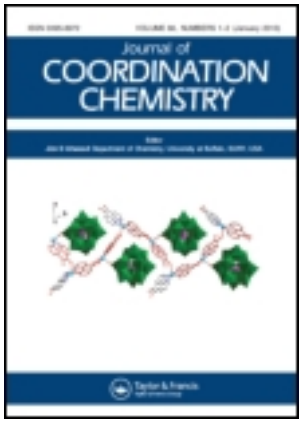

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Synthesis, spectral characterization, antioxidant, anticancer *in vitro*, and DNA cleavage studies of a series of ruthenium(II) complexes bearing Schiff base ligands

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Ruthenium(II) complexes with 2-acetylpyridine-thiosemicarbazones (L^1–L^4) were synthesized and characterized by analytical and spectral (FT-IR, UV–vis, NMR [^{1}H, ^{13}C and ^{31}P], and ESI-Mass) methods. Systematic biological investigations, free radical scavenging, anticancer activities, and DNA cleavage studies, were carried out for the complexes. Antioxidant studies showed that the complexes have significant antioxidant activity against DPPH, hydroxyl, nitric oxide radicals and hydrogen peroxide assay. The *in vitro* cytotoxicity of complexes against breast cancer (MCF-7) cell line was assayed showing high cytotoxicity with low IC_{50} values indicating their efficiency in destroying the cancer cells even at very low concentrations. The DNA cleavage studies showed that the complexes efficiently cleaved DNA.

Keywords: Ruthenium(II) complexes; Spectral studies; Antioxidant; Anticancer activity; DNA cleavage

1. Introduction

Biological activity of metal complexes depends upon the nature of metal ion, oxidation state, the types, and number of bound ligands and isomers present [1–3]. An understanding of how these factors affect biological activity should enable the design of metal complexes with specific medicinal properties. The wide spectrum of biological activity of platinum complexes [4–6] and the clinical success of cisplatin as anticancer drugs provide a good illustration of this point. Although 70% of all cancer patients receive cisplatin during cancer treatment, chemotherapy with cisplatin and its analogs still has several drawbacks, such as toxic side effects and lack of activity (drug resistance) against several types of cancer [7]. This has resulted in search among inorganic chemists in synthesizing new metal complexes for better anticancer activity with no side effects. Ruthenium, a transition metal of the platinum group, has emerged as an attractive alternative due to several favorable properties suited to rational anticancer drug design and biological applications. Biologically compatible ligand-exchange kinetics of ruthenium(II) complexes similar to those of

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platinum complexes, a higher coordination number that could potentially be used to fine-tune the properties of the complexes, and lower toxicity (than their platinum counterparts) towards normal cells by mimicking iron in binding to many biological molecules are some of the advantages of using ruthenium complexes over platinum complexes [8, 9]. A number of ruthenium complexes were recently shown to possess encouraging cytotoxic and antitumor properties in preclinical models [10, 11] and are now under investigation. Antioxidants have been extensively studied for their capacity to protect organisms and cells from damage induced by oxidative stress, and many new compounds have been synthesized or obtained from natural sources that could provide active components to prevent or reduce the impact of oxidative stress on cells [12]. Hence, the development of new synthetic complexes with good antioxidant properties has gained importance.

Studies on the interaction of metal complexes with DNA reveal useful information for the rational drug design and development of sensitive chemical probes for DNA since they are known to act on DNA by inhibiting its placation and transcription [13]. Though various transition metal complexes derived from Schiff bases [14] have been reported as good candidates, recently the DNA cleavage properties of ruthenium complexes have been actively investigated extensively [15]. The chemistry of ruthenium is receiving a lot of attention, primarily because of the fascinating electron transfer and energy transfer properties displayed by complexes of this metal [16]. Ruthenium offers a wide range of oxidation states and the reactivity of the ruthenium complexes depend on the stability and interconvertibility of these oxidation states, which in turn depends on the nature of the ligands bound to the metal. Complexation of ruthenium by ligands of different types has thus been of particular interest.

Thiosemicarbazones have emerged as an important class of sulfur donor ligands for transition metal ions [17–19] because of their mixed hard–soft donor character and versatile coordination behavior [20]. Biological activities of thiosemicarbazones are due to their ability to form chelates with heavy metals [21, 22] and they usually form chelates with transition metal ions by bonding through sulfur and azomethine nitrogen [23]. Biological activities of the metal complexes differ from those of either the ligand or the metal ion itself, and increased and or decreased biological activities are reported for several transition metal complexes [24, 25]. Various studies have also shown that the azomethine group having a lone pair of electrons in either p or sp^2 hybridized orbital on nitrogen has considerable biological and catalytic importance [26, 27]. Attachment of the thiosemicarbazide to the pyridine ring in 2nd position has more ability to coordinate with metal ions compared with 3rd or 4th position [28]. Thus synthesis of new ruthenium(II) complexes containing 2-acetylpyridine thiosemicarbazone ligands has gained importance. Hence, an attempt was made to synthesize a series of new class ruthenium(II) 2-acetylpyridine thiosemicarbazone complexes and to study their biological properties.

2. Experimental

2.1. *Materials and reagents*

All reagents were chemically pure and AR grade. The solvents were purified and dried according to standard procedures. $RuCl_3 \cdot H_2O$ was purchased from Loba Chemie Pvt Ltd. The starting complexes $[RuHCl(CO)(PPh_3)_3]$ [29], $[RuHCl(CO)(Py)(PPh_3)_2]$ [30] and

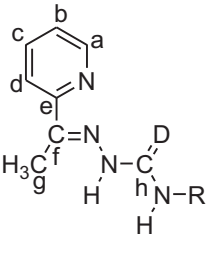
[RuHCl(CO)($AsPh_3$)$_3$] [31], and 2-acetylpyridine thiosemicarbazone/semicarbazone [32, 33] were prepared according to literature reports. The structure of the ligands are given in figure 1.

