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Ruthenium(II) carbonyl complexes containing *S*-methylisothiosemicarbazone based tetradentate ligand: synthesis, characterization and biological applications

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Abstract A series of hexa-coordinated ruthenium(II) complexes of the type $[Ru(CO)(B)L^n]$ ($n = 1$–4; B = PPh_3, $AsPh_3$ or Py) have been synthesized by reacting dibasic quadridentate Schiff base ligands H_2L^n ($n = 1$–4) with starting complexes $[RuHCl(CO)(EPh_3)_2(B)]$ (E = P or As; B = PPh_3, $AsPh_3$ or Py). The synthesized complexes were characterized using elemental and various spectral studies including UV–Vis, FT-IR, NMR (^1H, ^{13}C and ^{31}P) and mass spectroscopy. An octahedral geometry was tentatively proposed for all the complexes based on the spectral data obtained. The experiments on antioxidant activity showed that the ruthenium(II) *S*-methylisothiosemicarbazone Schiff base complexes exhibited good scavenging activity against various free radicals (DPPH, OH and NO). The in vitro cytotoxicity of these complexes has been evaluated by MTT assay. The results demonstrate that the complexes have good anticancer activities against selected cancer cell line, human breast cancer cell line (MCF-7) and human skin carcinoma cell line (A431). The DNA cleavage studies showed that the complexes have better cleavage of pBR 322 DNA.

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Introduction

Medicinal inorganic chemistry is continuously receiving interest in the field of biomedical applications (Ma et al. 2012; Hsu et al. 2013). This interest is largely due to the potential applications of metal containing compounds as antibacterial, antiviral, antifungal, antimalarial, and antitumor agents (Fricker 2007; Hartinger and Dyson 2009; Fry and Mascharak 2011; Jouad et al. 2002). It is a well known fact that cisplatin is arguably the most successful anticancer drug in the world. But, it exhibits high toxicity to normal cells leading to undesirable side-effects, although minimized by careful administration protocols, and also it is inactive against many cancer cell lines and metastasis (secondary) cancers (Wang and Lippard 2005). Therefore, attempts are being made to replace cisplatin with suitable alternatives and hence numerous transition metal complexes have been synthesized and tested for their anticancer activities. Among the metal atoms used in anticancer metal complexes, ruthenium is the most unique. It is a rare noble metal unknown to living systems and has strong complexation ability with numerous ligands (Demoro et al. 2012; Sathyadevi et al. 2012; Stringer et al. 2011).

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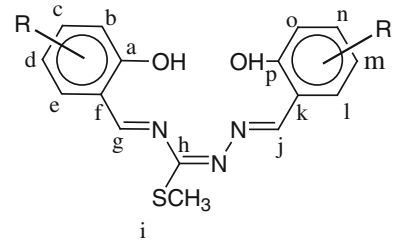
The chemistry of ruthenium is currently receiving a lot of attention, primarily because of the fascinating electron transfer and energy transfer properties displayed by the complexes of this metal (Hanson et al. 2013). Metal chelation is an important process useful to afford new chemical features to metal complexes in order to make them suitable for pharmacological applications. Many useful drugs containing metal-binding sites, which may alter the physiological profile of the free species. Some of them increase their activity by their ability to form chelates with specific metal ion. Metal complexes of sulphur containing Schiff bases have been the subject of current and growing interest because it has been shown that many of these complexes possess anticancer activity (Ramachandran et al. 2012; Wang et al. 2009). In particular, thiosemicarbazones, as a class of compounds, exhibit several interesting physical, chemical and wide range of biological properties (Senthil Raja et al. 2011).

First study on thiosemicarbazones activity against *Mycobacterium tuberculosis* (Da et al. 2009) many other biological activities of this type of molecules have been described, for instance antitumoral, antifungal and antiviral (Bal et al. 2007). Thiosemicarbazones, in their neutral or deprotonated form, behave as ONS/NNS tridentate or tetradentate chelate towards metal ions essential for life. Some thiosemicarbazones, such as marboran or triapine, are already used in medical practice. Recent discovery of the anticancer effects of metal complexes and their potential use in cancer diseases have received increasing attention (Alagesan et al. 2013; Su et al. 2013).

