CHAPTER II
Synthesis, characterization, DNA interaction, antioxidant and anticancer activities of new ruthenium(II) complexes of thiosemicarbazone/semicarbazone bearing 9,10-phenanthrenequinone

Though possible remedial measures are available at present to tackle any disease, continuous search in trying to find better and more effective drugs is on the increase. This is particularly true in the case of cancer where cisplatin and its analogs such as carboplatin and oxaliplatin during chemotherapy result in drawbacks including intrinsic or acquired resistance and toxicity. So the efforts to mitigate the drawbacks have prompted chemists to synthesize a variety of analogs, which is valuable not only for providing better drugs but also for offering useful tools in the study of the molecular mechanisms underlying tumor development. In this connection, ruthenium based coordination and organometallic complexes have shown enormous impact. Though a wide range of ruthenium complexes have been described in the literature, only few of them show outstanding anticancer activity\textsuperscript{1-6} and two of them, for instance NAMI-A and KP1019 are currently involved in clinical trials.\textsuperscript{7-9} It is known that ruthenium complexes are well suited for medical applications due to the fact that they have a very similar rates of ligand exchange to those of platinum(II) antitumor drugs,\textsuperscript{10} a wide range of accessible oxidation states under physiological environment and an ability of the ruthenium to mimic iron in binding to certain molecules of biological significance.\textsuperscript{11}

Similarly, quinonoidal compounds of natural or synthetic origin represent the second largest class of clinically approved anticancer agents.\textsuperscript{12-14} The presence of a 1,2-dione group embedded in a six-membered ring scaffold was essential for the anticancer activity of this class of compounds.\textsuperscript{15} One of the potential ligands of the \( o \)-quinone series is 9,10-phenanthrenequinone. Attention was drawn to phenanthrenequinone because of its planar structure resembling the phenanthroline compounds which are capable of undergoing intercalating interactions with DNA, resulting in cell antiproliferative activities.\textsuperscript{16,17} A combination of such planar
polycyclic compound with the potent cytotoxic thiosemicarbazone pharmacophore has been shown to produce synergistic effects on the antiproliferative activity of the parent ligands.\textsuperscript{18}

Based on the above discussions, in this chapter, the synthesis, characterization, DNA binding/cleavage, antioxidant and anticancer studies of ruthenium(II) complexes containing 9,10-phenanthrenequinone appended with derivatives of thiosemicarbazone/semicarbazone with PPh\textsubscript{3}/AsPh\textsubscript{3} as ancillary ligands were described.

**Experimental**

**Materials and instruments**

All the reagents used were chemically pure and AR grade. The solvents were purified and dried according to standard procedures. RuCl\textsubscript{3}.3H\textsubscript{2}O was purchased from Loba Chemie Pvt Ltd. The starting complexes [RuHCl(CO)(PPh\textsubscript{3})\textsubscript{3}] and [RuHCl(CO)(AsPh\textsubscript{3})\textsubscript{3}] and the ligand HL\textsubscript{4} were prepared according to literature procedures.\textsuperscript{19-21} Calf thymus DNA (CT-DNA) was purchased from Sigma and dissolved in 5 mM Tris HCl buffer (pH 7.2) containing 100 mM NaCl and 1 mM EDTA. It was dialyzed several times against 5 mM Tris HCl buffer. All experiments involving interactions of complexes with CT-DNA were carried out in Tris HCl buffer (pH 7.0). The concentration of DNA is determined spectrophotometrically using the extinction coefficient \(\epsilon_{258\text{nm}} = 6700 \text{ M}^{-1} \text{ cm}^{-1}\).

Microanalyses of carbon, hydrogen, nitrogen and sulfur were carried out using Vario EL III Elemental analyzer at SAIF-Cochin India. The IR spectra of the ligands and their complexes were recorded as KBr pellets on a Nicolet Avatar model spectrophotometer in 4000-400 cm\textsuperscript{-1} range. Electronic spectra of the complexes have been obtained in dichloromethane using a Shimadzu UV-1650 PC spectrophotometer in 800-200 nm range. \( ^1\text{H}, \ ^{13}\text{C} \) and \( ^{31}\text{P} \) NMR spectra were measured in Jeol GSX-400 instrument using DMSO-\(d_6\) as the solvent at room temperature. \( ^1\text{H}, \ ^{13}\text{C} \) and \( ^{31}\text{P} \) NMR spectra were obtained using TMS and \( o\)-phosphoric acid as a reference respectively. The ESI-Mass spectra were recorded by LC-MS Q-ToF Micro Analyzer (Shimadzu) in the SAIF, Panjab University, Chandigarh. DNA cleavage studies were carried out at Biogenics laboratory, Hubli.
Melting points were checked on a Technico micro heating table and were uncorrected.

**Preparation of ligands (HL\textsubscript{2}-HL\textsubscript{4})**

A solution of methylthiosemicarbazide hydrochloride/ phenylthiosemicarbazide hydrochloride/semicarbazide hydrochloride (1 mM) in ethanol (10 mL) was warmed on a steam bath for a few minutes and then added in one lot to a boiling solution of phenanthrenequinone (1 mM) in ethanol (100 mL). The mixture was refluxed at 70 °C for 2 h, a respective precipitate formed was separated, filtered off, washed with cold ethanol. Orange colored crystals, suitable for single crystal X-ray diffraction analysis, were obtained by slow evaporation of mixture of acetonitrile/dichloromethane solution of compounds.

**9,10-phenanthrenequinonethiosemicarbazone (HL\textsubscript{1})**

Yield: 92%; Color: Orange; M.p.: 218 °C. Anal. Calc. for C\textsubscript{15}H\textsubscript{11}N\textsubscript{2}OS: C, 64.04; H, 3.94; N, 14.94; S, 11.40%. Found: C, 64.36; H, 3.78; N, 14.73; S, 11.71%. IR (KBr, cm\textsuperscript{-1}): 3148 (N-H), 1630 (quinone C=O), 1596 (C=N), 838 (C=S). \textsuperscript{1}H NMR (DMSO-\textit{d}_6, ppm): 14.41 (s, 1H, hydrazinic N-H), 9.07, 9.36 (s, 2H, NH\textsubscript{2}), 7.45-8.69 (m, 8H, Ar-H).

**9,10-phenanthrenequinonemethylthiosemicarbazone (HL\textsubscript{2})**

Yield: 86%; Color: Orange; M.p.: 222 °C. Anal. Calc. for C\textsubscript{16}H\textsubscript{13}N\textsubscript{3}OS: C, 65.06; H, 4.44; N, 14.23; S, 10.86%. Found: C, 65.48; H, 4.77; N, 13.91; S, 10.49%. IR (KBr, cm\textsuperscript{-1}): 3120 (N-H), 1631 (quinone C=O), 1596 (C=N), 807 (C=S). \textsuperscript{1}H NMR (DMSO-\textit{d}_6, ppm): 14.65 (s, 1H, hydrazinic N-H), 8.35 (s, 1H, NH-CH\textsubscript{3}), 7.26-8.16 (m, 8H, Ar-H), 2.98 (s, 3H, CH\textsubscript{3}).

