The ruthenium(II) ferrocenyl heterocyclic thiosemicarbazone complexes of the type [RuCl(CO)(EPh$_3$)$_3$]$_2$L (where E = P/As; L = binucleating monobasic tridendate thiosemicarbazone ligand) have been investigated. Structural features were determined by analytical and spectral techniques. Binding of these complexes with CT-DNA by absorption spectral study indicates that the ruthenium(II) complexes form adducts with DNA and have intrinsic binding constant in the range of 3.3 × 10$^5$ - 1.2 × 10$^6$ M$^{-1}$. The complexes exhibit a remarkable DNA cleavage activity with CT-DNA in the presence of hydrogen oxide and the cleavage activity depends on dosage.

**Key Words:** Binuclear ruthenium(II) complexes, Benzothiazolyl thiosemicarbazide, DNA interaction, Oxidative DNA cleavage

**Introduction**

Over a decade, the DNA-binding metal complexes have been extensively studied as DNA structural probes, DNA dependent electron transfer probes, DNA foot printing and sequence specific cleaving agents and potential anticancer drugs.$^1$ Despite a considerable amount of reported materials, the knowledge of the nature of binding of these complexes to DNA and their binding geometries has remained relatively modest. Transition metal complexes can interact non-covalently with nucleic acids by intercalation, groove binding or external electrostatic binding.$^2$ There has been tremendous interest in studies related to the interaction of transition metal ions with nucleic acid because of their relevance in the development of new reagents in biotechnology and medicine.$^3$ These studies are also important to understand the toxicity of drugs containing metal ions.$^4$ In particular, ruthenium complexes are presently the objective of great attention in the field of medicinal chemistry with low systemic toxicity.$^5$ Also, ruthenium complexes appear to penetrate reasonably well into the tumour cells and bind effectively to DNA.$^6$ Moreover, the binuclear ruthenium complexes have been found to have better biological activity than mononuclear complexes.$^7$

In the efforts to develop drugs with such capabilities, scientists have focused upon many different aspects of biology during their research. Among the drugs discovered in recent years, various benzothiazoles$^8$ possess potent cytotoxicity properties. Thiazoles represent a very interesting class of compounds because of their wide applications in pharmaceutical, analytical and industrial aspects.$^9$ In addition, benzothiazolyl thiosemicarbazones and ferrocenyl thiosemicarbazones were found to be active against tumours and fungi.$^{10,11}$ The substitution of bulky group at the terminal nitrogen of the thiosemicarbazones considerably increases the biological activity.$^{12}$ With this aid, the incorporation of binucleating thiosemicarbazone into ruthenium tertiary phosphine/arsine complexes was initially aimed and promoted such compounds towards the DNA binding and DNA cleavage studies.

**Experimental**

**Reagents and Methods.** All the chemicals used were of AR grade. Solvents were purified and dried according to the standard procedure.$^{13}$ RuCl$_3$·3H$_2$O was purchased from Loba Chemie and was used without further purification. Calf-thymus (CT-DNA) was purchased from Bangalore Genei, Bangalore, India. Micro analyses (C, H, N & S) were performed on a Vario EL III CHNS analyser at STIC, Cochin University of Science and Technology, Kerala, India. IR spectra were recorded as KBr pellets in the 400-4000 cm$^{-1}$ region using Perkin Elmer FT-IR 8000 spectrophotometer. Electronic spectra were recorded in dimethylsulphoxide solution with a Systronics double beam UV-vis spectrophotometer 2202 in the range 200-800 nm. $^1$H, $^{13}$C and $^{31}$P NMR spectra were recorded on a Bruker AV III 500 MHz instrument using TMS and ortho phosphoric acid as an internal reference. EI mass spectrum of the complexes was recorded on a JEOL GCMA TE II mass spectrometer. Melting points were recorded on a Vego VMP-DS model heating table and were uncorrected. Benzothiazolyl thiosemicarbazide$^{14}$ and the metal precursors [RuHCl(CO)(PPh$_3$)$_3$]$^{15}$ and [RuHCl(CO)(AsPh$_3$)$_3$]$^{16}$ were prepared according to the reported
procedures.