The above discussions have stimulated much interest to investigate the biological studies of ruthenium(II) complexes with aromatic 2-hydroxy aldehydes containing thiosemicarbazones. Hence, in the present work, we describe the synthesis, characterization, bioactivities of ruthenium(II) complexes containing *S*-methylisothiosemicarbazone Schiff bases. The general structure of the ligands used in this study is given in Fig. 1.



Ligand	R
H_2L^1	H
H_2L^2	Cl
H_2L^3	OCH_3
H_2L^4	C_4H_4

Fig. 1 Structure of *S*-methylisothiosemicarbazone Schiff base ligands

Experimental

Physical measurements

Microanalyses of carbon, hydrogen and nitrogen were carried out using Vario EL III Elemental analyzer at SAIF, Cochin, India. The IR spectra of the ligand and their complexes were recorded as KBr pellets on a Nicolet Avatar model spectrophotometer in 4,000–400 cm^{-1} range. Electronic spectra of the ligand and their complexes have been recorded in methanol using a Shimadzu UV-1650 PC spectrophotometer in 800–200 nm range. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded in Jeol GSX-400 instrument using DMSO as the solvent. The ESI–MS spectra were recorded by using LC–MS Q-ToF Micro Analyzer (Shimadzu) in the SAIF, Panjab University, and Chandigarh. Melting points were recorded on a Technico micro heating table and are uncorrected.

Materials

All the reagents used were chemically pure and AR grade. The solvents were purified and dried according to standard procedures. $RuCl_3{\cdot}3H_2O$ was purchased from Loba Chemie Pvt Ltd. The starting complexes $[RuHCl(CO)(PPh_3)_3]$, $[RuHCl(CO)(AsPh_3)_3]$ and $[RuHCl(CO)(Py)(PPh_3)_2]$ were prepared according to literature procedures (Narayana prabhu and Ramesh 2012; Anitha et al. 2012). *S*-methylthiosemicarbazide and *S*-methylisothiosemicarbazone Schiff bases (Fig. 1) were prepared according to the literature procedures (Atasever et al. 2010).

Preparation of *S*-methylisothiosemicarbazone Schiff base ligands

A mixture of thiosemicarbazide (0.910 g, 10 mmol) and methyl iodide (1.505 g, 10.6 mmol) in absolute ethanol (20 ml) was refluxed for 45 min and allowed to cool at room temperature for 12 h. White crystals of *S*-methylisothiosemicarbazide separated was filtered, washed with ethanol and used for next step without further purification. Yield: 68 %. In the second step, a mixture of *S*-methylisothiosemicarbazide (0.525 g, 5 mmol) and corresponding aldehydes (salicylaldehyde/5-chloro salicylaldehyde/*o*-vanallin/2-hydroxynaphthaldehyde) (1.23, 1.57, 1.52, 1.72 g respectively, 10 mmol) in absolute ethanol was neutralized with aqueous $Na_2CO_3{\cdot}10H_2O$ solution. The pale yellow solid of the ligands obtained were filtered off and washed with water and finally with ethanol. Yield: 85–90 %.

Bis(salicylaldehyde) *S*-methylisothiosemicarbazone ligand

Yellow solid. Anal. Calc. (Found). ($C_{16}H_{15}O_2N_3S$): C, 61.32(61.46); H, 4.82(4.71); N, 13.41(13.21); S, 10.23(10.18). IR (KBr, cm^{-1}): 3280 (OH^a), 3459 (OH^p), 1617 ($C{=}N^g$), 1635 ($C{=}N^j$). ^1H NMR (DMSO-d_6, δ, ppm): 11.56 (s, $1H^a$, OH), 10.83 (s, $1H^p$, OH), 8.44 (s, $1H^g$, –CH=N), 8.31 (s, $1H^j$, –CH=N), 6.78–7.61 (m, aromatic), 2.31 (s, CH_3). UV λmax: 220, 298, 304, 332.