**9,10-phenanthrenequinonephenylthiosemicarbazone (HL\textsubscript{3})**

Yield: 87%; Color: Orange; M.p.: 193 °C. Anal. Calc. for C\textsubscript{21}H\textsubscript{15}N\textsubscript{3}OS: C, 70.57; H, 4.23; N, 11.76; S, 8.97%. Found: C, 70.15; H, 4.68; N, 11.30; S, 8.64%. IR (KBr, cm\textsuperscript{-1}): 3111 (N-H), 1634 (quinone C=O), 1598 (C=N), 843 (C=S). \textsuperscript{1}H NMR (DMSO-\textit{d}_6, ppm): 14.81 (s, 1H, hydrazinic N-H), 11.11 (s, 1H, NH-C\textsubscript{6}H\textsubscript{5}), 7.29-8.81 (m, 13H, Ar-H).
9,10-phenanthrenequinonesemicarbazone (HL₄)

Yield: 89%; Color: Yellowish orange; M.p.: 178 °C. Anal. Calc. for C₁₅H₁₃N₃O₃: C, 67.92; H, 4.18; N, 15.84%. Found: C, 67.51; H, 4.61; N, 15.47%. IR (KBr, cm⁻¹): 3139 (N-H), 1630 (quinone C=O), 1593 (C=N), 1674 (C=O). ¹H NMR (DMSO-d₆, ppm): 13.82 (s, 1H, hydrazinic N-H), 8.35, 8.57 (s, 2H, NH₂), 7.26-8.18 (m, 8H, Ar-H).

Synthesis of new ruthenium(II) complexes

All the new metal complexes were prepared according to the following general procedure. To a solution of [RuHCl(CO)(EP₃)₃] (E = P or As) (0.1 mM) in benzene (20 mL), the appropriate ligand (0.1 mM) was added in 1:1 molar ratio. The mixture was heated under reflux for 5 h on a water bath. Then the resulting solution was concentrated to 3 mL and the product precipitated by the addition of petroleum ether (60-80 °C) and was recrystallized using CH₂Cl₂. The complexes were dried under vacuum and the purity was checked by TLC.

[RuCl(CO)(PPh₃)(L₁)] (1)

Yield: 88%; Color: Brown; M.p.: 192 °C. Anal. Calc. for C₃₄H₂₅ClN₃O₂PRuS: C, 57.75; H, 3.56; N, 5.94; S, 4.53%. Found: C, 57.29; H, 3.21; N, 5.49; S, 4.98%. IR (KBr, cm⁻¹): 1957 (C=O), 1622 (quinone C=O), 1599 (C=N), 1588 (C=N), 756 (C-S). UV-Vis (λmax/nm): 465, 243. ¹H NMR (DMSO-d₆, ppm): 9.50 (s, 2H, NH₂), 7.32-8.86 (m, 23H, Ar-H). ¹³C NMR (DMSO-d₆, ppm): 204.61 (C=O), 180.13 (quinone C=O), 172.84 (C-S), 160.91 (C=N), 123.65-138.27 (Ar-C). ³¹P NMR (DMSO-d₆, ppm): 30.40. ESI-MS (m/z) = 707.0 [M⁺].

[RuCl(CO)(PPh₃)(L₂)] (2)

Yield: 81%; Color: Brown; M.p.: 178 °C. Anal. Calc. for C₃₄H₂₅ClN₃O₂PRuS: C, 58.29; H, 3.77; N, 5.83; S, 4.45%. Found: C, 58.70; H, 3.36; N, 5.41; S, 4.09%. IR (KBr, cm⁻¹): 1959 (C=O), 1620 (quinone C=O), 1600 (C=N), 1559 (C=N), 760 (C-S). UV-Vis (λmax/nm): 462, 245, 209. ¹H NMR (DMSO-d₆, ppm): 8.42 (s, 1H, NH-CH₃), 7.22-8.36 (m, 23H, Ar-H), 2.98 (s, 3H, CH₃). ¹³C NMR (DMSO-d₆, ppm): 203.42 (C=O), 179.90 (quinone C=O), 169.26 (C-S), 160.84 (C=N), 123.70-139.88 (Ar-C), 30.04 (NH-CH₃). ³¹P NMR (DMSO-d₆, ppm): 31.24. ESI-MS (m/z) = 721.89 [M⁺].
[RuCl(CO)(PPh₃)(L₃)] (3)

Yield: 85%; Color: Red; M.p.: 170 °C. Anal. Calc. for C₄₀H₂₉ClN₃O₂PRu: C, 61.34; H, 3.73; N, 5.36; S, 4.09%. Found: C, 61.79; H, 3.29; N, 5.80; S, 4.42%. IR (KBr, cm⁻¹): 1956 (C=O), 1609 (quinone C=O), 1596 (C=N), 1580 (C≡N), 746 (C=S). UV-Vis (λmax/nm): 508, 241. ¹H NMR (DMSO-d₆, ppm): 11.45 (s, 1H, NH-C₅H₅), 7.01-8.34 (m, 28H, Ar-H). ¹³C NMR (DMSO-d₆, ppm): 203.94 (C≡O), 182.73 (quinone C≡O), 172.26 (C=S), 160.59 (C≡N), 120.10-139.15 (Ar-C). ³¹P NMR (DMSO-d₆, ppm): 29.49. ESI-MS (m/z) = 783.6 [M⁺].

[RuCl(CO)(PPh₃)(L₄)] (4)

Yield: 86%; Color: Brown; M.p.: 172 °C. Anal. Calc. for C₃₄H₂₈ClN₃O₂PRu: C, 59.09; H, 3.65; N, 6.08%. Found: C, 59.55; H, 3.24; N, 6.51%. IR (KBr, cm⁻¹): 1954 (C=O), 1609 (quinone C=O), 1598 (C≡N), 1573 (C≡N), 1188 (C-O). UV-Vis (λmax/nm): 462, 232. ¹H NMR (DMSO-d₆, ppm): 8.50 (s, 2H, NH₂), 7.36-8.36 (m, 23H, Ar-H). ¹³C NMR (DMSO-d₆, ppm): 204.12 (C≡O), 180.62 (quinone C≡O), 168.80 (C≡O), 161.16 (C≡N), 122.42-137.74 (Ar-C). ³¹P NMR (DMSO-d₆, ppm): 30.42. ESI-MS (m/z) = 691.1 [M⁺].

