen complexes is strongly substituent dependent. A few manganese complexes have also been reported earlier to show anti-ROS activity, $^{24}$-$^{29}$ and Melvo et al. (2001) elegantly demonstrated the
superoxide scavenging antioxidant activity of Mn-salen complexes utilizing achiral ligands. However, the role of chirality and the structure activity relationship of manganese complexes was not explored for the disintegration of ROS.

The present chapter describes the synthesis of chiral Mn(III) salen complexes, viz. 7, 8, 9, 10, 11 and 12 having different substituents such as chloromethyl, triethylaminomethyl and tert-butyl at the 5,5’position of salen and examined their activity in calf thymus DNA (CT-DNA) binding by absorption spectroscopy, competitive binding study, viscosity measurements, CD measurements and thermal denaturation studies. We have also evaluated the role of the chirality of these complexes on antioxidant activity, e.g., DPPH and superoxide radical scavenging activity. Among the complexes studied here, the complex 7 showed highest DNA intercalation capacity and ~95% free radical inhibition activity, whereas its other enantiomer, complex 8 exhibited groove binding or external binding with DNA and 69% free radical inhibition activity.

3.2. Experimental Section

3.2.1. Materials and methods

Calf thymus DNA (CT-DNA), 1S,2S-(-)-cyclohexanediadimate and 1R,2R-(-)-cyclohexanediadimate, NBT (Nitroblue-tetrazolium) were purchased from Sigma Aldrich and used as received. EB (ethidium bromide), Riboflavin (vitamin B2), MET (L-methionine) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from s.d. Fine Chemicals. A solution of CT-DNA in phosphate buffer gave a ratio of UV absorbance at 260 and 280 nm of ca. 1.8-1.9, indicating that the DNA was sufficiently free of protein.

DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M⁻¹cm⁻¹) at 260 nm. Commercial grade solvents were distilled before use for the preparation of complexes, ligands and their intermediates. Elemental analysis of complexes was done on a CHNS Analyzer, Perkin Elmer model 2400 (USA). FTIR spectra were recorded by Perkin Elmer Spectrum GX spectrophotometer (USA) in KBr window. NMR spectra were recorded on a Bruker F113V spectrometer (500 MHz, Switzerland) and were referenced internally with TMS. High resolution mass spectra were recorded on a LC-MS (USA) (Q-TOF) LC (Waters), MS (Micromass) instruments using acetonitrile as mobile phase. The spray voltage, tube lens offset, capillary voltage and capillary temperature were set at 4.50 kV, 30.00 V, 23.00 V and 200 °C, respectively and the m/z values were quoted for the major peaks in
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the isotope distribution. UV-Vis and fluorescence spectra were recorded on Shimadzu UV 3101 PC NIR spectrophotometer (Japan) and LS 50B Perkin Elmer luminescence spectrophotometer (USA), respectively. Circular dichroism (CD) spectra were measured on J-815 CD spectrophotometer (Japan).

3.2.2. Synthesis of chiral salen ligands $S$-$1’–R$-$3’$

To a solution of aldehyde (2 mmol, 2 equivalent) in absolute EtOH (10 mL) was added in the 10 mL solution of $1S,2S$-$(+)$-cyclohexanediamine/ $1R,2R$-$(−)$-cyclohexanediamine (1 mM, 1 equivalent). The resulting mixture was allowed to reflux for 7-8 h. The progress of the reaction was monitored on TLC. The solvent was partially removed under reduced pressure on a rotary evaporator and the yellow products were precipitated by hexane (Scheme 3.1). The characterization data of chiral salen ligands $S$-$1’–R$-$3’$ is given below.

![Scheme 3.1 Structure of chiral salen ligands](image)

**Scheme 3.1** Structure of chiral salen ligands

**$S$-$1’$:** Yellow solid (82%); IR (KBr $\nu$/cm$^{-1}$): 3449, 2952, 2866, 1630, 1594, 1545, 1466, 1439, 1390, 1361, 1308, 1266, 1229, 1200, 1167, 1142, 1091, 1030, 974, 930, 871, 778, 753, 669; $^1$H NMR (500 MHz, CDCl$_3$, $\delta$ ppm): 13.68 (s, 2H, OH), 8.42 (s, 2H, H–C=N), 7.31 (d, 2H, aromatic H, J=2.0Hz), 7.26 (d, 2H, aromatic H, J=2.0Hz), 4.58 (s, 4H, CH$_2$Cl), 3.55–3.30 (m, 2H, CNCH), 1.89–1.44 (m, 8H, cyclohexyl-H), 1.42 (s, 18 H, $t$-
butyl); Analysis. Calcd for C_{30}H_{40}Cl_{2}N_{2}O_{2}: C, 67.79; H, 7.58; N, 5.27. Found: C, 67.71; H, 7.69; N, 5.32. MS (ESI): m/z = 531.56 [M]^+.