Bis(5-chloro salicylaldehyde) *S*-methylisothiosemicarbazone ligand

Yellow solid. Anal. Calc. (Found). ($C_{16}H_{13}O_2N_3Cl_2S$): C, 50.27(50.12); H, 3.43(3.15); N, 10.99(10.74); S, 8.39(8.14). IR (KBr, cm^{-1}): 3283 (OH^a), 3450 (OH^p), 1637 ($C{=}N^g$), 1661 ($C{=}N^j$). ^1H NMR (DMSO-d_6, δ, ppm): 11.63 (s, $1H^a$, OH), 10.75 (s, $1H^p$, OH), 8.42 (s, $1H^g$, –CH=N), 8.32 (s, $1H^j$, –CH=N), 6.80–7.81 (m, aromatic), 2.33 (s, CH_3). UV λmax: 225, 276, 337.

Bis(*o*-vanallin) *S*-methylisothiosemicarbazone ligand

Yellow solid. Anal. Calc. (Found). ($C_{18}H_{19}O4N_3S$): C, 57.89(57.66); H, 5.13(5.11); N, 11.25(11.07); S, 8.59(8.16). IR (KBr, cm^{-1}): 3313 (OH^a), 3414 (OH^p), 1622 ($C{=}N^g$), 1651 ($C{=}N^j$). ^1H NMR (DMSO-d_6, δ, ppm): 11.22 (s, $1H^a$, OH), 10.76 (s, $1H^p$, OH), 8.56 (s, $1H^g$, –CH=N), 8.23 (s, $1H^j$, –CH=N), 6.90–7.23 (m, aromatic), 2.32 (s, CH_3). UV λmax: 220, 264, 338.

Bis(2-hydroxynaphthaldehyde) *S*-methylisothiosemicarbazone ligand

Yellow solid. Anal. Calc. (Found). ($C_{24}H_{19}O_2N_3S$): C, 69.71(61.46); H, 4.63(4.24); N, 10.16(10.10); S, 7.75(7.34). IR (KBr, cm^{-1}): 3313 (OH^a), 3414 (OH^p), 1622 ($C{=}N^g$), 1651 ($C{=}N^j$). ^1H NMR (DMSO-d_6, δ, ppm): 12.99 (s, $1H^a$, OH), 12.67 (s, $1H^p$, OH), 9.28 (s, $1H^g$, –CH=N), 9.19 (s, $1H^j$, –CH=N), 6.81–9.35 (m, aromatic), 2.31 (s, CH_3). UV λmax: 238, 262, 328, 367, 382.

Synthesis of new ruthenium(II) *S*-methylisothiosemicarbazone Schiff base complexes

All the new metal complexes were prepared according to the following general procedure. To a benzene solution of 1 mmol (0.769–1.084 g) [RuHCl(CO)$(EPh_3)_2$(B)] (E = P or As; B = PPh_3, $AsPh_3$ or Py), 1 mmol (0.315–0.413 g) Schiff base ligand was added (mole ratio of ruthenium starting complex and ligand is 1:1 respectively) and the mixture was refluxed for 6 h and the reactions were monitored by TLC. The reaction mixture was reduced to 2–3 ml and the product was separated by the addition 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 yield obtained for all the complexes were 70–79 %.

[Ru(CO)$(PPh_3)L^1$]

Anal. Calc. (Found). ($C_{35}H_{28}O_3N_3SPRu$): C, 59.82 (59.62); H, 4.02(3.96); N, 5.98(5.53); S, 4.56(4.59). IR (KBr, cm^{-1}): 1956 (C≡O), 1603 ($C{=}N^g$), 1621 ($C{=}N^j$), 737 (C–S). ^1H NMR (DMSO-d_6, δ, ppm):8.23 (s, $1H^g$, –CH=N), 8.01 (s, $1H^j$, –CH=N), 7.12–7.81 (m, aromatic), 2.32 (s, CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 203.58 (C≡O), 173.45 (C–S), 168.01 (C–O), 160.59 ($C{=}N^g$), 161.23 ($C{=}N^j$), 120.14–139.76 (Ar–C), 23.09 (CH_3). ^{31}P NMR ($CDCl_3$, δ, ppm): 31.49. UV λmax: 230, 250, 303, 323, 402. ESI–MS, *m/e*: 702.60.