[RuCl(CO)(AsPh₃)(L₄)] (5)

Yield: 85%; Color: Brown; M.p.: 228 °C. Anal. Calc. for C₃₂H₂₅ClN₃O₂AsRuS: C, 54.37; H, 3.35; N, 5.59; S, 4.27%. Found: C, 54.92; H, 3.81; N, 5.16; S, 4.72%. IR (KBr, cm⁻¹): 1971 (C=O), 1627 (quinone C=O), 1598 (C≡N), 1581 (C≡N), 762 (C-S). UV-Vis (λmax/nm): 510, 454, 383, 233. ¹H NMR (DMSO-d₆, ppm): 9.57 (s, 2H, NH₂), 7.34-8.57 (m, 23H, Ar-H). ¹³C NMR (DMSO-d₆, ppm): 204.41 (C≡O), 181.90 (quinone C≡O), 171.69 (C=S), 162.28 (C≡N), 121.73-137.22 (Ar-C). ESI-MS (m/z) = 751.1 [M⁺].

[RuCl(CO)(AsPh₃)(L₂)] (6)

Yield: 82%; Color: Brown; M.p.: 178 °C. Anal. Calc. for C₃₃H₂₇ClN₃O₂AsRuS: C, 54.94; H, 3.56; N, 5.49; S, 4.19%. Found: C, 54.49; H, 3.12; N, 5.94; S, 4.66%. IR (KBr, cm⁻¹): 1939 (C=O), 1626 (quinone C=O), 1604 (C≡N), 1581 (C≡N), 739 (C-S). UV-Vis (λmax/nm): 464, 237. ¹H NMR (DMSO-d₆, ppm): 8.40 (s, 1H, NH-CH₃), 7.24-8.20 (m, 23H, Ar-H), 2.92 (s, 3H, CH₃). ¹³C NMR (DMSO-d₆, ppm): 204.10
(C=O), 182.56 (quinone C=O), 171.36 (C=S), 162.92 (C=N), 123.88-137.45 (Ar-C), 30.68 (NH-CH₃). ESI-MS (m/z) = 765.5 [M⁺].

[RuCl(CO)(AsPh₃)(L₃)] (7)

Yield: 81%; Color: Brown; M.p.: 180 °C. Anal. Calc. for C₄₀H₂₉ClN₅O₂AsRu: C, 58.08; H, 3.53; N, 5.08; S, 3.88%. Found: C, 58.48; H, 3.19; N, 5.51; S, 3.41%. IR (KBr, cm⁻¹): 1960 (C=O), 1613 (quinone C=O), 1581 (C=N), 1573 (C=N), 737 (C-S). UV-Vis (λmax/nm): 489, 247, 235, 222. ¹H NMR (DMSO-d₆, ppm): 11.71 (s, 1H, NH-C₆H₅), 7.35-8.57 (m, 28H, Ar-H). ¹³C NMR (DMSO-d₆, ppm): 204.98 (C=O), 183.09 (quinone C=O), 172.60 (C=S), 163.40 (C=N), 124.10-138.20 (Ar-C). ESI-MS (m/z) = 827.3 [M⁺].

[RuCl(CO)(AsPh₃)(L₄)] (8)

Yield: 78%; Color: Brown; M.p.: 158 °C. Anal. Calc. for C₃₃H₂₅ClN₅O₃AsRu: C, 55.56; H, 3.43; N, 5.72%. Found: C, 55.12; H, 3.78; N, 5.39%. IR (KBr, cm⁻¹): 1957 (C=O), 1626 (quinone C=O), 1596 (C=N), 1579 (C=N), 1188 (C-O). UV-Vis (λmax/nm): 368, 305, 267, 234. ¹H NMR (DMSO-d₆, ppm): 8.38 (s, 2H, NH₂), 7.02-8.18 (m, 23H, Ar-H). ¹³C NMR (DMSO-d₆, ppm): 203.48 (C=O), 180.98 (quinone C=O), 168.12 (C-O), 162.44 (C=N), 123.89-136.80 (Ar-C). ESI-MS (m/z) = 735.03 [M⁺].

X-ray Crystallography

Crystal data were collected on an Oxford/Agilent Gemini diffractometer. Structures were solved using the direct methods program SHELXL.²² All nonsolvent heavy atoms were located using subsequent difference Fourier syntheses. The structures were refined against F² with the program SHELXL,²³ in which all data collected were used including negative intensities. All nonsolvent heavy atoms were refined anisotropically. All nonsolvent hydrogen atoms were idealized using the standard SHELXL idealization methods.

DNA binding experiments

The experiments were carried out in 5 mM Tris-HCl buffer (pH 7.0) at ambient temperature and the complexes were dissolved in 5 mM Tris-HCl buffer containing 0.7% DMSO. The absorption spectral titration experiment was
performed by keeping the concentration of the ruthenium(II) complexes constant (10 μM) while varying the DNA concentration. The competitive studies of complexes with EB have been investigated with fluorescence spectroscopy in order to examine whether the complex is able to displace EB from its CT-DNA-EB complex. Changes in the fluorescence emission spectrum of the ethidium bromide-DNA complex were recorded under various complex concentrations (0-7 μM). Before measurements, the system was shaken and incubated at room temperature for 10 min. For all fluorescence measurements, the excitation and emission slits were maintained at 5 and 5 nm, respectively. The fluorescence spectra in the fluorimeter were obtained at an excitation wavelength of 522 nm and an emission wavelength of 584 nm. Thermal denaturation experiments were carried out by monitoring the absorption of CT DNA (100 μM) at 260 nm at various temperatures in the presence (10 μM) and the absence of each complex. Melting profiles were measured at 260 nm by a Cary 300 spectrophotometer. Readings were recorded for every 1.5 °C raise in temperature per minute. The T_m values were determined graphically from the plot of absorbance versus temperature. The viscosity measurement was carried out using an Ubbelodhe viscometer immersed in a thermostatic water bath maintained at 25 (±0.1) °C. DNA samples with approximately 200 base pairs in length were prepared by sonication in order to minimize complexities arising from DNA flexibility. Titrations were performed by addition of small volume of concentrated stock solutions of metal complex to a solution of calf thymus DNA in the viscometer. Flow times were measured with a digital stopwatch; each sample was measured three times and an average flow time was calculated. Relative viscosities for CT-DNA in the presence and absence of the complex were calculated from the relation \( \eta = (t - t_0)/t_0 \), where \( t \) is the observed flow time of DNA-containing solution and \( t_0 \) is the flow time of Tris–HCl buffer alone. Data are presented as \( (\eta/\eta_0)^{1/3} \) versus binding ratio \( (R = [\text{complex}]/[\text{DNA}] = 0.0-0.20) \), where \( \eta \) is the viscosity of CT-DNA in the presence of complex and \( \eta_0 \) is the viscosity of CT-DNA alone.