2.2. *Physical measurements*

Melting points were recorded on a Technico micro heating table and are uncorrected. Microanalyses of carbon, hydrogen and nitrogen were carried out using a Vario EL III Elemental analyzer at SAIF-Cochin India. IR spectra of the ligands and complexes were recorded as KBr pellets on a Nicolet Avatar model IR spectrophotometer from 4000 to 400 cm^{-1}. Electronic spectra of the ligands and complexes have been recorded in methanol using a Shimadzu UV–1650 PC spectrophotometer from 800 to 200 nm. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded with a Jeol GSX–400 nuclear magnetic resonance spectrometer using DMSO-d_6 as solvent. ESI-MS spectra were recorded using a LC-MS Q-ToF Micro analyzer (Shimadzu) in the SAIF, Panjab University, Chandigarh, India. Anticancer activities of the complexes were carried out at KMCH, Coimbatore, India. DNA cleavage studies were carried out at Biogenics, Hubli.

2.3. *Synthesis of ruthenium(II) Schiff base complexes [RuCl(CO)(B)L] (B = PPh_3, $AsPh_3$ or Py); L-Schiff base ligands*

The new metal complexes were prepared according to the following general procedure. To a solution of 0.1 mM [RuHCl(CO)(EPh_3)$_2$(B)] (E = P or As; B = PPh_3, $AsPh_3$ or Py) in benzene was added 0.1 mM Schiff base (mole ratio of ruthenium starting complex and ligand is 1:1, respectively) and the mixture was refluxed for 5 h while monitoring by TLC. The reaction mixture was reduced to 2–3 mL and the product was separated by the addition



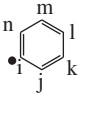
Ligand	D	R
L^1	S	H
L^2	S	$^{i}CH3$
L^3	S	
L^4	O	H

Figure 1. Structure of Schiff bases.

of small amount of petroleum ether at room temperature. The resulting complexes were recrystallized from CH_2Cl_2/petroleum ether and dried under vacuum. The overall yields obtained for the complexes were 70–82%.

3. Biological studies

3.1. *Antioxidant assays*

The ability of ruthenium complexes to act as hydrogen donors or free radical scavengers was explored by conducting a series of *in vitro* antioxidant assays involving DPPH radical, hydroxyl radical, nitric oxide radical, hydrogen peroxide assay and comparing the results with standard antioxidants, including the natural antioxidant vitamin C and the synthetic antioxidant BHT.

3.1.1. DPPH$^{\bullet}$ scavenging assay. The DPPH radical scavenging activity of the compounds was measured according to the method of Blois [34]. DPPH radical is a stable free radical. Because of the odd electron, DPPH shows a strong absorption at 517 nm in the visible spectrum. As this electron becomes paired in the presence of a free radical scavenger, the absorption vanishes and the resulting decolorization is stoichiometric with respect to the number of electrons taken up. Various concentrations of the experimental complexes were taken and the volume was adjusted to 100 mL with methanol. About 5 mL of a 0.1 mM methanolic solution of DPPH was added to the aliquots of samples and standards (BHT and vitamin C) and shaken vigorously. A negative control was prepared by adding 100 mL of methanol in 5 mL of 0.1 mM methanolic solution of DPPH. The tubes were allowed to stand for 20 min at 27 °C. The absorbance of the sample was measured at 517 nm against the blank (methanol).

3.1.2. OH$^{\bullet}$ scavenging assay. Hydroxyl radical scavenging activities of the complexes have been investigated using the Nash method [35]. *In vitro* hydroxyl radicals were generated by an Fe^{3+}/ascorbic acid system. Detection of hydroxyl radicals was carried out by measuring the amount of formaldehyde formed from oxidation with DMSO. The formaldehyde produced was detected spectrophotometrically at 412 nm. A mixture of 1.0 mL of iron–EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1.0 mL of DMSO (0.85% DMSO (v/v) in 0.1 M phosphate buffer, pH 7.4) were sequentially added in the test tubes. The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22%) and was incubated at 80–90 °C for 15 min in a water bath. After incubation, the reaction was terminated by addition of 1.0 mL of ice-cold trichloroacetic acid (17.5% w/v). Subsequently, 3.0 mL of Nash reagent was added to each tube and left at room temperature for 15 min. The intensity of color formed was measured spectrophotometrically at 412 nm against reagent blank.

3.1.3. NO$^{\bullet}$ scavenging assay. The assay of nitric oxide scavenging activity is based on a method [36] in which sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions.

These can be estimated using the Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) was mixed with a fixed concentration of the complex and standards and incubated at room temperature for 150 min. After the incubation period, 0.5 mL of Griess reagent containing 1% sulfanilamide, 2% H_3PO_4 and 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride was added. The absorbance of the chromophore formed was measured at 546 nm.

3.1.4. H_2O_2 scavenging assay. The ability of the complexes to scavenge hydrogen peroxide was determined using the method of Ruch *et al.* [37]. A solution of hydrogen peroxide (2.0 mM) was prepared in phosphate buffer (0.2 M, pH 7.4) and its concentration was determined spectrophotometrically from absorption at 230 nm with molar absorptivity 81 M^{-1} cm^{-1}. The complexes (100 μg mL^{-1}), BHT, and vitamin C (100 μg mL^{-1}) were added to 3.4 mL of phosphate buffer together with hydrogen peroxide solution (0.6 mL). An identical reaction mixture without the sample was taken as negative control. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against the blank (phosphate buffer).

For the four assays described above, all the tests were run in triplicate and the percentage of scavenging activity was calculated using the following formula: percentage of scavenging activity = $[A_0 - A_c/A_0] \times 100$ (A_0 and A_c are the absorbance in the absence and presence of the compound tested).