$[Ru(CO)(PPh_3)L^2]$

Anal. Calc. (Found). ($C_{35}H_{26}O_3N_3Cl_2SPRu$): C, 54.48(54.24); H, 3.40(3.32); N, 5.45(5.14); S, 4.16(4.15). IR (KBr, cm^{-1}): 1957 (C≡O), 1603 (C=N^g), 1622 (C=N^j), 737 (C–S). ^{1}H NMR (DMSO-d_6, δ, ppm): 8.12 (s, 1H^g, –CH=N), 7.99 (s, 1H^j, –CH=N), 7.18–7.83 (m, aromatic), 2.30 (s, CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 202.82 (C≡O), 175.02 (C–S), 168.91 (C–O), 162.72 (C=N^g), 163.23 (C=N^j), 121.28–137.76 (Ar–C), 25.87 (CH_3). ^{31}P NMR ($CDCl_3$, δ, ppm): 31.95. UV λmax: 232, 264, 345, 384. ESI–MS, *m/e*: 770.60.

$[Ru(CO)(PPh_3)L^3]$

Anal. Calc. (Found). ($C_{37}H_{32}O_5N_3SPRu$): C, 58.26 (58.02); H, 4.23(4.11); N, 5.51(5.40); S, 4.20(4.03). IR (KBr, cm^{-1}): 1928 (C≡O), 1575 (C=N^g), 1634 (C=N^j), 737 (C–S). ^{1}H NMR (DMSO-d_6, δ, ppm): 8.24 (s, 1H^g, –CH=N), 8.03 (s, 1H^j, –CH=N), 7.10–7.79 (m, aromatic), 2.29 (s, CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 202.96 (C≡O), 174.02 (C–S), 168.24 (C–O), 161.79 (C=N^g), 164.05 (C=N^j), 122.21–138.09 (Ar–C), 26.79 (CH_3). ^{31}P NMR ($CDCl_3$, δ, ppm): 33.51. UV λmax: 232, 255, 301, 408.

$[Ru(CO)(PPh_3)L^4]$

Anal. Calc. (Found). ($C_{43}H_{32}O_3N_3SPRu$): C, 64.33 (64.36); H, 4.02(3.98); N, 5.23(5.15); S, 3.99(4.10). IR (KBr, cm^{-1}): 1959 (C≡O), 1601 (C=N^g), 1616 (C=N^j), 738 (C–S). ^{1}H NMR (DMSO-d_6, δ, ppm): 8.20 (s, 1H^g, –CH=N), 8.09 (s, 1H^j, –CH=N), 7.13–7.80 (m, aromatic), 2.30 (s, CH_3). ^{13}C NMR (DMSO-d_6, δ, ppm): 203.58 (C≡O), 177.95 (C–S), 169.02 (C–O), 162.74 (C=N^g), 164.15 (C=N^j), 123.11–139.76 (Ar–C), 28.42(CH_3). ^{31}P NMR ($CDCl_3$, δ, ppm): 34.74. UV λmax: 231, 254, 329, 382, 436. ESI–MS, *m/e*: 803.52.

$[Ru(CO)(AsPh_3)L^1]$

Anal. Calc. (Found). ($C_{35}H_{28}O_3N_3SAsRu$): C, 56.30 (56.25); H, 3.78(3.45); N, 5.63(5.56); S, 4.29(4.10). IR (KBr, cm^{-1}): 1948 (C≡O), 1603 (C=N^g), 1621 (C=N^j), 745 (C–S). ^{1}H NMR (DMSO-d_6, δ, ppm): 8.15 (s, 1H^g, –CH=N), 7.95 (s, 1H^j, –CH=N), 7.15–7.78 (m, aromatic), 2.32(s, CH_3). UV λmax: 231, 270, 330, 382. ESI–MS, *m/e*: 745.72.

$[Ru(CO)(AsPh_3)L^2]$

Anal. Calc. (Found). ($C_{35}H_{26}O_3N_3Cl_2SAsRu$): C, 51.54(51.42); H, 3.21(3.33); N, 5.15(5.02); S, 3.93(3.66). IR (KBr, cm^{-1}): 1903 (C≡O), 1600 (C=N^g), 1630 (C=N^j), 744 (C–S). ^{1}H NMR (DMSO-d_6, δ, ppm): 8.21(s, 1H^g, –CH=N), 8.05 (s, 1H^j, –CH=N), 7.11–7.79 (m, aromatic), 2.31 (s, CH_3). UV λmax: 232, 264, 345, 384.231, 265, 360.