**DNA cleavage experiment**

For the gel electrophoresis experiment, supercoiled pBR 322 DNA (0.1 μg) was treated with the ruthenium(II) complexes with buffer (50 mM Tris-HCl, 18
mM NaCl, pH 7.2) and the solution was then irradiated at room temperature with a UV lamp (365 nm, 10 W). The samples were analyzed by electrophoresis for 1.5 h at 80 V on a 0.8% agarose gel in TBE (89 mM Tris-borate acid, 2 mM EDTA, pH 8.3). The gel was stained with 1 μg/mL ethidium bromide and observes the band under illuminator.

**Antioxidant assays**

The 2,2'-diphenyl-1-picrylhydrazyl (DPPH') radical scavenging activity of the compounds was measured according to the method of Blios. The DPPH radical is a stable free radical having λ_{max} at 517 nm. Various concentration of the experimental complexes were added to solution of DPPH in methanol (125 μM, 2 mL) and the final volume was made up to 4 mL with doubly distilled water. The solution was incubated at 37 °C for 30 min in the dark. The decrease in absorbance of DPPH was measured at 517 nm.

The hydroxyl (OH') radical scavenging activity of the compounds has been investigated using the Nash method. In *vitro* hydroxyl radicals were generated by a Fe^{3+}-ascorbic acid system. The detection of hydroxyl radicals was performed by measuring the amount of formaldehyde formed from the oxidation reaction with DMSO. The formaldehyde produced was detected spectrophotometrically at 412 nm. A mixture of 1.0 mL iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL EDTA solution (0.018%) and 1.0 mL DMSO (0.85% v/v DMSO in 0.1 M phosphate buffer, pH 7.4) were sequentially added to the test tubes. The reaction was initiated by adding 0.5 mL ascorbic acid (0.22%) and the mixture was incubated at 80-90 °C for 15 min in a water bath. After incubation, the reaction was terminated by the addition of 1.0 mL ice-cold trichloroacetic acid (17.5% w/v). Subsequently, 3.0 mL Nash reagent was added to each tube and the tubes were left at room temperature for 15 min. The intensity of the color formed was measured spectrophotometrically at 412 nm against a reagent blank.

For the two assays described above, all the tests were run in triplicate and various concentration of the complexes was used to fix a concentration at which the compounds showed around 50% of scavenging activity. In addition, the
percentage of scavenging activity was calculated using the following formula:

\[
\text{percentage of scavenging activity} = \left[ \frac{(A_0 - A_C)}{A_0} \right] \times 100 \quad (A_0 \text{ and } A_C \text{ are the absorbance in the absence and presence of the complexes respectively}).
\]

The 50% of scavenging activity (IC\textsubscript{50}) can be calculated using the percentage of scavenging activity results.

**In vitro anticancer activity evaluation by MTT assay**

Cytotoxicity studies of the complexes were carried out on human breast cancer cell lines (MCF-7, MDA-MB-468) and skin cancer cell line (A431) which were obtained from National Centre for Cell Science (NCCS), Pune, India. Cell viability was tested out using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay method.\textsuperscript{26} The cells were grown in Dulbecco’s Modified Eagles Medium containing 10% fetal bovine serum (FBS). For screening experiments, the cells were seeded into 96-well plates in 100 µL of the respective medium containing 10% FBS, at a plating density of 10000 cells/well and incubated at 37 °C, 5% CO\textsubscript{2}, 95% air and 100% relative humidity for 24 h prior to the addition of compounds. The compounds were dissolved in DMSO and diluted in the respective medium containing 1% FBS. After 24 h, the medium was replaced with the respective medium with 1% FBS containing the compounds at various concentrations and incubated at 37 °C, 5% CO\textsubscript{2}, 95% air and 100% relative humidity for 48 h. Experiments were performed in triplicate and the medium without the compounds served as control. After 48 h, 10 µL MTT (5 mg/mL) in phosphate-buffered saline was added to each well and the wells were incubated at 37 °C for 4 h. The medium with MTT was then flicked off and the formazan crystals that had formed were dissolved in 100 µL DMSO. Then the absorbance at 570 nm was measured using a microplate reader. The percentage of cell growth inhibition was determined using the following formula:

\[
\text{% cell Inhibition} = 100 - \frac{\text{Abs (sample)}}{\text{Abs (control)}} \times 100.
\]

A graph of the percentage of cell inhibition versus concentration was plotted and from this the IC\textsubscript{50} value was calculated.
Results and Discussion

The synthetic route for the ligands and their ruthenium(II) complexes was shown in scheme 1. All the complexes were stable at room temperature, non-hygroscopic in nature and highly soluble in common organic solvents such as dichloromethane, benzene, acetonitrile, chloroform and DMSO. The analytical data was in good agreement with proposed molecular formula of the complexes.

Scheme 1. The synthetic route for the ligands and their corresponding ruthenium(II) complexes

X-ray crystallography

The ORTEP representation of ligands, \textbf{HL}_{1-4} was shown in figure 1. Relevant data collection and details of the structure refinement were summarized in table 1. The selected bond lengths and bond angles for compounds \textbf{HL}_{1-4} were given in table 2. The ligands \textbf{HL}_{1} and \textbf{HL}_{4} crystallized in a monoclinic form with space group P2/n, C2/c respectively whereas the ligands \textbf{HL}_{2} and \textbf{HL}_{3} crystallized in a triclinic form with space group P-1. The ligands existence in the thione/ketone form were confirmed by the C=S/C=O bond length, 1.6782(8), 1.6770(9) and 1.6705(6) Å for \textbf{HL}_{1-3} and 1.2353(15) for \textbf{HL}_{4}. The azomethine bond length, C(14)-N(1), 1.3145(10), 1.3089(11), 1.3085(7) and 1.3073(15) Å for \textbf{HL}_{1-4} was in conformity with a formed C≡N double bond length (1.30 Å).
Figure 1. ORTEP view of ligands HL$_1$-4. Thermal ellipsoids were drawn at 30% probability level. The hydrogen atoms were omitted for clarity.
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<td>c = 13.8228(3)</td>
<td>c = 25.7238(6)</td>
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<tr>
<td>α = 90</td>
<td>α = 72.804(6)</td>
<td>α = 87.849(2)</td>
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<tr>
<td>β = 93.802(3)</td>
<td>β = 83.088(5)</td>
<td>β = 88.137(2)</td>
<td>β = 108.001(2)</td>
<td></td>
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<tr>
<td>γ = 90</td>
<td>γ = 89.132(4)</td>
<td>γ = 87.272(2)</td>
<td>γ = 90</td>
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<td>Volume Å³</td>
<td>1270.21(8)</td>
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<td>Z</td>
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<td>2</td>
<td>8</td>
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<tr>
<td>Calculated density (Mg/m³)</td>
<td>1.471</td>
<td>1.428</td>
<td>1.425</td>
<td>1.156</td>
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<td>Absorption coefficient (mm⁻¹)</td>
<td>0.253</td>
<td>0.237</td>
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<td>F(000)</td>
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<td>308</td>
<td>372</td>
<td>1096</td>
</tr>
<tr>
<td>Theta range for data collection (°)</td>
<td>2.698 to 41.149</td>
<td>3.059 to 41.009</td>
<td>3.458 to 40.966</td>
<td>3.538 to 75.554</td>
</tr>
<tr>
<td>Data/restraints/parameters</td>
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<td>8814/0/199</td>
<td>10798/0/243</td>
<td>3086/0/181</td>
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<tr>
<td>Goodness-of-fit on F²</td>
<td>1.060</td>
<td>1.036</td>
<td>1.071</td>
<td>1.036</td>
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<tr>
<td>R indices [I&gt;2σ(I)]</td>
<td>R₁ = 0.0450, wR₂ = 0.1203</td>
<td>R₁ = 0.0508, wR₂ = 0.1106</td>
<td>R₁ = 0.0396, wR₂ = 0.1035</td>
<td>R₁ = 0.0417, wR₂ = 0.1263</td>
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<tr>
<td>R indices (all data)</td>
<td>R₁ = 0.0595, wR₂ = 0.1316</td>
<td>R₁ = 0.0882, wR₂ = 0.1301</td>
<td>R₁ = 0.0500, wR₂ = 0.1088</td>
<td>R₁ = 0.0473, wR₂ = 0.1337</td>
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Table 2. Selected bond lengths (Å) and angles (°) of the ligands $\text{HL}_{1-4}$