**R-1':** Yellow solid (82%); IR (KBr u/cm⁻¹): 3449, 2952, 2866, 1630, 1545, 1466, 1439, 1390, 1361, 1308, 1266, 1229, 1200, 1167, 1142, 1091, 1030, 974, 930, 871, 778, 753, 669; ¹H NMR (500 MHz, CDCl₃, δ ppm): 13.68 (s, 2H, OH), 8.42 (s, 2H, H–C=N), 7.31 (d, 2 H, aromatic H, J=2.0Hz), 7.26 (d, 2H, aromatic H, J=2.0Hz), 4.58 (s, 4H, CH₂Cl), 3.55–3.30 (m, 2H, CNCH), 1.89–1.44 (m, 8 H, cyclohexyl-H), 1.42 (s, 18 H, t-butyl); Analysis. Calcd for C_{30}H_{40}Cl_{2}N_{2}O_{2}: C, 67.79; H, 7.58; N, 5.27. Found: C, 67.71; H, 7.69; N, 5.32. MS (ESI): m/z = 531.56 [M]^+.

**S-2':** Yellow solid (90%); IR (KBr u/cm⁻¹): 3553, 3477, 3415, 3238, 2934, 2860, 2709, 2659, 1726, 1629, 1597, 1534, 1467, 1441, 1360, 1319, 1266, 1232, 1207, 1160, 1097, 1049, 1028, 985, 946, 873, 799, 774, 743, 708, 566, 467, 408; ¹H NMR (500 MHz, CDCl₃, δ ppm): 13.7-13.6 (m, 2H), 8.27 (s, 2H), 7.30 (s, 2H), 6.97(s, 2H), 4.28 (s, 4H), 3.9-3.8 (m, 2H), 3.29 (q, J=7.2Hz, 12H), 2.7-2.6 (m, 2H), 2.10-1.92 (m, 2H), 1.60-1.44 (m, 4H), 1.42 (s, 18H), 1.45-1.25 (t-butyl, J=7.2Hz, 18H); Analysis. Calcd for C_{42}H_{70}Cl_{2}N_{4}O_{2}: C, 68.76; H, 9.55; N, 7.64. Found: C, 68.72; H, 9.51; N, 7.60. MS (ESI): m/z = 733.94 [M]^+.

**R-2':** Yellow solid (90%); IR (KBr u/cm⁻¹): 3553, 3477, 3415, 3238, 2934, 2860, 2710, 2658, 1725, 1626, 1595, 1534, 1468, 1442, 1361, 1319, 1266, 1233, 1207, 1161, 1097, 1049, 1025, 986, 944, 871, 799, 774, 743, 710, 565, 466, 409; ¹H NMR (500 MHz, CDCl₃, δ ppm): 13.7-13.5 (m, 2H), 8.27 (s, 2H), 7.31 (s, 2H), 6.97 (s, 2H), 4.26 (s, 4H), 3.9-3.8 (m, 2H), 3.28 (q, J=7.2Hz, 12H), 2.7-2.6 (m, 2H), 2.10-1.90 (m, 2H), 1.60-1.42 (m, 4H), 1.42 (s, 18H), 1.45-1.24 (t-butyl, J=7.2Hz, 18H); Analysis. Calcd for C_{42}H_{70}Cl_{2}N_{4}O_{2}: C, 68.76; H, 9.55; N, 7.64. Found: C, 68.72; H, 9.51; N, 7.60. MS (ESI): m/z = 733.94 [M]^+.

**S-3':** Yellow solid (90%); IR (KBr u/cm⁻¹): 2960, 2869, 1631, 1595, 1468, 1439, 1362, 1271, 1174, 829; ¹H NMR (500 MHz, CDCl₃, δ ppm): 13.76 (s, 2H), 8.34 (s, 2H), 7.34 (d, 2H, J=2.2Hz), 7.02 (d, 2H, J=2.2Hz), 3.31-3.70 (m, 2H), 1.46 (s, 18 H), 1.4-2.0 (m, 6 H), 1.32-1.54 (m, 2H); Analysis. Calcd for C_{38}H_{54}N_{2}O_{2}: C, 79.07; H, 9.95; N, 5.12. Found: C, 79.05; H, 9.94; N, 5.10. MS (ESI): m/z = 546.83 [M]^+.
**R-3**: Yellow solid (90%); IR (KBr u/cm⁻¹): 2961, 2868, 1632, 1469, 1440, 1363, 1273, 1175, 830; ¹H NMR (500 MHz, CDCl₃, δ ppm): 13.75 (s, 2H), 8.35 (s, 2H), 7.33 (d, 2H, J=2.2Hz), 7.01 (d, 2H, J=2.2Hz), 3.31-3.71 (m, 2H), 1.45-2.0 (m, 6H), 1.32-1.51 (m, 2H); Analysis. Calcd for C₃₆H₅₄N₂O₂: C, 79.07; H, 9.95; N, 5.12. Found: C, 79.05; H, 9.94; N, 5.10. MS (ESI): m/z = 531.56 [M]+.