$[Ru(CO)(AsPh_3)L^3]$

Anal. Calc. (Found). ($C_{37}H_{32}O_5N_3SAsRu$): C, 55.09 (55.04); H, 4.00(3.76); N, 5.21(5.24); S, 3.97(3.87). IR (KBr, cm^{-1}): 1930 (C≡O), 1568 (C=N^g), 1592 (C=N^j), 740 (C–S). ^{1}H NMR (DMSO-d_6, δ, ppm): 8.19 (s, 1H^g, –CH=N), 8.01 (s, 1H^j, –CH=N), 7.09–7.85 (m, aromatic), 2.31 (s, CH_3). UV λmax: 235, 273, 331, 380. ESI–MS, *m/e*: 806.49.

$[Ru(CO)(AsPh_3)L^4]$

Anal. Calc. (Found). ($C_{43}H_{32}O_3N_3SAsRu$): C, 60.99 (60.91); H, 3.81(3.62); N, 4.96(4.78); S, 3.79(3.57). IR (KBr, cm^{-1}): 1951 (C≡O), 1601 (C=N^g), 1616 (C=N^j), 745 (C–S). ^{1}H NMR (DMSO-d_6, δ, ppm): 8.20 (s, 1H^g, –CH=N), 7.99 (s, 1H^j, –CH=N), 7.16–7.80 (m, aromatic), 2.29 (s, CH_3). UV λmax: 232, 260, 327, 388, 460.

$[Ru(CO)(Py)L^1]$

Anal. Calc. (Found). ($C_{22}H_{18}O_3N_4SRu$): C, 50.86(50.91); H, 3.49(3.36); N, 10.78(10.71); S, 6.17(6.07). IR (KBr, cm^{-1}): 1949 (C≡O), 1602 (C=N^g), 1620 (C=N^j), 746 (C–S). ^{1}H NMR (DMSO-d_6, δ, ppm): 8.11 (s, 1H^g, –CH=N), 7.94 (s, 1H^j, –CH=N), 7.10–7.79 (m, aromatic), 2.29 (s, CH_3). UV λmax: 230, 271, 330, 381.

$[Ru(CO)(Py)L^2]$

Anal. Calc. (Found). ($C_{22}H_{16}O_3Cl_2N_4SRu$): C, 44.91 (44.96); H, 2.74(2.71); N, 9.52(9.31); S, 5.45(5.38). IR (KBr, cm^{-1}): 1957 (C≡O), 1601 (C=N^g), 1619 (C=N^j), 745 (C–S). ^{1}H NMR (DMSO-d_6, δ, ppm):

8.15 (s, $1H^g$, –CH=N), 7.98 (s, $1H^j$, –CH=N), 7.16–7.85 (m, aromatic), 2.31 (s, CH_3). UV λmax: 235, 337, 370. ESI–MS, *m/e*: 588.33.

[Ru(CO)(Py)L^3]

Anal. Calc. (Found). ($C_{24}H_{22}O_5N_4SRu$): C, 49.73 (49.61); H, 3.83(3.39); N, 9.67(9.54); S, 5.53(5.69). IR (KBr, cm^{-1}): 1931 (C≡O), 1588 ($C=N^g$), 1611 ($C=N^j$), 742 (C–S). ^{1}H NMR (DMSO-d_6, δ, ppm): 8.13 (s, $1H^g$, –CH=N), 8.00 (s, $1H^j$, –CH=N), 7.12–7.89 (m, aromatic), 2.31 (s, CH_3). UV λmax: 228, 269, 331, 380.

[Ru(CO)(Py)L^4]

Anal. Calc. (Found). ($C_{30}H_{22}O_3N_4SRu$): C, 58.15(58.01); H, 3.58(3.53); N, 9.04(9.12); S, 5.17(5.04). IR (KBr, cm^{-1}): 1949 (C≡O), 1601 ($C=N^g$), 1616 ($C=N^j$), 744 (C–S). ^{1}H NMR (DMSO-d_6, δ, ppm): 8.16 (s, $1H^g$, –CH=N), 7.95 (s, $1H^j$, –CH=N), 7.09–7.86 (m, aromatic), 2.29 (s, CH_3). UV λmax: 230, 262, 323, 383, 459.