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<th>Bond/Distance</th>
<th>$\text{HL}_1$</th>
<th>$\text{HL}_2$</th>
<th>$\text{HL}_3$</th>
<th>$\text{HL}_4$</th>
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<td>C(15)-S(1)/C(15)-O(2)</td>
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<td>1.6770(9)</td>
<td>1.6705(6)</td>
<td>1.2353(15)</td>
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<tr>
<td>C(1)-O(1)</td>
<td>1.2397(9)</td>
<td>1.2380(10)</td>
<td>1.2367(7)</td>
<td>1.2331(15)</td>
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<tr>
<td>C(14)-N(1)</td>
<td>1.3145(10)</td>
<td>1.3089(11)</td>
<td>1.3085(7)</td>
<td>1.3073(15)</td>
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<td>N(1)-N(2)</td>
<td>1.3411(9)</td>
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<tr>
<td>C(15)-N(2)</td>
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<td>1.3212(12)</td>
<td>1.3411(8)</td>
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<td>C(1)-C(14)</td>
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<td>1.4812(18)</td>
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<td>N(3)-C(15)-S(1)/N(3)-C(15)-O(2)</td>
<td>125.33(6)</td>
<td>125.30(7)</td>
<td>127.73(4)</td>
<td>125.43(11)</td>
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<tr>
<td>N(2)-C(15)-S(1)/N(2)-C(15)-O(2)</td>
<td>117.15(6)</td>
<td>118.10(7)</td>
<td>117.61(4)</td>
<td>117.31(11)</td>
</tr>
</tbody>
</table>

Infrared spectra

The ligands behave as monoanionic tridentate moiety forming two five-membered chelate rings around the central metal through a donor atom set comprising of the quinone carbonyl oxygen, imine nitrogen and the thiolate sulfur/enolate oxygen as revealed from the corresponding shifts in IR frequencies of the respective vibrations.\textsuperscript{27} The bands assigned to azomethine $\nu$(C=N) and quinone carbonyl $\nu$(C=O) vibrations appeared at 1593-1598 and 1630-1634 cm\textsuperscript{-1} respectively in the spectra of free ligands were shifted to lower wave numbers while the bands at 3111-3148 and 807-843/1674 cm\textsuperscript{-1} ascribed to the $\nu$(N–H) and $\nu$(C=S)/$\nu$(C=O) stretchs respectively disappeared on metal complexation confirming the thio/keto enolization nature of the ligands and subsequent coordination through the deprotonated sulfur/oxygen.\textsuperscript{28-30} This fact was further confirmed by the appearance of two new bands in the range 1581-1604 and 737-762/1188 cm\textsuperscript{-1}.
corresponds to $\nu(C=\!N\!\!-\!N=\!C)$ and $\nu(C-S)$/$\nu(C-O)$ stretching vibrations respectively.\textsuperscript{31} In all the ruthenium complexes, the band due to terminal $\nu(C=O)$ group appeared at 1939-1971 cm\textsuperscript{-1}. In addition to the above vibrations, the characteristic bands due to triphenylphosphine and triphenylarsine were also present in the expected region.\textsuperscript{32}

**Electronic spectra**

All the ruthenium complexes showed two to four intense absorptions in the ultraviolet and visible region 209-510 nm. The less intense absorption in the visible region at 368-510 nm was probably due to metal-to ligand charge transfer transition.\textsuperscript{33} In addition, the high intensity bands around 300 nm region were characterized of ligand-centered transitions, which were likely due to the chelating tridentate ligands. The pattern of the electronic spectra of all the complexes indicated the presence of an octahedral environment around ruthenium(II) ion, similar to that of other ruthenium complexes.\textsuperscript{34}

**NMR spectra**

The singlet at 13.82-14.81 ppm was assigned to hydrazinic N-H proton indicates that the ligands exist in thionic/ketonic forms. This peak was not found in the spectra of complexes, which was consistent with deprotonation of these ligands upon metal complexation. The terminal NH\textsubscript{2} protons in ligands $\text{HL\textsubscript{1}}$ and $\text{HL\textsubscript{4}}$ were magnetically non-equivalent, have shown two singlets in the region 8.35-9.36 ppm. These protons became equivalent upon formation of ruthenium complexes and observed as a singlet in the region 8.38-9.57 ppm.\textsuperscript{35} The ligands $\text{HL\textsubscript{2}}$, $\text{HL\textsubscript{3}}$ and their corresponding complexes show singlet in the region 8.35-11.71 ppm was assigned to NH methyl and NH phenyl protons. In the spectra of all the complexes, the multiplet observed at around 7.01-8.86 ppm was assigned to aromatic protons of the ligands, phenyl group of triphenylphosphine/triphenylarsine. Further, the methyl protons appeared in the region of 2.92-2.98 ppm (Figure 2).
Figure 2. (i) $^1$H-NMR spectra
Figure 2. (ii) $^1$H-NMR spectra
Figure 2. (iii) Representative $^1$H-NMR spectra
The $^{13}$C NMR spectra (Figure 3) of the complexes showed a peak at 203.42-204.98 ppm region was due to terminal C≡O carbon. The presence of a peak at
179.90-183.09 ppm region was assigned to quinone carbonyl (C=O) carbon. The azomethine (C=N) carbon exhibited a peak in the region of 160.59-163.40 ppm. A sharp singlet around 29.82-30.68 ppm was assigned to methyl carbon. In addition, two peaks in the region 168.12-168.80 and 169.26-172.84 ppm were assigned to semicarbazone C-O and thiosemicarbazone C-S respectively. The aromatic carbons appeared in the region of 120.10-139.88 ppm.