**Synthesis of Thiosemicarbazone Ligands.** To a methanolic solution (20 mL) of 6-nitro/H-2-benzothiazolyl thiosemicarbazide (20 mmol), diacetyl ferrocene (10 mmol) in methanol was added and stirred along with a few drops of glacial acetic acid. The mixture was then refluxed for about 8 h. The resultant product was washed with methanol and the purity of the ligands was checked by TLC and was further purified by column chromatography using petroleum ether and ethyl acetate mixture (60:40). The outline of synthesis of thiosemicarbazone ligands is given in Scheme 1.

**Scheme 1.** Synthesis of binucleating thiosemicarbazone ligands.

![Scheme 1](image1)

**H$_2$L$_1$:** Yield 82%; Colour: Dark brown; mp 298 °C. Anal. Calc for C$_{36}$H$_{32}$N$_8$S$_2$FeO: C, 52.78; H, 3.84; N, 16.41; S, 18.79. Found: C, 52.70; H, 3.82; N, 16.42; S, 18.89. IR (cm$^{-1}$): 1631 (C=N), 807 (C=S), 1543 (C=N thiazole ring). UV-vis (in DMSO); $\lambda_{max}$) nm; 308, 368, 432, 471. 1H NMR (ppm); 7.28-8.24 (m, Ar-H), 8.68 (s, NH), 11.9 (s, SH), 4.60-4.80 (m, Cp ring-H). 13C NMR (ppm); 119-131 (Ar, C), 140 (C-S), 157 (C=N imine), 165 (thiazole C=N), 71-81 (C, Cp ring).

**H$_2$L$_2$:** Yield 79%; Colour: Black; mp 289 °C. Anal. Calc for C$_{50}$H$_{40}$N$_{10}$Ru$_2$FeO$_3$: C, 52.78; H, 3.84; N, 16.41; S, 18.79. Found: C, 52.70; H, 3.82; N, 16.42; S, 18.89. IR (cm$^{-1}$): 1631 (C=N), 807 (C=S), 1543 (C=N thiazole ring). UV-vis (in DMSO); $\lambda_{max}$) nm; 305, 368, 430. 1H NMR (ppm); 7.39-8.23 (m, Ar-H), 2.28 (s, CH$_3$), 8.67 (s, NH), 11.3 (s, SH), 4.59-4.79 (m, Cp ring-H). 13C NMR (ppm); 117-132 (Ar, C), 27 (CH$_3$), 141 (C-S), 159 (C=N imine), 165 (thiazole C=N), 71-81 (C, Cp ring).

**Synthesis of Binuclear Ruthenium(II) Thiosemicarbazone Complexes.** All the reactions were carried out under anhydrous condition. The monobasic tridentate thiosemicarbazone (0.1 mmol) were added to a solution of [RuHCl(CO)(PPh$_3$)$_3$] (0.1 mmol), (E=P/As) in 1:2 molar ratio in benzene-methanol/tetrahydrofuran (50 mL) mixture, and then refluxed for 10 h. The resulting solution was concentrated to about 3 cm$^3$ and the complexes were precipitated by the addition of small quantity of petroleum ether (60-80 °C). The complexes were then filtered off, washed with petroleum ether and recrystallized from tetrahydrofuran/CH$_2$Cl$_2$/petroleum ether and dried under vacuum. Schematic route for synthesis of binuclear ruthenium(II) thiosemicarbazone complexes is given in Scheme 2.

**Scheme 2.** Structure of binuclear ruthenium(II) thiosemicarbazone complexes.

![Scheme 2](image2)