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

The human breast cancer cell line (MCF-7) was obtained from National Center for Cell Science (NCCS), Pune, and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). Cells were maintained at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1×10^5 cells/mL. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat DMSO to prepare the stock (200 mM) and stored frozen prior to use. At the time of drug addition, the frozen concentrate was thawed and an aliquot was diluted to twice the desired final maximum test concentration with serum free medium. Additional three, 10-fold serial dilutions were made to provide a total of four drug concentrations. Aliquots of 100 μL of these different drug dilutions were added to the appropriate wells already containing 100 μL of medium formed the required final drug concentrations. Following the drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity.

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15 μL of MTT (5 mg/mL) in PBS was

added to each well and incubated at 37 °C for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were dissolved in 100 µL of DMSO and then the absorbance at 570 nm measured using a micro plate reader [38, 39]. Experiments were performed in triplicate and the medium without the compounds served as control. The % cell inhibition was determined using the following formula:

$$\%\ \text{Cell inhibition} = \frac{100 - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

Nonlinear regression graph was plotted between % cell inhibition and Log_{10} concentration and IC_{50} was determined using GraphPad Prism software.

3.3. *DNA cleavage experiments*

DNA cleavage experiments were carried out according to reported procedure [40]. For the gel electrophoresis experiment, CT-DNA was treated with the ruthenium(II) complexes in TEA buffer (10 mM Tris acetate, 10 mM EDTA, pH 8.0) and the solution was then incubated at 37 °C for 2 h. DNA sample (20 µL mixed with bromophenol blue dye, 1:1 ratio) was carefully loaded into the wells, along with standard DNA marker. The samples were analyzed by electrophoresis for 30 min at 50 V on a 0.8% agarose gel in TEA (4.84 g Tris-acetate, 0.5 M EDTA/1 L, and pH 8.0). The gel was stained with 10 µg/mL ethidium bromide and the bands were observed under illuminator.

4. Results and discussion

The following ligands are used for this study and the synthetic route for the new complexes are given in scheme. The ruthenium(II) Schiff base complexes, [RuCl(CO)(B)L] (B = PPh_3, $AsPh_3$ or Py; L = Schiff base ligands), were synthesized in quantitative yield from reaction of [RuHCl(CO)$(EPh_3)_2$(B)] (E = P or As; B = PPh_3, $AsPh_3$ or Py) with Schiff base ligands in dry benzene in 1:1 M ratio (figure 2). In these reactions the Schiff base is a mononegative tridentate ligand replacing two molecules of triphenylphosphine or triphenylarsine and one hydride from the starting complex.

All the complexes were isolated in high yields and are stable both in solid state and in solution. The analytical data (table 1) are in agreement with the formulas proposed. ESI-Mass spectra of [RuCl(CO)$(AsPh_3)L^1$], [RuCl(CO)$(PPh_3)L^1$], and [RuCl(CO)$(AsPh_3)L^2$] confirmed the molecular weights of their proposed structures with m/e values 663.1 (figure S17), 621.2 (figure S18), and 677.0 (figure S19), respectively. The complexes were obtained as powders. All the ligands and complexes are stable at room temperature, non-hygroscopic and highly soluble in common solvents such as chloroform, dichloromethane, methanol, and DMSO.

4.1. *Infrared spectroscopic analysis*

IR spectral bands are useful for determining coordination of the ligands to the metal ion in new complexes. In IR spectra of the ligands, bands due to $\upsilon_{C=N}$ of azomethine and of pyridine were at 1614–1578 and 1600–1523 cm^{-1}, respectively. In spectra of the new

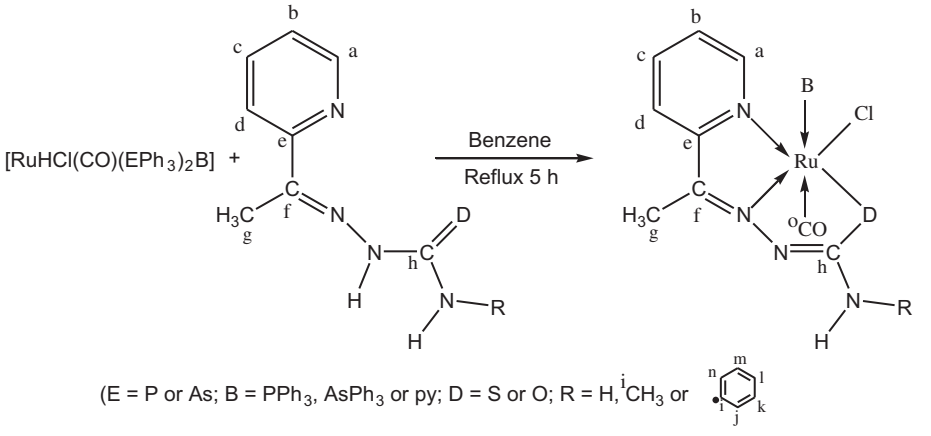


Figure 2. General scheme for the synthesis of new ruthenium(II) complexes.