**Figure 3.** Representative $^{13}$C NMR spectra
31P NMR spectra of 1, 2, 3 and 4 were recorded to confirm the presence of triphenylphosphine group in the complexes (Figure 4). A sharp singlet was observed around 29.49–31.24 ppm due to the presence of triphenylphosphine ligand in the complexes.

![Figure 4](image)

**Figure 4.** Representative 31P NMR spectra

**ESI-Mass spectra**

ESI-Mass spectra were also employed to check the composition of the complexes (Figure 5). The molecular ion peak observed for the complexes (1-8) at m/z = 707.0, 721.89, 783.6, 691.1, 751.1, 765.5, 827.3, 735.03 respectively confirmed the stoichiometry of the complexes.
Figure 5. (i) Representative ESI-Mass spectra
DNA binding studies

Electronic absorption spectroscopy

Electronic absorption spectroscopy was performed to examine the binding mode of DNA with metal complexes. The change observed in the absorption
spectra of the complexes in the presence of increasing concentration of DNA is used for determining the interaction of complexes with duplex DNA. The absorption spectra of complexes in the absence and presence of CT-DNA (at a constant concentration of complexes, [Ru] = 10 μM) were given in figure 6. The UV-Vis absorption spectra of complexes showed two well resolved bands at 250 nm and about 500 nm. The sharp and intense peak in the 250 nm region was due to the intra ligand n-π* transition. More broad and less intense band appeared in the range of 500 nm attributed to the metal to ligand charge transfer (MLCT) transition. Upon titration with CT-DNA, the complex 3 exhibited dramatic changes in their spectroscopic properties. In particular, the characteristic visible absorption band underwent hypochromism (19%) with slight red shift at a ratio of [Ru]/[DNA] of 0.17. The addition of further amounts of DNA to the complex 3 did not cause any more absorption changes, due to the binding saturation of complex 3 with DNA. Generally, extensive hypochromism and stronger red shift was considered as a concrete indication of intercalation of the small molecules in DNA.\textsuperscript{36,37} Hence, the observed small hypochromism and slight red shift of complex 3, suggest that the existed interaction is not intercalation binding mode and it should be groove binding.\textsuperscript{38,39} One distinct isobestic point at 300 nm appeared for complex 3 suggest that atleast two spectroscopically distinct chromophores (free and bound) were present in the solution and also evidences for occurrence of a single binding mode of complex 3 with DNA. The magnitude of the hypochromism and red shift depend on the strength of the interaction between the DNA and the complex. The less pronounced hypochromism with slight red shift suggested that there was a meager association of complex 3 with DNA and also it was feasible that 3 may bind to the DNA double helix by one of the grooves. The mixed spectral changes were observed in the MLCT transition band of complex 2 upon addition of CT-DNA implies that relatively weak association of complex 2 to the helix. For complexes 1 and 4 the band centred at 465 nm shows an initial hyperchromism with mixed spectral changes in the presence of increasing amounts of CT-DNA. The interaction may be primarily through external contact (electrostatic binding)\textsuperscript{40,41} and on further increment of DNA, involving different binding modes between 1 and 4 with DNA. The hyperchromism obtained from 1 and 4 can probably related to the role played
by the terminal amine group of thiosemicarbazone moiety of the ligand in the binding to DNA.\[^{42,43}\]

**Figure 6.** UV spectra of ruthenium complexes in the absence (---) and in the presence of CT-DNA in increasing amounts, [Complex] = 10 \(\mu\)M, [DNA] = 0-60 \(\mu\)M

No attempt was made to calculate the binding constant for the complexes 1, 2 and 4 because of the mixed spectral behavior. The binding constant of complex 3 with CT-DNA was obtained by monitoring the changes in absorbance of the MLCT (503 nm) with increasing concentration of DNA (Figure 7). The intrinsic binding constant \(K_b\) of the complex with CT-DNA was determined according to following equation,\[^{44}\] through plots of [DNA]/(\(\varepsilon_a - \varepsilon_I\)) versus [DNA]:

\[
[\text{DNA}]/(\varepsilon_a - \varepsilon_I) = [\text{DNA}]/(\varepsilon_b - \varepsilon_I) + 1/(K_b (\varepsilon_b - \varepsilon_I))
\]

Where [DNA] is the concentration of DNA in base pairs, \(\varepsilon_a, \varepsilon_f,\) and \(\varepsilon_b\) are the apparent-, free- and bound-metal-complex extinction coefficients respectively. \(K_b\) is the equilibrium binding constant of complex binding to DNA. The apparent binding constant value (\(K_b\)) of complex 3 at room temperature was calculated to be
2.27 \times 10^3 \text{ M}^{-1}. The observed \( K_b \) value of complex 3 to CT-DNA is obviously smaller than that of the classical intercalators, \([\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}\), \text{dppz = dipyrido-[3,2-d: 2',3'-f]-phenazine,} \( K_b > 10^6 \text{ M}^{-1}\), \([\text{Ru(bpy)}_2(\text{appo})]^{2+} (1 \times 10^6 \text{ M}^{-1})^{45,46}\) and comparable with the values reported for groove binding metal complexes containing thiosemicarbazone ligands\(^{47}\) and other ligands.\(^{48}\)

![Graph](image)

**Figure 7.** Plot of \([\text{DNA}]/(\square_{\text{DNA}} - \square_{\text{DNA}}) \) vs \([\text{DNA}]\) for the titration of DNA with the complex 3 and solid line is linear fitting of the data