**[RuCl(CO)(PPh$_3$)$_3$]L$_1$:** Yield 62%; Colour: Brown; mp > 300 °C. Anal. Calc for C$_{50}$H$_{40}$N$_{10}$Ru$_2$FeO$_3$: C, 50.28; H, 3.23; N, 8.62; S, 7.90. Found: C, 50.18; H, 3.13; N, 8.61; S, 7.93. EI-MS: Found m/z = 1624 (M$^+$) (calculated m/z = 1624.30 for M$^+$. IR (cm$^{-1}$): 1603 (C=N), 745 (C=S), 1461 (C=N thiazole ring), 1952 (CO). UV-vis (in DMSO); $\lambda_{max}$, nm; 305, 368, 430, 468. 1H NMR (ppm); 7.20-8.25 (m, Ar-H), 2.34 (s, CH$_3$), 8.67 (s, NH), 4.55-4.78 (m, Cp ring-H). 13C NMR (ppm); 116-134 (Ar, C), 27 (CH$_3$), 136 (C-S), 179 (C=N), 201 (CO), 69-79 (C, Cp ring). 31P NMR (ppm); 42 (P, PPh$_3$).

**[RuCl(CO)(AsPh$_3$)$_3$]L$_2$:** Yield 59%; Colour: Black; mp > 300 °C. Anal. Calc for C$_{50}$H$_{40}$N$_{10}$Ru$_2$FeO$_3$:C, 47.70; H, 3.06; N, 8.18; S, 7.49. Found: C, 47.81; H, 3.26; N, 8.28; S, 7.39. EI-MS: Found m/z = 1712 (M$^+$) (calculated m/z = 1712.20 for M$^+$. IR (cm$^{-1}$): 1619 (C=N), 737 (C=S), 1469 (C=N thiazole ring), 1954 (CO). UV-vis (in DMSO); $\lambda_{max}$, nm; 308, 368, 432, 471. 1H NMR (ppm); 7.28-8.24 (m, Ar-H), 2.35 (s, CH$_3$), 8.69 (s, NH), 4.56-4.78 (m, Cp ring-H). 13C NMR (ppm); 117-133 (Ar, C), 27 (CH$_3$), 136 (C-S), 178 (C=N), 201 (CO), 69-79 (C, Cp ring).

**[RuCl(CO)(PPh$_3$)$_3$]L$_3$:** Yield 58%; Colour: Black; mp > 300 °C. Anal. Calc for C$_{50}$H$_{40}$N$_{10}$Ru$_2$FeO$_3$:C, 53.23; H, 3.55; N, 7.30; S, 8.36. Found: C, 53.21; H, 3.65; N, 7.32; S, 8.37. EI-MS: Found m/z = 1534 (M$^+$) (calculated m/z = 1534.09 for M$^+$. IR (cm$^{-1}$): 1619 (C=N), 737 (C=S), 1469 (C=N thiazole ring), 1954 (CO). UV-vis (in DMSO); $\lambda_{max}$, nm; 308, 368, 432, 471. 1H NMR (ppm); 7.28-8.24 (m, Ar-H), 2.35 (s, CH$_3$), 8.69 (s, NH), 4.56-4.78 (m, Cp ring-H). 13C NMR (ppm); 117-133 (Ar, C), 27 (CH$_3$), 136 (C-S), 178 (C=N), 201 (CO), 69-79 (C, Cp ring).
m/z = 1534.31 for M⁺. IR (cm⁻¹): 1609 (C=N), 748 (C–S), 1461 (C=N thiazole ring), 1963 (CO). UV-vis (in DMSO); λmax nm; 305, 340, 464. ¹H NMR (ppm); 7.13-8.24 (m, Ar-H), 2.30 (s, CH₃), 8.68 (s, NH), 4.61-4.81 (m, Cp ring-H). ¹³C NMR (ppm); 117-135 (Ar, C), 137 (C-S), 172 (C=N), 198 (CO), 69-73 (C, Cp ring). ³¹P NMR (ppm); 42 (P, PPh₃).

[RuCl(CO)(EPh₃)₂]L²⁺: Yield 61%; Colour: Brown; mp > 300 °C. Anal. Calc for C₅₅H₆₃N₆RuFeO₄AsS₅Cl:C, 53.23; H, 3.55; N, 7.30; S, 8.36. Found: C, 53.21; H, 3.65; N, 7.32; S, 8.37. EI-MS: Found m/z = 1622 (M⁺) (calculated m/z = 1622.20 for M⁺). IR (cm⁻¹): 1613 (C=N), 738 (C–S), 1461 (C=N thiazole ring), 1963 (CO). UV-vis (in DMSO); λmax nm; 305, 365, 431, 469. ¹H NMR (ppm); 7.27-8.24 (m, Ar-H), 2.08 (s, CH₃), 8.69 (s, NH), 4.60-4.85 (m, Cp ring-H). ¹³C NMR (ppm); 118-133 (Ar, C), 31 (CH₃), 136 (C-S), 172 (C=N), 199 (CO), 68-73 (C, Cp ring).