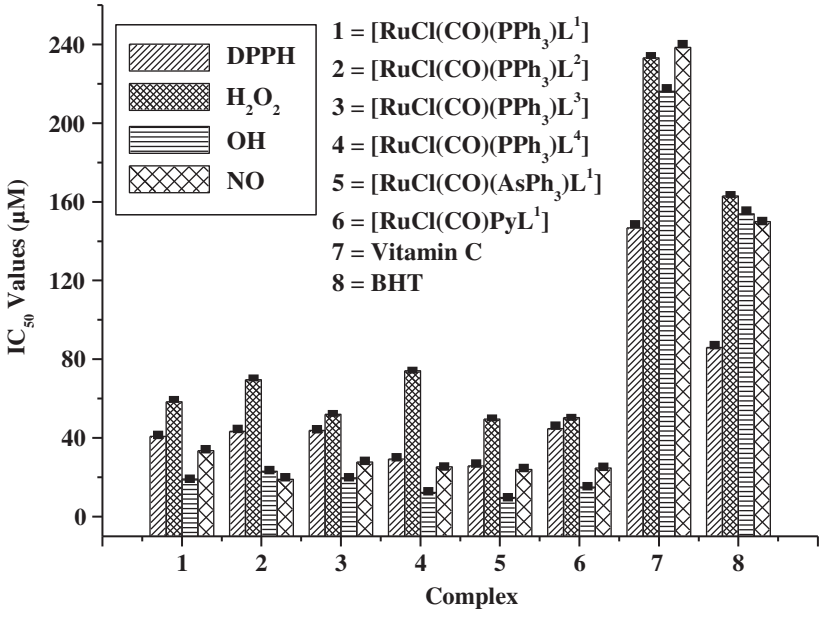


Figure 3. Scavenging effect of ruthenium(II) complexes on various radicals compared with standard vitamin C and BHT.

complexes, these bands appeared at 1608–1527 and 1580–1510 cm^{-1} indicating coordination of azomethine and pyridine. A band at 836–801 cm^{-1} due to $\upsilon_{C=S}$ in spectra of the ligands shifted to 747–741 cm^{-1} in spectra of the complexes, indicating coordination of sulfur after thioenolization of the –NH–C=S followed by deprotonation prior to coordination of sulfur [41, 42]. In IR spectra of the semicarbazone L^4, the band due to $\upsilon_{C=O}$ at 1658 cm^{-1} disappeared on complexation and a new band appeared at 1326–1323 cm^{-1}, indicating coordination through O after keto enolization followed by deprotonation [43].

Table 1. Analytical data of free ligands and ruthenium(II) complexes.

Compound	M. Pt (°C)	Elemental analyzes calculated (found) (%)			
		C	H	N	S
L^1	181	49.46(49.98)	5.19(5.24)	28.84(28.61)	16.51(16.42)
L^2	172	51.90(51.42)	5.81(5.50)	26.90(26.61)	15.39(15.77)
L^3	186	62.20(62.76)	5.22(5.46)	20.72(20.54)	11.86(11.73)
L^4	211	53.92(54.14)	5.66(5.58)	31.44(31.71)	–
$[RuCl(CO)(PPh_3)L^1]$	192	52.30(52.84)	3.90(3.62)	9.04(8.93)	5.17(5.33)
$[RuCl(CO)(PPh_3)L^2]$	165	53.04(53.48)	4.13(4.33)	8.84(8.64)	5.06(5.58)
$[RuCl(CO)(PPh_3)L^3]$	164	56.93(56.59)	4.05(4.21)	8.05(8.41)	4.61(4.92)
$[RuCl(CO)(PPh_3)L^4]$	225	53.69(53.93)	4.01(3.89)	9.28(9.48)	–
$[RuCl(CO)(AsPh_3)L^1]$	190	48.84(48.31)	3.64(3.39)	8.44(8.75)	4.83(4.55)
$[RuCl(CO)(AsPh_3)L^2]$	179	49.60(49.86)	3.87(3.99)	8.26(8.32)	4.73(4.88)
$[RuCl(CO)(AsPh_3)L^3]$	196	53.55(53.97)	3.81(3.60)	7.57(7.77)	4.33(4.18)
$[RuCl(CO)(AsPh_3)L^4]$	175	50.05(50.47)	3.73(3.51)	8.65(8.87)	–
$[RuCl(CO)PyL^1]$	164	36.63(36.93)	7.90(7.73)	15.26(15.53)	6.98(6.67)
$[RuCl(CO)PyL^2]$	168	39.96(39.54)	3.58(3.46)	15.53(15.31)	7.11(7.49)
$[RuCl(CO)PyL^3]$	162	46.83(46.32)	3.54(3.69)	13.65(13.81)	6.25(6.06)
$[RuCl(CO)PyL^4]$	171	39.96(41.31)	3.35(3.64)	16.64(16.85)	–

The strong absorption at 1955–1947 cm^{-1} has been assigned to terminal carbonyl in the new ruthenium complexes [44]. Characteristic bands due to triphenylphosphine and triphenylarsine (1430 and 696 cm^{-1}) were also present in spectra of all complexes [45]. The important IR absorption frequencies of the ligands and their metal complexes along with their assignments are listed in table 2.

4.2. *Electronic spectroscopic analysis*

Electronic spectra of the complexes in methanol showed two to five bands from 408 to 201 nm (table 2). Bands at 399–327 nm have been assigned to charge transfer transitions arising from the metal t_{2g} to the unfilled molecular orbitals derived from the π* level of the ligands [46–49] based on their extinction coefficient values. Bands below 300 nm are

Table 2. IR absorption frequencies (cm^{-1}) and electronic spectral data (nm) of free ligands and ruthenium(II) complexes.