**Competitive studies with EB**

The binding of ruthenium(II) complexes with CT-DNA cannot be directly presented in the emission spectra, since the ruthenium(II) complexes were non-emissive both in the presence and absence of CT-DNA. It has been studied by competitive Ethidium bromide (EB) binding experiments. The binding influence of ruthenium(II) complexes of phenanthrenequinone thiosemicarbazones on the fluorescence intensity of EB-DNA complex has been indirectly studied. The emission spectra of the EB-DNA system in the presence of increasing amount of complexes 1 and 3 were shown in figure 8. The fluorescence intensity of EB around 600 nm showed a remarkable decreasing trend with increasing concentration of complex 3, indicated that some EB molecules were released from EB-DNA after an exchange with complex 3 which results in the fluorescence quenching of EB. This decrease of fluorescence (20%) indicates the competition with EB in binding mode to DNA. Interestingly, the extent of quenching was relatively smaller than the well
known intercalater.\textsuperscript{49} A similar fluorescence quenching effect of EB bonded to DNA has been observed for the addition of several groove-binding compounds such as netropsin and distamycin A.\textsuperscript{50} Groove binding mode of the complexes at some circumstances may lead to quenching of EB emission.\textsuperscript{51} This supports that the complex 3 bound to DNA by groove binding mode. This fluorescence quenching process may be mainly controlled by a static quenching mechanism rather than dynamic, because dynamic quenching results from collisions between excited state and quencher whereas static quenching by association, only luminophore-quencher associations result in reduction in emission. The affinities of 1, 2 and 4 for DNA were low as evident from the slight increases of fluorescence intensity of EB-DNA system. Since intercalated EB is the only fluorescent species, the fluorescence increase indicates that the complexes (1, 2 and 4) can make a contraction in the helix axis of DNA by electrostatic binding to the phosphate group of the DNA backbone. This kind of spectral feature has been also emerged for some copper(II), magnesium(II) and iron(II) complexes\textsuperscript{52-54} showing electrostatic interaction with DNA and it causes a contraction of DNA which drives a few EB molecules out of DNA.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure8.png}
\caption{Emission spectra of EB bound to DNA in the absence (…..) and in the presence of complexes 1, 3; [EB] = 20 \textmu M, [DNA] = 26 \textmu M}  
\end{figure}
In order to understand quantitatively, the magnitude of the binding strength of complex 3 with CT-DNA, the quenching behavior was then analyzed by Stern-Volmer equation. The quenching plots illustrates the quenching of EB bound to DNA by the complex which was in good agreement with the linear Stern-Volmer equation, \( F_0/F = 1 + K_{sv}r \), where \( F_0 \) and \( F \) are fluorescence intensities in the absence and presence of the complex respectively and \( r \) is the concentration ratio of the complex to DNA. \( K_{sv} \) is the linear Stern-Volmer quenching constant. From the plot of \( F_0/F \) versus \([\text{Complex}]/[\text{DNA}]\), \( K_{sv} \) was given by the ratio of slope to the intercept (Figure 9). The calculated \( K_{sv} \) value for the complex 3 was \( 1.88 \pm 0.66 \text{ M}^{-1} \), in fine correlation with the non-intercalaters. The EB displacement result of the complex 3 was accordance with those observed for many mononuclear complexes and lends strong support for their binding with DNA in groove binding mode. The difference in binding mode of these ruthenium(II) complexes can be probably correlated to their substituents on terminal nitrogen atom of the thiosemicarbazone and its capability of co-planarity with phenanthrenequinone ring and thus the complex 3 was contain good DNA binding affinity than other complexes due to the presence of phenyl ring.

![Figure 9. Stern-Volmer quenching plot of EB bound to CT-DNA by the complex 3](image)

75
Viscosity studies on CT-DNA interactions

The nature of the interaction between the ruthenium(II) complexes and DNA was further explicated by viscosity measurements. A classical intercalative mode causes a significant increase in viscosity of DNA solution whereas drug molecules that bind exclusively in the DNA groove (e.g., netropsin and distmycine) under the same conditions typically cause less pronounced (positive or negative) change in DNA viscosity.\(^55\) As a validation of the above verdict, viscosity measurement of complexes 1-4 were carried out and the effects of complexes on the viscosity of DNA were shown in figure 10.

\[\text{Figure 10. Effect of increasing amount of complexes 1 (▲), 2 (●), 3 (■) and 4 (▼) on the relative viscosities of CT-DNA in 5 mM Tris-HCl buffer (pH, 7).}\]

On increasing the amount of the complex 3, the relative viscosity of DNA randomly changed, which is similar to the behavior of the non-intercalaters.\(^56\) The results suggested that the complex 3 bound through one of the grooves of DNA double helix. It was clear that the groove binding mode of the complex 3 would be expected to aromatic phenyl ring in the amine group of chelating ligand and it was facilitates to approach the DNA freely than other substituents. The complexes (1, 2 and 4) did not change the relative viscosity of DNA in a manner consistent with previously reported electrostatic binding [Ru(bpy)\(3\)]\(^{2+}\) complex.\(^57\) On the basis of
the spectroscopic and viscosity results, it seems that the complexes (1, 2 and 4) can interact with DNA through non-specific electrostatic binding mode.

**Thermal denaturation studies**

The binding of small molecules into the double helix is known to increase the helix melting temperature, at which the double helix is denatured into single-stranded DNA.\(^5\) The extinction coefficient of DNA bases at 260 nm in the double-helical form is much smaller than that of the single stranded form. Hence melting of the helix leads to an increase in the absorption at this wavelength.\(^6\) The melting temperature (Tm) is strongly related to the stability of the double-helical structure. When the complex intercalates into DNA, the stability of double stranded DNA will increase and melting temperature increases sharply. When the complex binds with DNA outside the melting temperature will increase slightly. The melting curve of CT-DNA in the absence and presence of complexes was presented in figure 11. In the absence of metal complexes, the Tm of CT-DNA was determined to be 60 ± 0.16 °C. In the presence of 17μM of complex 3, a concentration sufficient to saturate the DNA lattice, the Tm was 62 ± 0.16 °C. The slight change in Tm of CT-DNA upon binding with complex 3, suggesting that they have no capability to stabilize the DNA duplex because they do not significantly increase Tm.\(^7\) The results suggest that the binding of complex 3 to DNA involves exclusively any one of the grooves of DNA and an intercalative behavior can be excluded. In contrast, no significant changes were observed in the denatured temperature of the CT-DNA upon addition of 1, 2 and 4. It seems that CT-DNA interacts with the complexes (1, 2 and 4) electrostatically by DNA-phosphate backbone. Since intercalative mode of binding is hydrophobically bound between the base pairs of DNA, the non intercalative thiosemicarbazone (groove or electrostatic) ligands bound to outside of DNA through the hydrophilic contacts and confirm outside binding. The structure, size, hydrophobicity and relative disposition of the ligands in the coordination sphere of the central metal atom, gives the opportunity to determine how these factors contribute to the affinity in DNA binding.\(^8\) It has been observed that the substitutions on the terminal N (aminic nitrogen) position of the thiosemicarbazone chain can affect coordination and biological properties of metal complexes.\(^9\)
Moreover, differences of their DNA binding selectivity and affinity could also correlate to the effects of structural alterations on thiosemicarbazone moiety.\textsuperscript{50,51} Obviously, the differences of DNA-binding property between complexes (1, 2, 3 and 4) should also be caused by their different substituent in the terminal part of thiosemicarbazone ligands. This observation may be explained by the fact that in complex 3 the phenyl substitution of the thiosemicarbazone moiety would be considered to be coplanar with the phenanthrenequinone part owing to create intimacy with DNA. On the other hand, rest of complexes (1, 2 and 4) the substitution of the thiosemicarbazone moiety may lie out of the plane of the phenanthrenequinone ring, which may produce unfavorable steric clash and it would restrict the complex to approach DNA. The absorption, EB displacement and viscosity studies concluded that ruthenium(II) complexes can bind to CT-DNA in an non-intercalative mode and that the order of complexes binding to CT-DNA is $3 > 1 \sim 2 \sim 4$.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure11.png}
\caption{Thermal denaturation plots of 100 $\mu$m CT- DNA alone (●) and in the presence of complexes 1 (●), 2 (▼), 3 (■) and 4 (▲) at $R = [\text{Ru}]/[\text{DNA}] = 0.17$}
\end{figure}
DNA cleavage studies by gel electrophoresis