DNA-Binding and Cleavage Assay.

Electronic Absorption Spectroscopy: All the experiments involving the binding of binuclear ruthenium(II) complexes with CT-DNA were carried out in a doubly distilled water buffer with tris(hydroxymethyl)-aminomethane (Tris, 5 mM) and sodium chloride (50 mM) and adjusted to pH 7.2 with hydrochloric acid. A solution of CT-DNA in the buffer gave a ratio of UV absorbance of about 1.8-1.9 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein. The DNA concentration per nucleotide was determined spectrophotometrically by employing an extinction coefficient of 6600 M⁻¹ cm⁻¹ at 260 nm. The complexes were dissolved in a mixed solvent of 5% DMSO and 95% Tris-HCl buffer. Stock solutions were stored at 4 °C and used within 4 days. Absorption titration experiments were performed with fixed concentration of the complexes (25 µM) with varying concentration of DNA (0-40 µM). While measuring the absorption spectra, an equal amount of DNA was added to both the test solution and the reference solution to eliminate the absorbance of DNA itself.

DNA Cleavage: The DNA cleavage activity of the ligands and ruthenium(II) complexes were monitored by agarose gel electrophoresis on CT-DNA. The tests were performed under aerobic condition with H₂O₂ as an oxidant. Each reaction mixture contained 30 µM of CT-DNA, 30 and 60 µM of each compound in DMSO and 60 µM of hydrogen peroxide in 50 mM Tris-HCl (pH 7.2). The reaction was incubated at 37 °C for 2 h. After incubation, 1 µL of loading buffer (0.25% bromphenol blue, 0.25% xylene cyanol and 0.05% glycerol) was added to the reaction mixture and loaded onto a 1% agarose gel containing 1.0 µg/mL of ethidium bromide. The electrophoresis was carried out for 2 h at 50 V in Tris-acetate acid-EDTA buffer. The bands were visualized under UV light and photographed.

Results and Discussion

Structure of the Complexes. A new series of binuclear ruthenium(II) thiosemicarbazone complexes of the type [RuCl(CO)(EPh₃)₂]L (E=P/As; L = binucleating monobasic tridentate thiosemicarbazone ligands) were synthesized by reacting ruthenium(II) precursors [RuHCl(CO)(EPh₃)] with binucleating monobasic tridentate thiosemicarbazone ligands in 2:1 molar ratio, respectively in benzene-methanol/tetrahydrofuran mixture. The synthesized binuclear ruthenium(II) thiosemicarbazone complexes are stable in air at room temperature, non-hygroscopic in nature and soluble in common solvents such as dichloromethane, dimethylformamide, dimethylsulphoxide, etc. The analytical data of the complexes are in good agreement with the calculated values thus confirming the proposed hetero bimetallic composition for all the complexes.

IR Spectra. The important IR spectral data of the ligands were compared with those of the binuclear ruthenium(II) complexes in order to confirm the binding mode of the thiosemicarbazone ligands to ruthenium atom in the complexes. In principle, the thiosemicarbazone ligands exhibit thione-thiol tautomerism, since it contains a thioamide –NH-C=S functional group. The free ligands display ν(C=S) absorptions in the region 807-826 cm⁻¹, shifts to 737-748 cm⁻¹ in the spectra of the complexes. This observation may be attributed to the enolization of –NH-C=S and subsequent coordination through the deprotonated sulphur. A strong band in the range 1631-1649 cm⁻¹ due to the azomethine group (C=N) of free ligands was shifted to lower frequency in the spectra of complexes in the range 1603-1619 cm⁻¹ indicating that the other coordination is through azomethine nitrogen atom. IR spectrum of the ligands revealed a medium intensity band in the region 1511-1543 cm⁻¹ ν(C=N) thiazole ring, which is shifted to lower frequency in the range 1461-1469 cm⁻¹ after complexation, which also indicates that it has been affected upon coordination to the metal ion.