**3.2.3. Synthesis of chiral Mn(III) salen complexes 7-12**

A solution of Mn(CH₃COO)₂·4H₂O (4 mmol) in ethanol (10 mL) was added to a solution of desired chiral salen ligands viz., (1S,2S)-N,N’-bis-[3-tert-butyl-5-chloromethyl-salicylidene]-1,2-cyclohexanediamine S-1’/ (1R,2R)-N,N’-bis-[3-tert-butyl-5-chloromethyl-salicylidene]-1,2-cyclohexanediamine R-1’ / (1S,2S)-N,N’-bis-[3-tert-butyl-5-N,N’triethylaminomethyl-salicylidene]-1,2-cyclohexanediamine dichloride S-2’/ (1R,2R)-N,N’-bis-[3-tert-butyl-5-N,N’triethylaminomethyl-salicylidene]-1,2-cyclohexanediamine dichloride R-2’ / (1S,2S)-N,N’-bis-[3,5-di-tert-butylsalicylidene]-1,2-cyclohexanediamine S-3’ and (1R,2R)-N,N’-bis-[3,5-di-tert-butylsalicylidene]-1,2-cyclohexanediamine R-3’ (2 mmol) in absolute ethanol (25 mL) and the resulting brown solution was refluxed for 7-8 h under nitrogen atmosphere. The reaction mixture was cooled to room temperature and solid LiCl (6 mM) was added to it with stirring for 4 h under aerobic condition. The resulting mass was filtered and the solvent was removed from the filtrate till dryness on a rotary evaporator. The resulting residue was extracted with dichloromethane (100 mL) and the organic phase was washed with water and dried over anhydrous Na₂SO₄. The resulting solution was filtered and evaporated to give dark brown colored Mn(III) salen complexes 7, 8, 9, 10, 11 and 12 (Scheme 3.2). The characterization data of the complexes are as follows.

**Complex 7**: Brown solid (70%); IR (KBr u/cm⁻¹): 3437, 2942, 2864, 1610, 1533, 1431, 1339, 1308, 1239, 1177, 1027, 888, 821, 779, 731, 644, 566; Analysis. Calcd. for C₃₀H₅₈N₂O₂Cl₃Mn: C, 58.12; H, 6.18; N, 4.52. Found: C, 57.92; H, 6.09; N, 4.42; MS (ESI): m/z = 620.35 [M]+; UV-Vis. λ_max: 318 (8156), 400 (3610) nm.

**Complex 8**: Brown solid (68%); IR (KBr u/cm⁻¹): 3433, 2948, 2868, 1612, 1530, 1432, 1341, 1311, 1241, 1177, 1029, 889, 825, 780, 734, 644, 570; Analysis. Calcd. for C₃₀H₅₈N₂O₂Cl₃Mn: C, 58.12; H, 6.18; N, 4.52. Found: C, 57.88; H, 6.11; N, 4.49; MS (ESI): m/z = 619.95 [M]+; UV-Vis. λ_max: 318 (13760), 400 (5680) nm.
**Complex 9:** Brown solid (90%); IR (KBr \( \nu/cm^{-1} \)): 3431, 2952, 2865, 1796, 1717, 1613, 1541, 1435, 1388, 1341, 1309, 1267, 1237, 1204, 1169, 1095, 1029, 940, 867, 827, 781, 735, 658, 567, 482; Analysis. Calcd. for C\(_{42}\)H\(_{68}\)N\(_4\)O\(_2\)Cl\(_3\)Mn: C, 61.35; H, 8.28; N, 6.82. Found: C, 61.29; H, 8.22; N, 6.79. MS (ESI): \( m/z = 823.75 \) [M]+; UV-Vis. \( \lambda_{\text{max}} \): 325 (7170), 411 (3422) nm.