The cleavage reaction on plasmid DNA with complexes 1-4 can be monitored by agarose gel electrophoresis. When circular plasmid DNA is subject to electrophoresis, relatively fast migration will be observed for the intact supercoil form (Form I). If scission occurs on one strand (nicking), the supercoil will relax to generate a slower moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Form I and Form II will be generated. The gel electrophoresis separation of pBR 322 DNA after incubation with the ruthenium(II) complexes and irradiation at 365nm for 30 minutes was shown in figure 12. No obvious DNA cleavage was observed for controls in which the complex was absent. With increasing concentration of the ruthenium(II) complexes 1-4, the amount of Form-I of pBR 322 DNA diminishes gradually whereas Form-II increases slowly. These results indicated that the scission occurs on one strand (nicked).

[Image of gel electrophoresis diagram]

Figure 12. Agarose gel electrophoresis diagram showing the cleavage of pBR 322 DNA by ruthenium(II) complex. Lane C, Control DNA (without complex). Lane 1, 2 by complex 1; Lane 3, 4 by complex 2; Lane 5, 6 by complex 3; Lane 7, 8 by complex 4 at 10 & 20 µg/mL respectively

Antioxidative activity

Experiments were carried out in order to explore the free radical scavenging ability of the new complexes along with standard antioxidant Vitamin C with the hope of developing potential antioxidants and therapeutic reagents for respiratory diseases such as asthma, emphysema and asbestosis. The 50% inhibitory concentration (IC₅₀) value of complexes varies from 15.9 to 30.3 µM and 14.7 to
22.2 µM against DPPH radical and OH radical respectively, whereas standard antioxidant Vitamin C showed their IC$_{50}$ value 148.4 and 191.1 µM respectively (Table 3). From the above results, it can be concluded that a much less scavenging activity was exhibited by Vitamin C when compared to that of ruthenium complexes. The complex 3 showed better activity when compared to the other complexes, which may be due to the electron withdrawing effect of phenyl group on terminal nitrogen atom of the ligand. Further, the results obtained in the two different radical assays confirmed that the ruthenium(II) complexes were more effective to arrest the formation of OH radical than the DPPH radical. The lower IC$_{50}$ values observed in antioxidant assays did demonstrate that these complexes have a strong potential to be applied as scavengers to eliminate the radicals.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$(µM)</th>
<th>(\text{IC}_{50}^{\text{DPPH}})</th>
<th>(\text{IC}_{50}^{\text{OH}^-})</th>
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<tr>
<td>1</td>
<td>19.1 ± 0.2</td>
<td>17.4 ± 0.2</td>
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</tr>
<tr>
<td>2</td>
<td>30.3 ± 0.4</td>
<td>22.2 ± 0.9</td>
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</tr>
<tr>
<td>3</td>
<td>15.9 ± 0.3</td>
<td>14.7 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>23.7 ± 0.4</td>
<td>21.2 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>148.4 ± 0.9</td>
<td>191.1 ± 0.6</td>
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</table>

**Cytotoxic activity evaluation by MTT assay**

The cytotoxicity assays of ruthenium(II) complexes and cisplatin against MCF-7(breast), MDA-MB-468(breast) and A431 (skin) cancer cell lines were shown in table 4. The results indicated that the complexes have potent cytotoxic effects against the three cancer cell lines and the inhibitory rate of the complex 3 was much higher than that of the complexes 1, 2 and 4. The cytotoxicity of the complexes was found to be concentration dependent and the cell viability decreased with increasing concentration of ruthenium(II) complexes. Furthermore, it was noteworthy that the measured cytotoxic activity was less than that of cisplatin, but
the inhibition of cell proliferation produced by the ruthenium(II) complexes on the same batch of cell lines under identical experimental conditions was still rather active with IC₅₀ values falling in the 4.5-31.71 µM range. In addition, the IC₅₀ value of complexes for NIH 3T3 mouse embryonic fibroblasts (normal cells) was above 280 µM, which confirmed that the complexes act very specifically on cancer cells.

**Table 2.** In vitro anticancer activities of complexes and cisplatin against various cancer cell line

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCF-7</td>
</tr>
<tr>
<td>1</td>
<td>9.3 ± 0.08</td>
</tr>
<tr>
<td>2</td>
<td>21.98 ± 0.12</td>
</tr>
<tr>
<td>3</td>
<td>4.5 ± 0.07</td>
</tr>
<tr>
<td>4</td>
<td>15.91 ± 0.09</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>3.19 ± 0.03</td>
</tr>
</tbody>
</table>

**Conclusion**

The ruthenium(II) complexes of the type [RuCl(CO)(EPh₃)(L)] (E = P or As; L = monobasic tridentate ONS/ONO ligand) have been synthesized and characterized by analytical and spectroscopic methods (FT-IR, electronic, ¹H, ¹³C, ³¹P NMR and ESI-Mass). The ligands were coordinated to ruthenium through quinone oxygen, imine nitrogen and thiolato sulfur/enolate oxygen. Furthermore, they were characterized with regard to drug-like properties such as DNA binding, DNA cleavage, radical scavenging ability and tumor-inhibiting potential in various cancer cell line. The DNA-binding properties of the complexes 1, 2, 3 and 4 were comprehensively studied by different methods, including electronic absorption spectroscopy, EB displacement, viscosity and thermal denaturation experiments. The experimental results showed that the complexes 1, 2 and 4 could bind to CT-DNA, probably to the phosphate groups by electrostatic interaction. However, the complex 3 with the phenyl substituted phenanthrenequinone thiosemicarbazone showed interaction with the DNA by groove binding. The trend in the DNA-
binding affinities of this series of complexes can be reasonably explained by the presence of different substitutions on the terminal part of thiosemicarbazone moiety. The complexes also efficiently cleaved the pBR 322 DNA. The results on antioxidant activity indicated that the complex 3 displayed very high scavenging activity against DPPH radical and OH radical. In addition, cytotoxicity evaluation showed that the complex 3 displayed higher cytotoxic activity than other complexes against human breast carcinoma cell line (MDA-MB-468, MCF-7) and human skin carcinoma cell line (A431). These may be due to the electron withdrawing nature of phenyl group on terminal nitrogen atom of the ligand.
References