Compound	ν_{NH}	$\nu_{C=N}$	$\nu_{C=N(Py)}$	ν_{CO}	$\nu_{C=O}$	$\nu_{C=S}$	ν_{C-S}	λ_{max}
L^1	3185	1607	1575	–	–	836	–	202,310
L^2	3240	1578	1539	–	–	834	–	203,310
L^3	3241	1581	1523	–	–	801	–	201,226,297,309,341
L^4	3167	1614	1600	–	1658	–	–	203,307
$[RuCl(CO)(PPh_3)L^1]$	–	1596	1568	1955	–	–	746	393,341,204
$[RuCl\ CO)(PPh_3)L^2]$	–	1557	1510	1952	–	–	746	384,342,203
$[RuCl(CO)(PPh_3)L^3]$	–	1528	1497	1951	–	–	741	408,345,259,203
$[RuCl(CO)(PPh_3)L^4]$	–	1608	1581	1950	1323	–	–	395,340,230,204
$[RuCl(CO)(AsPh_3)L^1]$	–	1597	1569	1953	–	–	742	380,327,202
$[RuCl(CO)(AsPh_3)L^2]$	–	1558	1510	1948	–	–	741	380,329,204
$[RuCl(CO)(AsPh_3)L^3]$	–	1527	1496	1955	–	–	747	399,258,204
$[RuCl(CO)(AsPh_3)L^4]$	–	1600	1582	1951	1324	–	–	378,328,202
$[RuCl(CO)PyL^1]$	–	1596	1567	1954	–	–	746	389,342,309,203
$[RuCl(CO)PyL^2]$	–	1556	1515	1947	–	–	745	389,340,309,203
$[RuCl(CO)PyL^3]$	–	1564	1496	1954	–	–	747	408,341,259,203
$[RuCl(CO)PyL^4]$	–	1604	1580	1950	1326	–	–	385,302,203

Table 3. ^{1}H NMR data of Schiff base ligands and their complexes.

Compound	^{1}H NMR
L^1	10.31(S,1H,NH), 8.59(d,1H^{a}Py), 8.38(d,1H^{d},Py), 8.19(S,2H,NH_2), 7.79(t,2H^{b},Py), 7.35 (t,2H^{c},Py), 2.38(S,3H,CH_3)
L^2	10.38(S,1H,NH), 8.65(S,1H,R-NH), 8.58(d,1H^{a},Py), 8.41(d,1H^{d},Py), 7.81(t,2H^{b},Py), 7.35 (t,2H^{c},Py), 2.36(S,3H,CH_3), 3.08(S,3H,CH_3)
L^3	10.65(S,1H,R-NH), 10.25(S,1H,NH), 8.61(d,1H^{a},Py), 8.49(d,1H^{d},Py), 7.85(t,2H^{b},Py), 7.61(d,1H^{j,n},Ph), 7.54(t,2H^{k,m},Ph), 7.35(t,2H^{c},Py), 7.21(t,2H^{l},Ph), 2.4(S,3H,CH_3)
L^4	10.32(S,1H,NH), 8.56(d,1H^{a},Py), 8.43(d,1H^{d},Py), 8.14(S,2H,NH_2), 7.75(t,2H^{b},Py), 7.36 (t,2H^{c},Py), 2.38(S,3H,CH_3)
[RuCl(CO)(PPh_3)L^1]	8.21(S,2H,NH_2), 7.85(d,1H^{a},Py), 7.75(d,1H^{d},Py), 7.39(t,2H^{b},Py), 7.21(t,2H^{c},Py), 7.15–6.90(m,15H,PPh_3), 2.25(S,CH_3)
[RuCl(CO)(PPh_3)L^2]	8.70(S,R-NH), 7.82(d,1H^{a},Py), 7.78(d,1H^{d},Py), 7.61(t,2H^{b},Py), 7.41(t,2H^{c},Py), 7.39–7.12 (m,15H,PPh_3), 2.37(S,CH_3), 2.92(S,CH_3)
[RuCl(CO)(PPh_3)L^3]	9.92(S,R-NH), 8.10–7.85(m,15H,PPh_3), 7.72(d,1H^{a},Py), 7.64(d,1H^{d},Py), 7.45(t,2H^{b},Py), 7.38(t,2H^{c},Py), 7.27(d,1H^{j,n},Ph), 7.15(t,2H^{k,m},Ph), 6.96(t,2H^{l},Ph), 2.32(S,CH_3)
[RuCl(CO)($AsPh_3$)L^2]	8.72(S,R-NH), 7.85(d,1H^{a},Py), 7.79(d,1H^{d},Py), 7.59(t,2H^{b},Py), 7.43(t,2H^{c},Py), 7.35–7.15 (m,15H,PPh_3), 2.36(S,CH_3), 2.92(S,CH_3)
[RuCl(CO)($AsPh_3$)L^3]	9.91(S,R-NH), 8.12–7.86(m,15H,PPh_3), 7.74(d,1H^{a},Py), 7.61(d,1H^{d},Py), 7.46(t,2H^{b},Py), 7.37(t,2H^{c},Py), 7.29(d,1H^{j,n},Ph), 7.13(t,2H^{k,m},Ph), 6.90(t,2H^{l},Ph), 2.34(S,CH_3)
[RuCl(CO)(PPh_3)L^4]	8.19(S,NH_2), 7.89(d,1H^{a},Py), 7.75(d,1H^{d},Py), 7.39(t,2H^{b},Py), 7.21(t,2H^{c},Py), 7.18–6.95 (m,15H,PPh_3), 2.27(S,CH_3)

assigned to intra-ligand charge transfer transitions. Electronic spectra of the complexes are very similar to those observed for other octahedral ruthenium(II) complexes [50].

4.3. *^{1}H NMR spectroscopic analysis*

The ^{1}H NMR spectra of the ligand and the corresponding ruthenium(II) Schiff base complexes were recorded to confirm the presence of coordinated ligand in the complexes. The spectral data and their assignments are given in table 3. Signals due to NH in the free ligands at 10.38–10.25 ppm were absent in the complexes [51] revealing thioenolization of the –NH–C=S group and subsequent deprotonation prior to coordination through sulfur. Signals at 8.61–8.38 and 7.85–7.35 ppm assigned to protons of pyridine shifted slightly to the upfield at 7.85–7.39 ppm in spectra of the complexes, confirming that the third coordination is through pyridine nitrogen. Multiplets at 7.27–6.90 ppm in spectra of the complexes are assigned to aromatic protons. The methyl protons are at 2.2–2.42 ppm.