![Scheme 3.2 Structure of chiral Mn(III) salen complexes](image)

**Complex 10:** Brown solid (92%); IR (KBr \( \nu/cm^{-1} \)): 3430, 2954, 2863, 1796, 1719, 1612, 1540, 1435, 1385, 1340, 1310, 1265, 1237, 1204, 1171, 1096, 1030, 940, 869, 824, 779, 734, 658, 566, 485; Analysis. Calcd. for C\(_{42}\)H\(_{68}\)N\(_4\)O\(_2\)Cl\(_3\)Mn: C, 61.35; H, 8.28; N, 6.82. Found: C, 61.41; H, 8.25; N, 6.76. MS (ESI): \( m/z = 823.38 \) [M]+; UV-Vis. \( \lambda_{\text{max}} \): 325 (6200), 411 (3058) nm.

**Complex 11:** Brown solid (90%); IR (KBr \( \nu/cm^{-1} \)): 3482, 2954, 2866, 1613, 1534, 1462, 1433, 1389, 1311, 1249, 1173, 1029, 868, 836, 780, 747, 670, 641, 569; Analysis. Calcd. for C\(_{38}\)H\(_{52}\)N\(_2\)O\(_2\)Cl Mn: C, 68.07; H, 5.58; N, 4.41. Found: C, 67.42; H, 5.69; N, 4.55; MS (ESI): \( m/z = 635.15 \) [M]+; UV-Vis. \( \lambda_{\text{max}} \): 315 (12720), 415 (5148) nm.
**Complex 12**: Brown solid (91%); IR (KBr $\nu$/cm$^{-1}$): 3483, 2954, 2866, 1614, 1534, 1462, 1433, 1388, 1311, 1249, 1172, 1030, 867, 836, 779, 746, 670, 640, 568; Analysis. Calcd. for $C_{36}H_{52}N_2O_2\text{Cl}$ Mn: C, 68.07; H, 5.58; N, 4.41. Found: C, 67.48; H, 5.64; N, 4.49. MS (ESI): $m/z = 634.95$ [M]$^+$. UV-Vis. $\lambda_{\text{max}}$: 315 (13270), 415 (5428) nm.

### 3.2.4. DNA binding study

In order to evaluate the quantitative DNA binding affinity with chiral Mn(III) salen complexes 7-12, the intrinsic binding constant was obtained by an absorption spectroscopy method. Initially, 1000 mL solution of blank buffer (1 mM phosphate buffer, pH 7.0) was placed in the reference cell and the respective chiral Mn(III) salen complex solution (50 $\mu$M) in phosphate buffer having 0.01% DMSO in sample cuvettes (1 cm path length) and the spectra were recorded at 280-450 nm. The incremental concentration of buffered DNA solution (0-300 $\mu$M) was added to a fixed concentration of chiral Mn(III) salen complexes 7, 8, 9, 10, 11 and 12 and spectra were recorded.

The DNA competitive binding study with EB was carried out in phosphate buffer (1 mM, pH 7.0) by keeping a fixed concentration of EB (30 $\mu$M), DNA (100 $\mu$M) and different concentrations of chiral Mn(III) salen complexes 7, 8, 9, 10, 11 and 12 (0–110 $\mu$M). The emission spectra were recorded at 500-700 nm, where the excitation wavelength was kept at 478 nm.

Viscosity measurements were performed on Ostwald’s viscometer at 30 ± 0.01 °C by using fixed a concentration of DNA solution (50 $\mu$M) and increasing the concentration of chiral Mn(III) salen complexes 7, 8, 9, 10, 11 and 12 (0-60 $\mu$M) in phosphate buffer (1 mM, pH 7.0). Each sample was measured in triplicate and the average flow time was calculated with a digital stopwatch. Data were presented as $(\eta/\eta_0)^{1/3}$ versus the ratio of the concentration of the compound and DNA where $\eta$ is the viscosity of DNA in the presence of the complex, and $\eta_0$ is the viscosity of DNA alone.36

To determine the stability of DNA, thermal denaturation experiments were performed on a TCC-260 temperature controller programmer UV-3101 PC spectrophotometer by mixing solutions of 7, 8, 9, 10, 11 and 12 (20 $\mu$M) in phosphate buffer (1 mM, pH 7.0) with a solution of CT-DNA (0.4 mM). The resulting solutions were incubated for 2 min at different temperatures (35-95 °C) and the absorption
intensity was recorded at 260 nm. The T<sub>m</sub> value was determined from the graph at the midpoint of temperature curve.