Electronic Spectra. The absorption of free ligands showed two types of transitions appeared in the range 305-308 nm and at 368 nm are due to π–π* and n-π* transitions involving molecular orbital of the cyclopentadienyl ring, C=N and enolic -SH chromophore respectively. For all the ligands and complexes, the shoulder in the region 428-430 nm is attributed to the transition of 3d electron on iron to either non-bonding or antibonding orbitals of the cyclopentadienyl ring. These bands are shifted in the spectra of the complexes, indicating the involvement of imine group nitrogen and thiol sulphur in coordination with central metal atom. The spectra of the complexes showed five bands in the region 305-471 nm. The ground state of ruthenium(II) in an octahedral environment is ¹A₁g, arising from the t₂g⁵ configuration, and the excited states corresponding to the t₂g⁴e² configuration are ³T₁g, ³T₂g, ⁴T₁g, and ⁴T₂g. Hence, four
bands corresponding to the transitions $^1\text{A}_{1g} \rightarrow ^3\text{T}_{1g}$, $^1\text{A}_{1g} \rightarrow ^3\text{T}_{2g}$, $^1\text{A}_{1g} \rightarrow ^1\text{T}_{1g}$ and $^1\text{A}_{1g} \rightarrow ^1\text{T}_{2g}$ are possible in order of the increasing energy. The lower wavelength bands are characterized as ligand centered transitions occurring within the ligand orbitals. The bands appearing in the region 464-471 nm have been assigned to charge transfer transitions arising from excitation of an electron from the metal $t_{2g}$ level to an unfilled molecular orbital derived from the $\pi^*$ level of the ligands. The pattern of the electronic spectra for the complexes indicated the presence of an octahedral environment around ruthenium(II) atom similar to that of the other ruthenium(II) octahedral complexes.\textsuperscript{11,23}

\textbf{\textsuperscript{1}H, \textsuperscript{13}C and \textsuperscript{31}P NMR Spectra.} The formation of ligands and complexes were conveniently monitored by peak ratios in the \textsuperscript{1}H, \textsuperscript{13}C and \textsuperscript{31}P NMR spectra. The \textsuperscript{1}H NMR spectra of H\textsubscript{2}L\textsubscript{1}, \{RuCl(CO)(PPh\textsubscript{3})\}\textsubscript{2}L\textsubscript{1} and \textsuperscript{31}P NMR spectrum of \{$[\text{RuCl(CO)(PPh\textsubscript{3})}_2L_1]\$} are given in Figure S1-S3 respectively. The signal due to the $-\text{SH}$ proton in the ligands at 11.3-11.9 ppm disappears in the case of metal complexes, showing the bonding of thiolic sulphur to the metal after the deprotonation of the functional group.\textsuperscript{24} The signal observed at 8.67-8.69 ppm is due to $-\text{NH}$ proton for free ligands and the complexes.\textsuperscript{25} For ligands and complexes, the chemical shift at 4.55-4.85 ppm is due to cyclopentadienyl ring protons.\textsuperscript{26} Of the slight chemical shifts were observed for ferrocene in the complexes when compared to ligands, reflect extension of the delocalized $\pi$-system of the thiosemi-carbazide chain into cyclopentadienyl ring.\textsuperscript{27} The ligands and complexes shows signal at 2.08-2.35 ppm due to methyl group.