4.4. *^{13}C NMR spectroscopic analysis*

The ^{13}C NMR spectra of some of the complexes showed (table 4) a peak at 204.11–201.12 ppm due to C≡O. A peak at 181.83–177.45 ppm is assigned to C–S. The azomethine (> C=N) exhibited its peak at 153.64–151.33 ppm and pyridine C=N had its resonance at 160.80–153.48 ppm. The multiplets around 119–142 ppm region are assigned to aromatic carbons. A sharp singlet at 13.71–14.53 ppm is assigned to methyl.

4.5. *^{31}P NMR spectroscopic analysis*

^{31}P NMR spectra of some of the complexes were recorded to confirm the presence of triphenylphosphine groups in the complexes and the spectral data are shown in table 4. A sharp singlet was observed at 37.21–37.01 ppm due to presence of triphenylphosphine in the complexes.

Table 4. ^{13}C NMR and ^{31}P NMR data of ruthenium(II) complexes.

Complex	^{13}C NMR (ppm)	^{31}P NMR (ppm)
[RuCl(CO)$(PPh_3)L^1$]	201.40(C^i,C≡O), 181.83(C^h,C–S), 160.24(C^a,Py), 153.48(C^e,Py), 151.33(C^f,C=N), 138.69(C^b,Py), 134.92(C^d,Py), 133.21(C^c,Py), 129.97–128.73(C,PPh_3), 13.7(C^g,CH_3)	37.21
[RuCl(CO)$(PPh_3)L^2$]	201.91(C^j,C≡O), 181.54(C^h,C–S), 160.80(C^a,Py), 159.13(C^e,Py), 153.46(C^f,C=N), 138.59(C^b,Py), 134.64(C^d,Py), 133.23(C^c,Py), 129.97–128.70(C,PPh_3), 14.04(C^g,CH_3), 13.66(C^i,CH_3)	Not recorded
[RuCl(CO)$(PPh_3)L^3$]	204.11(C^o,C≡O), 178.17(C^h,C–S), 159.93(C^a,Py), 155.53(C^e,Py), 153.64(C^f,C=N), 140.91(C^b,Py), 138.86(C^d,Py), 134.80(C^c,Py), 133.13(C^i,Ph), 129.97–128.74(C,PPh_3), 125.34(C^j,Ph), 125.07(C^n,Ph), 122.05(C^l,Ph), 119.01(C^k,Ph), 119.49(C^m,Ph), 14.15(C^g,CH_3)	37.01
[RuCl(CO)$(AsPh_3)L^3$]	201.12(C^o,C≡O), 177.45(C^h,C–S), 160.22(C^e,Py), 155.10(C^e,Py), 153.68(C^f,C=N), 141.00(C^b,Py), 138.95(C^d,Py), 134.65(C^c,Py), 133.64(C^i,Ph), 130.16–128.52(C,PPh_3), 25.31(C^j,Ph), 125.09(C^n,Ph), 122.04(C^l,Ph), 119.05(C^k,Ph), 119.54(C^m,Ph), 14.51(C^g,CH_3)	Not recorded

4.6. *Antioxidant activity*

Free radicals can induce DNA damage in humans and such damage has been suggested to contribute to aging and various diseases, including cancer and chronic inflammation [52]. Hence, we carried out experiments to explore the free radical scavenging ability of the complexes to develop antioxidants and therapeutic reagents for respiratory diseases, such as asthma, emphysema and asbestosis [53]. The antioxidant potential of ruthenium(II) complexes against DPPH radical, OH radical, H_2O_2 radical, and NO radical assay were investigated with respect to different concentrations of the test compounds varying from 0 to 50 μM and the results are shown in table 5 (figure 3). The 50% inhibitory concentration (IC_{50}) value of complexes varies from 9.78 to 23.00 μM against OH radical. The complexes showed their IC_{50} values against NO, DPPH, and H_2O_2 radicals of 19.00–33.52 μM, 25.74–44.67 μM, and 49.65–74.19 μM, respectively. Among all free radicals, the hydroxyl radical ($OH^{\bullet}$) is by far the most potent, and therefore the most dangerous oxygen metabolite; elimination of this radical is a major aim of antioxidant administration. From the results, the synthesized complexes were more reactive against OH radical. The suppression ratio of all radicals increases with increasing amount of the complex concentrations from 0 to 50 μg. The IC_{50} values of the complexes obtained from different assay experiments revealed that they possess excellent antioxidant activities, better than those of standard antioxidants, including the natural antioxidant vitamin C and the synthetic antioxidant BHT

Table 5. Antioxidant activity of ruthenium(II) complexes, vitamin C and BHT against various radicals.

Compound	IC_{50} (μM)			
	DPPH	H_2O_2	OH	NO
[RuCl(CO)$(PPh_3)L^1$]	40.8 ± 0.8	58 ± 1	19.1 ± 0.1	33.5 ± 0.8
[RuCl(CO)$(PPh_3)L^2$]	43 ± 1	69.6 ± 0.9	23.0 ± 0.7	19 ± 1
[RuCl(CO)$(PPh_3)L^3$]	43.7 ± 0.6	52.0 ± 0.4	19.7 ± 0.3	27.7 ± 0.7
[RuCl(CO)$(PPh_3)L^4$]	29.2 ± 0.9	74.2 ± 0.2	12.3 ± 0.6	25.2 ± 0.2
[RuCl(CO)$(AsPh_3)L^1$]	25 ± 1	49.6 ± 0.6	9.8 ± 0.1	23.9 ± 0.9
[RuCl(CO)PyL^1]	45 ± 1	50.3 ± 0.1	15.0 ± 0.4	24.7 ± 0.5
Vitamin C	147 ± 2	233 ± 1	216 ± 1	239 ± 2
BHT	86 ± 1	162.9 ± 0.8	154 ± 2	150.0 ± 0.3