3.2.5. Antioxidant activity assessment

3.2.5.1. DPPH radical scavenging activity

The determination of the DPPH scavenging activity was carried out by using a methanolic solution of DPPH (25 mg L<sup>-1</sup>) mixed with different concentrations (0.65-20 μM) of chiral Mn(III) salen complexes 7, 8, 9, 10, 11 and 12 in methanol. The above mixture was stirred vigorously for 5 min and was allowed to stand for 1 h at room temperature before its absorbance was measured at 517 nm. The percentage of radical activity was calculated by using following equation.

\[
\text{DPPH scavenging activity (\%)} = \left( \frac{A_0 - A_i}{A_0} \right) \times 100
\]  

3.2.5.2. Superoxide radicals scavenging activity

The superoxide radical was generated in the test system by using NBT/VitB<sub>2</sub>/MET, and determined spectrometrically by the Nitroblue tetrazolium photoreduction method with a minor modification.<sup>1,16</sup> The suppression of superoxide radical was calculated by measuring the absorbance at 560 nm. Accordingly, the test compounds (chiral Mn(III) salen complexes 7-12; 0.65-20 μM) were dissolved in 0.01% DMSO and phosphate buffer (1 mM, pH 7.0) and the resulting solution were added to a mixture containing NBT (65 μM), L-MET (13 μM), VitB<sub>2</sub> (1.5 μM), EDTA (0.1 mM) and phosphate buffer (10 mM, pH 7.0). The above mixture was illuminated with a white fluorescence lamp (15 W) for 15 min and its absorbance (A<sub>i</sub>) was measured at 560 nm. The above mixture without the test compounds was used as the control and its absorbance under the same conditions was taken as A<sub>0</sub>. All the experiments were conducted in triplicate and data were expressed as mean a standard deviation. The suppression ratio was calculated by using the following equation.

\[
\text{O}_2^{•−} \text{ scavenging activity (\%)} = \left( \frac{A_0 - A_i}{A_0} \right) \times 100
\]  

3.3. Results and Discussion

Chiral salen complexes, viz., (1<sup>S</sup>,2<sup>S</sup>)-N,N’-bis-[3-<i>tert</i>-butyl-5-chloromethyl-salicylidine]-1,2-cyclohexanediaminato manganese(III) chloride 7 / (1<sup>R</sup>,2<sup>R</sup>)-N,N’-bis-[3-
*tert*-butyl-5-chloromethyl-salicylidene]-1,2-cyclohexanediaminato manganese(III) chloride 8 / (1S,2S)-N,N’-bis-[*3-tert*-butyl-5-N,N’-triethylaminomethyl-salicylidine]-1,2-cyclohexanediaminato manganese(III) trichloride 9 / (1R,2R)-N,N’-bis-[*3-tert*-butyl-5-N,N’-triethyl-aminomethyl-salicylidine]-1,2-cyclohexanediaminato manganese(III) trichloride 10 / (1S,2S)-N,N’-bis-[3,5-di-*tert*-butylsalicylidene]-1,2-cyclohexanediaminato manganese(III) chloride 11 and (1R,2R)-N,N’-bis-[3,5-di-*tert*-butyl-salicylidene]-1,2-cyclohexanediaminato manganese(III) chloride 12 were prepared by the interaction of their respective chiral salen ligands with manganese(II) acetate under an inert atmosphere followed by addition of LiCl under aerobic condition (Scheme 3.2). All the chiral metal complexes were characterized by microanalysis, IR, LC-MS, UV-vis. and CD spectroscopy (data is given in the experimental section).

### 3.3.1. DNA binding

#### 3.3.1.1. Electronic absorption spectroscopy

Electronic absorption spectroscopy was used to study the interaction of metal complexes 7, 8, 9, 10, 11 and 12 with DNA. The change in absorbance and shift in wavelength upon addition of increasing concentrations of DNA solution in a fixed concentration of chiral metal complexes gives valuable information on the mode of interaction. The metal complex binding with DNA through intercalation usually results in hypochromism and bathochromism due to the strong stacking interaction between the aromatic chromophore and the base pairs of DNA. The absorption spectra of chiral Mn(III) salen complexes 7, 8, 9, 10, 11 and 12 with DNA are shown in Figure 3.1-3.3. Upon increasing the concentration of CT-DNA in the solution of the complexes 7 and 8, the bands at 318 and 400 nm showed hypochromism while complexes 9 and 10 showed hyperchromism for the 325 and 411 nm bands. Interestingly, complexes 11 and 12 showed both hypochromism and hyperchromism for the 315 and 415 nm bands, respectively. These spectroscopic characteristics suggest that all the chiral Mn(III) salen complexes tested had some interaction with DNA. Among these, complex 7 showed strongest interaction.
Figure 3.1 Absorption spectra of chiral Mn(III) salen complexes (50 μM) (A) Complex 7 (B) Complex 8 in phosphate buffer (1 mM, pH 7.0) in the presence of increasing concentration of DNA. DNA = (0-300 μM). Inset plot is [DNA]/(ε_a − ε_f) vs. DNA.