\textsuperscript{13}C NMR spectra of all the ligands displayed a single resonance at 157-159 ppm due to the azomethine carbon atoms, which also confirms the structure of the ligands.\textsuperscript{28} The signal at 165 ppm corresponds to thiazolic C=N carbon.\textsuperscript{28} The downfield shift of these two signals at 172-179 ppm clearly indicates that both the C=N carbons are affected by coordination.\textsuperscript{30} The thiolic carbon of the thiosemicarbazones appeared at 140-141 ppm. Upon coordination, this signal was shifted to upfield and appeared at 136-137 ppm.\textsuperscript{24} The signal due to methyl carbon of the ligands and complexes appears at 27-31 ppm. The aromatic carbons of free ligands and the complexes show signal in the region 116-135 ppm. For ligands and complexes, the signal at 68-81 ppm is assigned to cyclopentadienyl ring carbons.\textsuperscript{31} For all the complexes, the terminal carbonyl group, CO appeared in the range 198-201 ppm.\textsuperscript{32}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{EI-Mass spectra of the complexes, \{RuCl(CO)(PPh\textsubscript{3})\}_2L_1, \{RuCl(CO)(AsPh\textsubscript{3})\}_2L_1, \{RuCl(CO)(PPh\textsubscript{3})\}_2L_2 and \{RuCl(CO)(AsPh\textsubscript{3})\}_2L_2.}
\end{figure}
DNA Interaction of Binuclear Ruthenium(II) Complexes

{[RuCl(CO)(PPh₃)]L¹}

Figure 2. Absorption spectral traces of the complexes, {[RuCl(CO)(PPh₃)]L¹}, {[RuCl(CO)(AsPh₃)]L¹}, {[RuCl(CO)(PPh₃)]L²} and {[RuCl(CO)(AsPh₃)]L²} with CT-DNA.

P NMR spectrum of the complexes [RuCl(CO)(PPh₃)L¹] and [RuCl(CO)(PPh₃) L²] were recorded in order to confirm the presence of triphenylphosphine group. The observation of a sharp singlet at 42 ppm confirms the presence of only one triphenylphosphine group in the complexes.

Mass Spectral Analysis. The mass spectra of the binuclear ruthenium(II) complexes were in good agreement with the proposed structure and are shown in Figure 1. The molecular ion peak, M⁺ appeared at m/z = 1624, 1712, 1534 and 1622 confirms the stoichiometry of the complexes, {[RuCl(CO)(PPh₃)]L¹}, {[RuCl(CO)(AsPh₃)]L¹}, {[RuCl(CO)(PPh₃)]L²} and {[RuCl(CO)(AsPh₃)]L²}, respectively.

DNA-Binding and Cleavage Assay.

Electronic Absorption Spectroscopy: Electronic absorption spectroscopy is one of the most common techniques for the investigation of the mode of interaction of metal complexes. Absorption spectra of the complexes in the absence and presence of CT-DNA are equal to 1/(ε₁ + ε₂), respectively. This is good agreement with the reported literature. From the results obtained, it has been found that the complex, {[RuCl(CO)(PPh₃)]L¹} strongly bound with CT-DNA than that of other complexes.

DNA Cleavage Activity. After binding to DNA, duly designed metal complexes can induce several changes in DNA conformation. Metal complexes, which could induce DNA deformation, such as bending, ‘local denaturation’ (overwinding and underwinding), intercalation, micro loop
is generally monitored by agarose gel electrophoresis and in gel under the influence of electric field. This result suggests that the nuclease activity of the CT-DNA (30 µM) with different concentration of the complexes; Lane 1: DNA control; Lane 2: 30 µM \([\text{RuCl(CO)}(\text{PPh}_3)]\text{L}^1\) + DNA + 60 µM H\(_2\)O\(_2\); Lane 3: 60 µM \([\text{RuCl(CO)}(\text{PPh}_3)]\text{L}^1\) + DNA + 60 µM H\(_2\)O\(_2\); Lane 4: 30 µM \([\text{RuCl(CO)}(\text{AsPh}_3)]\text{L}^1\) + DNA + 60 µM H\(_2\)O\(_2\); Lane 5: 60 µM \([\text{RuCl(CO)}(\text{AsPh}_3)]\text{L}^1\) + DNA + 60 µM H\(_2\)O\(_2\); Lane 6: 30 µM \([\text{RuCl(CO)}(\text{PPh}_3)]\text{L}^2\) + DNA + 60 µM H\(_2\)O\(_2\); Lane 7: 60 µM \([\text{RuCl(CO)}(\text{PPh}_3)]\text{L}^2\) + DNA + 60 µM H\(_2\)O\(_2\); Lane 8: 30 µM \([\text{RuCl(CO)}(\text{AsPh}_3)]\text{L}^2\) + DNA + 60 µM H\(_2\)O\(_2\); Lane 9: 60 µM \([\text{RuCl(CO)}(\text{AsPh}_3)]\text{L}^2\) + DNA + 60 µM H\(_2\)O\(_2\).