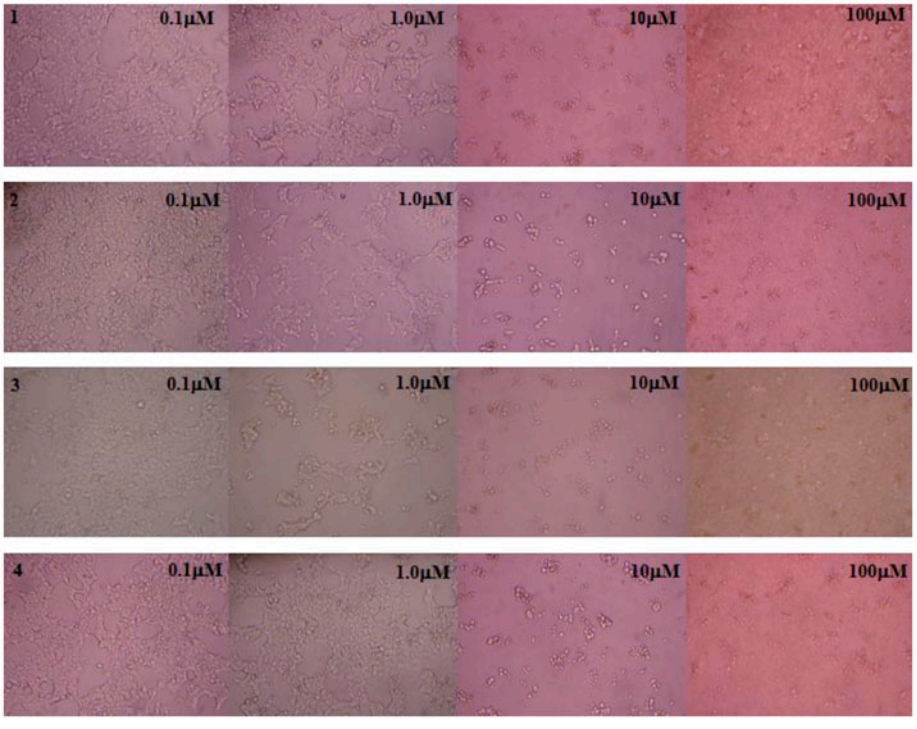


Figure 4. Cytotoxic effect of ruthenium(II) complexes against MCF-7 at different concentrations (0.1, 1.0, 10, and 100 μM). (1-[RuCl(CO)(PPh_3)L^1]; 2-[RuCl(CO)(PPh_3)L^2]; 3-[RuCl(CO)(PPh_3)L^3]; 4-[RuCl(CO)($AsPh_3$)L^3]). Cell viability decreased with increasing concentrations of complexes.

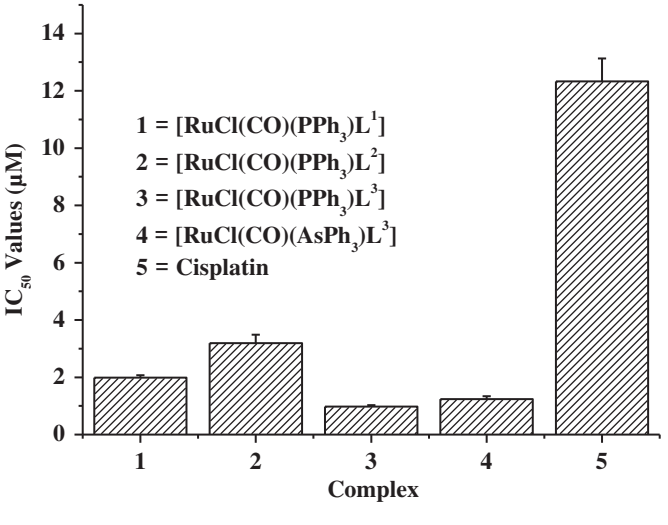


Figure 5. Cytotoxic effect of ruthenium(II) complexes compared with cisplatin.

(butylated hydroxytoluene). Moreover, the ruthenium(II) complexes showed higher antioxidant activity when compared to that of other metal complexes [54].

According to Meyerstein [55], the assay used to determine the hydroxyl radical scavenging properties does not measure the antioxidant properties in biological samples. The antioxidant activities of the ruthenium complexes are due to their role in shortening of the radical chain processes. The detailed mechanism is unclear, but probably due to the redox recycling processes of ruthenium(II)/(III); the new complexes scavenge the radicals produced in the chain processes and thereby quench their propagation.

$$\mathrm{Ru(II)} + \mathrm{RO_2^{\bullet}} \rightarrow \mathrm{Ru(III)^{-}O_2R}$$

$$\mathrm{Ru(III)^{-}O_2R} + \mathrm{RO_2^{\bullet}} \rightarrow \mathrm{Ru(II)} + \mathrm{products}$$

4.7. *Anticancer activity evaluation by MTT assay*

MTT assay was performed on human breast cancer cell line (MCF-7) to check the anticancer activity of the complexes. Ruthenium(II) species are generally more reactive compared to that of ruthenium(III) due to a high propensity for ligand exchange reactions, and may therefore interact with target molecules more rapidly [56–58]. Upon increasing the concentration of complexes from 0.1 to 100 μm, the % cell inhibition also increased. It is evident from figures 4–6 that the number of cells decreased with an increase in the concentration of the complexes. Analysis on the effect of complexes over the cell inhibition tendency clearly revealed that complex containing L^3 exhibited higher inhibition capacity