Figure 3.2 Absorption spectra of chiral Mn(III) salen complexes (50 μM) (A) Complex 9 (B) Complex 10 in phosphate buffer (1 mM, pH 7.0) in the presence of increasing concentration of DNA. DNA = (0-300 μM). Inset plot is [DNA]/(ε_a − ε_f) vs. DNA.
Figure 3.3 Absorption spectra of chiral Mn(III) salen complexes (50 μM) (A) Complex 11 (B) Complex 12 in phosphate buffer (1 mM, pH 7.0) in the presence of increasing concentration of DNA. DNA = (0-300 μM). Inset plot is [DNA]/ (εₐ−ε₇) vs. DNA.

The intrinsic binding constant of all the complexes with DNA was obtained by monitoring the changes in absorbance of the LMCT band of complexes with increasing concentration of DNA using the following functional equation.

$$\frac{[DNA]}{(εₐ−ε₇)} = \frac{[DNA]}{(ε₈−ε₇)} + \frac{1}{K₈ (ε₈−ε₇)} \quad (3)$$

Where [DNA] is the concentration of DNA in base pairs, εₐ, ε₇ and ε₈ correspond to Aₐ/[Mn], the extinction coefficient of the free chiral Mn(III) salen complex, and the extinction coefficient of the chiral Mn(III) salen complex in the fully bound form, respectively. K₈ was obtained from the ratio of the slope to intercept by using the plot of [DNA]/ (εₐ−ε₇) vs. [DNA] (Table 3.1). The results in Table 3.1 indicates that more polar complexes 7, 8, 9 and 10 bind quite strongly with DNA due to the presence of chloromethyl and triethylaminomethyl groups at the 5 5' positions of these complexes. On the other hand complexes 11 and 12 which bear hydrophobic groups (tert-butyl) at these locations bind relatively less strongly with DNA. Further, among complexes 7-12 the S enantiomer of the complexes bind more strongly than their R counterparts, however, the complex 7 showed strong binding through intercalation while 8, 9, 10, 11 and 12 bind through groove or external binding with DNA. These results are significantly
superior as compared to the earlier report, where different aldehyde moieties on Mn(III) salen complexes with no substituents on the catalyst were used for DNA binding studies, which gives $K_b$ for the $R$ enantiomer as $(1.26 \pm 0.12) \times 10^4$ M$^{-1}$ and the $S$ enantiomer as $(1.09 \pm 0.13) \times 10^4$ M$^{-1}$.

Table 3.1 Binding constant of $S$ and $R$ enantiomer of chiral Mn(III) salen complexes

<table>
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</table>

3.3.1.2. Luminescence spectroscopy study

Chiral Mn(III) salen complexes 7, 8, 9, 10, 11 and 12 showed no luminescence upon excitation of the CT and LMCT bands, irrespective of the presence or absence of a solvent or CT-DNA. Hence, competitive binding studies for these complexes were carried out by fluorescence spectral method using the emission intensity of ethidium bromide (EB) as a probe. In control experiments, when chiral metal complex solutions of increasing concentration were added to ethidium bromide solution, there was no change in the emission intensity of EB in the absence of DNA, indicating that there is no interaction between the EB and the complex. EB is known to emit intense fluorescence in the presence of DNA due to the strong intercalation between the base pairs of DNA. It has been reported that the enhanced fluorescence can be quenched by the addition of the second molecule. In the present study it can be clearly seen from Figure 3.4-3.6 that there is a reduction in the emission intensity of the DNA bound EB on the addition of increasing concentrations of chiral Mn(III) salen complexes suggesting its competitive binding with DNA–EB. Based on the above data, the Stern-Volmer quenching constant can be calculated by using the following equation

$$I_0/I = 1 + K_r r \quad (4)$$
Figure 3.4 The emission spectra of DNA bound EB in the presence of (A) Complex 7 (B) Complex 8 in phosphate buffer (1 mM, pH 7.0) in the presence of increasing concentration of the complexes. DNA = 100μM.

Figure 3.5 The emission spectra of DNA bound EB in the presence of (A) Complex 9 (B) Complex 10 in phosphate buffer (1 mM, pH 7.0) in the presence of increasing concentration of the complexes. DNA = 100μM.

In the above equation $I_0$ and $I$ are the emission intensities in the absence and presence of chiral Mn(III) salen complexes and $r$ is the ratio of the total concentration of chiral complexes to DNA. Accordingly, the plot Figure 3.7 of $I_0/I$ vs. [Mn]/[DNA], $K$ is given by the ratio of the slope to intercept. The $K$ values thus calculated (Table 3.2)
suggest that the Δλ/nm and Stern–Volmer constant values are highest for complex 7. However, in general, the S enantiomer showed stronger binding with DNA than the R enantiomer, suggesting that the DNA, which is essentially chiral in nature, indeed shows a stereochemical preference for binding with external chiral molecules and is also affected by the steric and electronic properties of the complexes. Hence, among all the complexes studied, complex 7 binds with DNA more efficiently through intercalation, while the complexes 8, 9, 10, 11 and 12 either bind through groove or external binding with DNA. These results are in consonance with the electronic absorption titration studies given earlier in the section.