The ability of metal complexes to perform DNA cleavage is generally monitored by agarose gel electrophoresis and in the present work CT DNA was chosen to investigate its cleavage. The cleavage experiments were carried out in the absence and presence of activating agent, H\(_2\)O\(_2\) under aerobic condition. The hydrolytic cleavage activity of the binuclear ruthenium(II) complexes does not show any significant cleavage of DNA (not shown) when applied separately. This result suggests that the nuclease activity of the complexes does not involve by hydrolytic pathway. Further, the ferrocenyl thiosemicarbazone ligands were subjected to nuclease activity in the presence of H\(_2\)O\(_2\) and are shown in Figure 3. A control experiment using DNA with H\(_2\)O\(_2\) does not show any significant cleavage of DNA (Lane 1). Upon electrophoresis, there is no considerable difference in the intensity of the bands observed for the ligands bound DNA as compared to control DNA. This reveals that the ferrocenyl thiosemicarbazone ligands are not active to cleave DNA.

These experimental facts demonstrate that, the binuclear ruthenium(II) thiosemicarbazone complexes with an activating agent, H\(_2\)O\(_2\) are required to show effective cleavage of CT DNA. Figure 4 shows that the oxidative cleavage of CT DNA induced by binuclear ruthenium(II) complexes in the presence of H\(_2\)O\(_2\). Control experiment using DNA alone (Lane 1) does not show any significant cleavage of CT-DNA even on longer exposure time. When the DNA is allowed to interact with the complexes at various concentrations (30 and 60 µM), a substantial decrease in the intensities of the bands for the metal bound DNA as compared to untreated control DNA was observed, which suggests the cleavage of DNA by ruthenium(II) complexes. When the concentration was increased to 60 µM for all the complexes, the bands (Lane 3, 5, 7 & 9) were completely disappeared, indicating the sufficient cleavage of DNA. The results showed that \([\text{RuCl(CO)}(\text{PPh}_3)]\text{L}^1\) has more cleavage activity than the other complexes and the efficiency of the complexes are comparable to those of other hetero bimetallic ruthenium complexes. This phenomenon indicates that the complexes with H\(_2\)O\(_2\) are capable to cleave the DNA; as a consequence, these complexes are better suited for therapeutic applications particularly, in cancer chemotherapeutics.

**Conclusion**

The binuclear ruthenium(II) complexes of benzothiazole substituted ferrocenyl thiosemicarbazone were synthesized and characterized by various physico-chemical and spectroscopic methods. The complexes are tentatively assigned for an octahedral geometry. The DNA binding study suggests that all the complexes interact with DNA most likely through a mode that involves a stacking interaction between the aromatic chromophore and the base pairs of DNA via electrostatically. The magnitudes of intrinsic binding constant, K\(_s\) of the binuclear ruthenium(II) complexes has been estimated in the range 3.3×10\(^4\) M\(^{-1}\)-1.2×10\(^5\) M\(^{-1}\). The binding affinity of the complexes were in the order \([\text{RuCl(CO)}(\text{PPh}_3)]\text{L}^1\) > \([\text{RuCl(CO)}(\text{AsPh}_3)]\text{L}^1\) > \([\text{RuCl(CO)}(\text{PPh}_3)]\text{L}^2\) > \([\text{RuCl(CO)}(\text{AsPh}_3)]\text{L}^2\). DNA cleavage studies reveal that the complexes have the ability to cleave nucleic acids and the extent of the cleavage was found to be dose dependent. The complex, \([\text{RuCl(CO)}(\text{PPh}_3)]\text{L}^1\) has more cleavage activity than the other complexes.

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