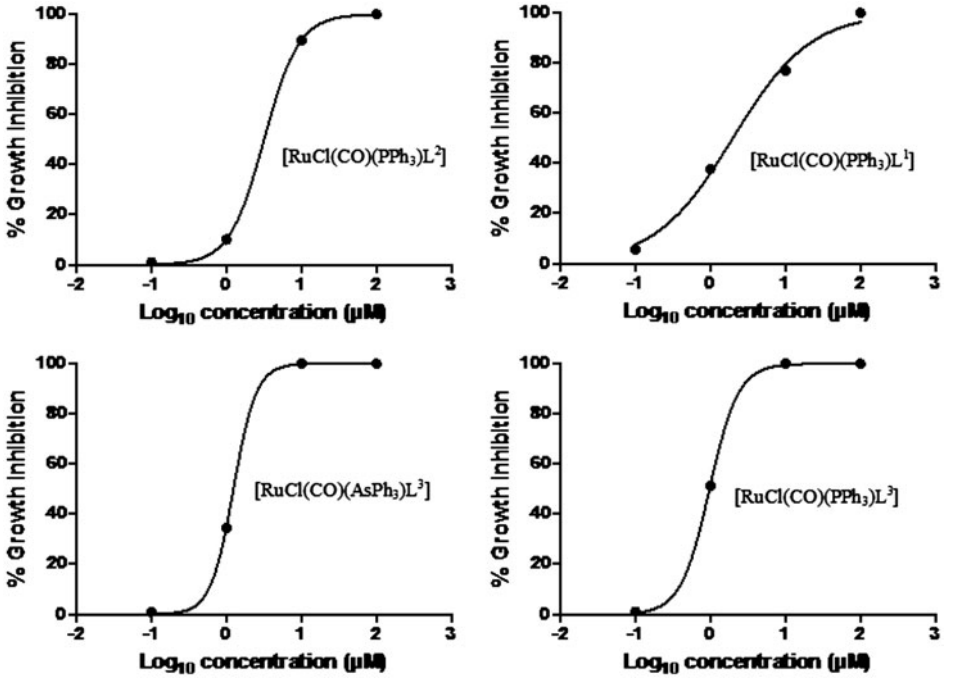


Figure 6. The % growth inhibition against $\log_{10}$ concentrations of different complexes on breast cancer cell line (MCF-7).

Table 6. IC_{50} (μM) value of ruthenium(II) complexes and cisplatin against breast cancer cell line (MCF-7).

Complex	IC_{50} (μM)[a]
$[RuCl(CO)(PPh_3)L^1]$	1.99 ± 0.08
$[RuCl(CO)(PPh_3)L^2]$	3.2 ± 0.3
$[RuCl(CO)(PPh_3)L^3]$	0.98 ± 0.05
$[RuCl(CO)(AsPh_3)L^3]$	1.2 ± 0.1
Cisplatin	12.3 ± 0.8

Note: [a]Fifty percent inhibitory concentration after exposure for 48 h in the MTT assay.

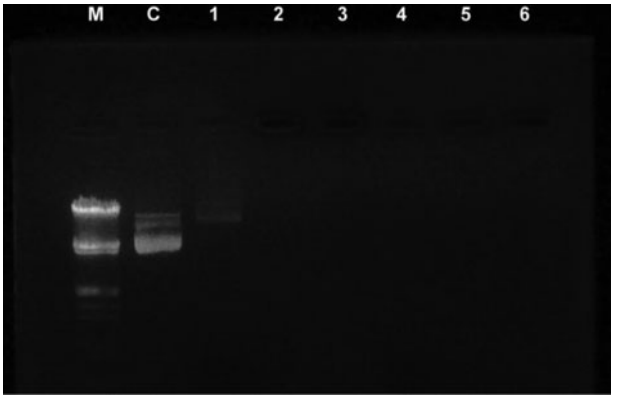


Figure 7. Agarose gel electrophoresis diagram showing the cleavage CT-DNA by ruthenium(II) complex in TEA buffer (4.84 g Tris base, pH = 8, 0.5 M EDTA/1 L). Lane M, DNA alone; lane C, control DNA (untreated complex). Lanes 1, 2 and 3 by $[RuCl(CO)(PPh_3)L^1]$ at 10, 50 and 100 μg/mL, respectively; lanes 4, 5, and 6 by $[RuCl(CO)(PPh_3)L^3]$ at 10, 50, and 100 μg/mL, respectively.

when compared to others. This may be due to terminal phenyl substitution in L^3. The IC_{50} values of complexes and standard are given in table 6. The IC_{50} values observed for the synthesized complexes were less than those of the standard drugs like cisplatin. Further, ruthenium arene complexes which were published recently have shown lesser cytotoxic activity compared to our complexes [59]. The higher pharmacological potential of our complexes may be due to the higher penetrating power of ruthenium(II) complexes with five-membered chelates through the cell membrane.

4.8. *DNA cleavage studies by gel electrophoresis*

DNA cleavage studies of the new complexes have been carried out using gel electrophoresis. Figure 7 shows the interaction of complexes with DNA at different concentrations (10, 50, and 100 μg). No obvious DNA cleavage was observed for controls in which the complexes were absent. $[RuCl(CO)(PPh_3)L^1]$ has shown partial cleavage of CT–DNA at 10 μg concentration and complete cleavage at the other two concentrations. $[RuCl(CO)(PPh_3)L^3]$ has shown complete cleavage of DNA at all concentrations. By increasing

concentration of complexes the DNA band completely disappears, which shows effective DNA cleavage by the complexes.

5. Conclusion

A series of new ruthenium(II) complexes were synthesized and characterized using spectral and elemental analysis. An octahedral geometry was proposed for all the complexes from the spectral data. Antioxidant, anticancer, and DNA cleavage properties of the synthesized complexes were studied. All the ruthenium(II) complexes possess excellent antioxidant properties, especially more activity against dangerous OH radical and are better than standard antioxidants vitamin C and BHT. The complexes were also found to efficiently cleave DNA and to have better anticancer activity.

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Supplemental data

Supplemental data for this article can be accessed http://dx.doi.org/10.1080/00958972.2013.858135.

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