![Figure 3.6](image)

**Figure 3.6** The emission spectra of DNA bound EB in the presence of (A) Complex 11 (B) Complex 12 in phosphate buffer (1 mM, pH 7.0) in the presence of increasing concentration of the complexes. DNA = 100μM.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>( \lambda_{\text{max, DNA + EB}} )</th>
<th>( \lambda_{\text{max, DNA+ EB + Complex}} )</th>
<th>( \Delta \lambda/\text{nm} )</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>612</td>
<td>620</td>
<td>8</td>
<td>0.77 ± 0.012</td>
</tr>
<tr>
<td>8</td>
<td>612</td>
<td>616</td>
<td>4</td>
<td>0.41 ± 0.013</td>
</tr>
<tr>
<td>9</td>
<td>612</td>
<td>617</td>
<td>5</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>10</td>
<td>612</td>
<td>616</td>
<td>4</td>
<td>0.39 ± 0.017</td>
</tr>
<tr>
<td>11</td>
<td>612</td>
<td>616</td>
<td>4</td>
<td>0.51 ± 0.009</td>
</tr>
<tr>
<td>12</td>
<td>612</td>
<td>615</td>
<td>3</td>
<td>0.30 ± 0.013</td>
</tr>
</tbody>
</table>
3.3.1.3. Circular dichroism spectroscopy

The CD spectral analysis is a powerful tool which gives valuable information on
the binding mode of chiral metal complexes with DNA.\textsuperscript{15,19} The CD spectra of
complexes 7, 8, 9, 10, 11 and 12 in DMSO are shown in Figure 3.8–3.10. The CD
spectra of free complexes 7, 9 and 11 and their respective enantiomers 8, 10 and 12
showed bands of opposite configuration at ~320 and ~415 nm. Complexes 7 and 8
showed increase in spectral strength of 32% on interaction with CT-DNA in the CT
region, whereas the LMCT band of 7 had a red shift of 3 nm in the presence of CT-DNA
with an increase in spectral strength by 26%. On the contrary, the LMCT band of 8
showed a decrease in spectral strength by 21% with a 1 nm blue shift. These results
clearly indicate the different matching of enantiomers of the complex with DNA. On the
other hand, complex 9 in the presence of DNA showed a blue shift of 1 nm in the LMCT
region with a decrease in spectral strength (23%), while 10 showed an increase in
spectral strength (22%) with a blue shift of 1 nm in this region. Paradoxically, complexes
11 and 12 showed no significant change in the CD spectra in the presence of DNA. These
results show that both substituents at the 5, 5’position on the salen moiety and the
chirality of the complexes used in the present study have profound effects on the binding
efficiency with DNA.
Figure 3.8 CD spectra of chiral Mn(III) salen complexes 7 and 8 in the presence and absence of DNA. Complex concentration = 50 µM. Inset graph is LMCT region changes in the presence of CT-DNA.

Figure 3.9 CD spectra of chiral Mn(III) salen complexes 9 and 10 in the presence and absence of DNA. Complex concentration = 50 µM. Inset graph is LMCT region changes in the presence of CT-DNA.
3.3.1.4. Viscosity study

To further strengthen our understanding of the interactions of chiral Mn(III) salen complexes 7, 8, 9, 10, 11 and 12 with DNA, viscosity measurements were carried out. Hydrodynamic measurements that are sensitive to length change are regarded as the least ambiguous and most critical test of binding mode in the absence of crystallographic structural data. A partial and non-classical intercalation ligand could bend the DNA helix, thereby reducing its effective length and its viscosity. On the contrary, a classical intercalation model often causes lengthening of the DNA helix, as base pairs are separated to accommodate the binding ligand, leading to the increase in DNA viscosity. Figure 3.11 shows the changes in relative viscosity of CT-DNA in the presence of S and R enantiomers of chiral Mn(III) salen complexes. The results clearly show that the complex 7 induced the maximum increase in the density, indicating strong intercalative binding between the base pairs of DNA as against its enantiomer 8. A similar trend of increases in density was also observed with complexes 9 and 10, although the extent of binding was less. However, complexes 11 and 12 showed no significant increase in
density, possibly due to poor binding with DNA. Thus, overall binding affinity to DNA follows the order of $7 > 8 > 9 > 11 > 10 > 12$.