Antioxidant assays

DPPH· scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the compounds was measured according to the method of Blios (Sathiya Kamatchi et al. 2012). The DPPH radical is a stable free radical. Because of the odd electron, DPPH shows a strong absorption band at 517 nm in the visible spectrum. As this electron becomes paired off 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 µl 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 µl 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).

OH· scavenging assay

The hydroxyl radical scavenging activity of the compounds has been investigated by using the Nash method (Sathiya Kamatchi et al. 2012). In vitro hydroxyl radicals were generated by a Fe^{3+}/ascorbic acid system. The detection of hydroxyl radicals was carried out 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 of iron-EDTA solution (0.13 % ferrous ammonium sulphate 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 the 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 the colour formed was measured spectrophotometrically at 412 nm against reagent blank.

NO· scavenging assay

The assay of nitric oxide (NO) scavenging activity is based on a method (Sathiya Kamatchi et al. 2012) where 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 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.

In vitro cytotoxicity activity evaluation by MTT assay

Cytotoxicity studies of the complexes were carried out on human breast cancer cell line (MCF-7) and human skin carcinoma cell line (A431) which was obtained from National Centre for Cell Science (NCCS), Pune, India. Cell viability was carried out using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium \bromide) assay method (Blagosklonny and EI-diery 1996). The MCF-7 cells were grown in Eagles minimum essential medium containing 10 % fetal bovine serum (FBS) and A431 were grown in Dulbecco's Modified Eagles Medium (DMEM) containing 10 % fetal bovine serum (FBS). For screening experiments, the cells were seeded into 96-well plates in 100 mm^3 of the respective medium containing 10 % FBS, at a plating density of 10,000 cells/well and incubated at 37 °C, 5 % CO_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_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, 15 mm^3 of MTT (5 mg/cm^3) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4 h. The medium with MTT was then removed and the formed formazan crystals were dissolved in 100 mm^3 of DMSO and the absorbance measured at 570 nm using a micro plate reader. The % cell inhibition was determined using the following formula and a graph was plotted between % of cell inhibition and concentration. From this plot, the IC_{50} value was calculated.

$$\%\ \text{inhibition} = [\text{mean OD of untreated cells (control)} / \text{mean OD of treated cells (control)}] \times 100.$$

DNA cleavage experiments

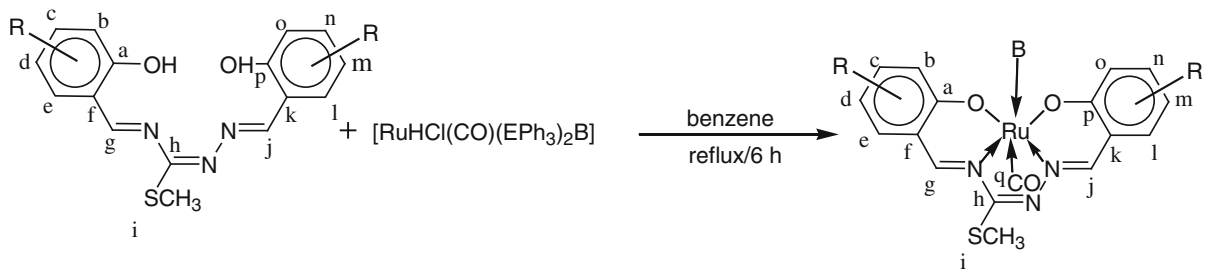
For the gel electrophoresis experiment, supercoiled pBR 322 DNA (0.1 μg) was treated with the ruthenium(II) complexes with buffer (50 mM Tris–HCl, 18 mM NaCl, pH = 7.2) and the solution was then irradiated at room temperature with a UV lamp (365 nm, 10 W). The samples were analyzed by electrophoresis 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 bands under illuminator.