![Figure 3.11](image)

**Figure 3.11** The relative viscosity of DNA (50 μM) in the presence of chiral $S$ and $R$ enantiomer of Mn(III) salen complexes (0-60 μM).

### 3.3.1.5. Thermal denaturation study

The thermal behaviour of DNA in the presence of complexes can give an insight into their conformational changes and also information about the interaction affinity of complexes. It is well known that on increasing the temperature, the double stranded DNA shows hyperchromism due to its gradual dissociation to form single strands. The melting curves of CT-DNA in the presence and absence of the chiral Mn(III) salen complexes are shown in **Figure 3.12**. The $T_m$ of CT-DNA was found to be 70 °C in the absence of a complex. The $T_m$ of the CT-DNA in the presence of complex 7 increased to 75 °C showing retardation in the dissociation of the DNA helix due to strong binding with the metal complex. Other complexes, e.g., 8, 9, 10, and 11 do not alter the $T_m$ value of CT-DNA, which remains at 70 °C. On the contrary, 12 showed a decrease in $T_m$ value (65 °C) of CT-DNA, possibly due to its instability in the presence of this complex. Based on the above observations, it can be concluded that complex 7 binds with DNA through intercalation whereas complexes 8, 9, 10, 11 and 12 had groove or external binding with DNA.

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3.3.2. Antioxidant activity

3.3.2.1. DPPH radical scavenging activity

DPPH is a stable free radical, which has been widely used to estimate the radical scavenging activity of antioxidants.\textsuperscript{42} Generally, it has the ability to accept an electron or hydrogen radical to become a stable diamagnetic molecule. Thus, it shows a decrease in absorbance at 517 nm whose intensity depends on the number of electrons it has taken up. DPPH radical scavenging activity for 7, 8, 9, 10, 11 and 12 was found to be 95, 67, 74, 50, 59 and 56% at 20 μM concentration, respectively (Figure 3.13). These results indicate some correlation between DNA binding ability as described in the preceding sections and free radical scavenging activity. The complex 7 that showed the strongest DNA binding ability has also demonstrated the highest DPPH radical scavenging activity. This study has further strengthened the fact that for the present set of complexes, the S enantiomer is more effective than their R counterpart.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{tikzexample}
\caption{Thermal denaturation graph of CT-DNA (0.4 mM) in the presence of chiral S and R enantiomer of Mn(III) salen complexes (20 μM).}
\end{figure}
Figure 3.13 DPPH scavenging effect by 7, 8, 9, 10, 11 and 12 chiral Mn(III) salen complexes

3.3.2.2. Superoxide radical scavenging activity

Superoxide (O$_2^{•−}$) and hydrogen peroxide (H$_2$O$_2$) are highly reactive compounds among the reactive oxygen species (ROS). These species can disintegrate cell membranes and damage protein and DNA structures and are largely responsible for many diseases, such as cancer, liver injury and cardiovascular complications.$^{16}$ As shown in Figure 3.14, the superoxide radical scavenging abilities of the test chiral Mn(III) salen complexes (7-12) increased with the increasing concentration of the complexes ranging from 0.65-20 μM. In the superoxide radical scavenging activity, the chiral Mn(III) salen complexes capable of oxidizing O$_2^{•−}$ anion will compete with NBT and slow down its reduction, i.e. formation of blue formazon color, and thus, decreases the absorbance at 560 nm. The results shown in Figure 3.14 clearly indicate the highest superoxide scavenging behaviour for the complex 7 with 81% scavenging effect, as compared to 8 with 57% at a concentration of 20 μM. Complexes 9 and 10 also showed a high scavenging activity with 60% and 51%, respectively. In contrast, the complexes 11 and 12 having tert-butyl groups at the 3,3’ and 5,5’ positions showed almost negligible scavenging activity at all concentrations tested. These results indicate that the complex 7 showed highest superoxide scavenging activity.
Figure 3.14 Scavenging effect of the chiral Mn(III) salen complexes on O$_2^\cdot$ by MET/VitB2/NBT system.

3.4. Conclusion

Chiral Mn(III) salen complexes 7, 8, 9, 10, 11 and 12 having different substituents at the 3,3’and 5,5’ positions of the salen unit were prepared and characterized by appropriate physico-chemical methods. Binding of these complexes to CT-DNA was investigated by electronic absorption titrations, competitive binding experiments, circular dichroism, thermal denaturation and viscosity measurement studies. Complexes with polar groups at the 5,5’ positions of the salen unit interact with DNA more strongly than those with hydrophobic groups at these positions. Among all the chiral Mn(III) salen complexes, complex 7 demonstrated highest DNA binding affinity as well as antioxidant activities.
3.5. References


Chapter 3