Results and discussion

The reactions of $[RuHCl(CO)(EPh_3)_2(B)]$ (E = P or As; B = PPh_3, $AsPh_3$ or Py) with *S*-methylisothiosemicarbazone Schiff base ligands in 1:1 molar ratio in dry benzene afforded new hexa-coordinated low-spin ruthenium(II) complexes (Scheme 1). The analytical data are in good agreement with proposed molecular formula of the complexes. In addition, ESI-Mass spectra of complexes $[Ru(CO)(PPh_3)L^1]$, $[Ru(CO)(AsPh_3)L^1]$, $[Ru(CO)(PPh_3)L^2]$, $[Ru(CO)(Py)L^2]$, $[Ru(CO)(AsPh_3)L^3]$ and $[Ru(CO)(PPh_3)L^4]$ have also confirmed the molecular weights of their proposed structures with *m/e* values 702.60, 745.72, 770.60, 588.33, 806.49 and 803.52 respectively. In all the reactions, the Schiff base ligand behaves as dibasic quadridentate ligands by replacing two molecules of triphenylphosphine or triphenylarsine, one hydride and one chloride ion from the precursors. All the complexes are stable in air at room temperature, brown in color, non-hygroscopic in nature and highly soluble in common organic solvents such as dichloromethane, methanol, acetonitrile and DMSO.

Infrared spectroscopic analysis

The IR spectra of the complexes have been examined in comparison with that of the ligand. The spectra of free ligands showed two intense bands in the region 1,635–1,665 and 1,604–1,637 cm^{-1} characteristic of two $\upsilon_{C=N}$ imine groups. These bands shifted towards lower frequencies 1,592–1,634 and 1,568–1,603 cm^{-1} in complexes indicated that the coordination of ligand with metal through azomethine nitrogens (Pal 2002). The two bands appeared in ligand spectra very close to each other in the region 3,253-3,459 cm^{-1} were due to the stretching frequency of two phenolic OH groups. These bands disappeared in complexes showed that the binding of phenolic oxygen with metal via deprotonation. Further the strong absorption around the 1,920–1,957 cm^{-1} has been assigned to the terminally coordinated carbonyl



Scheme 1 General scheme for the synthesis of new ruthenium(II) complexes

group in the new ruthenium complexes (Nareshkumar and Ramesh 2005). In addition, the other characteristic bands due to triphenylphosphine and triphenylarsine (around 695 and 1,435 cm^{-1}) were also present in the spectra of all complexes (Ramachandran and Viswanathamurthi 2013).

Electronic spectroscopic analysis

The electronic spectra of all the complexes in methanol showed three to five bands in the region 460–220 nm. The bands around 408–323 nm have been assigned to charge transfer transitions arising from the metal t_{2g} level to the unfilled molecular orbitals derived from the π* level of the ligands based on their extinction coefficient values. The bands below 300 nm were characterized by intra-ligand charge transfer. The electronic spectra are similar to those observed for other octahedral ruthenium(II) complexes (Muthu Tamizh et al. 2012).

^{1}H NMR spectral studies

The ^{1}H NMR spectra of the ligands and the corresponding ruthenium(II) complexes were recorded in DMSO to confirm the presence of coordinated ligand in the complexes. The two singlets at 10.75–13.01 ppm are assigned to two phenolic OH groups of the ligands. The bands are not found in the spectra of complexes, which is consistent with deprotonation of these ligands upon metal complexation. The spectra of ligands have shown two peaks for two azomethine proton (–CH=N–) in the range of 8.31–9.28 ppm, which are shifted slightly to the up field and appeared at 7.95–8.23 ppm in complexes indicated that the coordination of ligand with metal through azomethine nitrogens. The multiplets at 7.12–7.91 ppm in the spectra of complexes are assigned to aromatic protons. In addition, the methyl group protons appeared at 2.31 ppm.

^{13}C NMR spectroscopic analysis

The ^{13}C NMR spectra of the complexes have showed a peak at 203.58–201.30 ppm region is due to C≡O carbon. The presence of a peak at 177.95–173.45 ppm region is assigned to C–S carbon. The azomethine (>C=N) carbons exhibited its peak in the region of 164.15–160.59 ppm. In addition, the peak in the region of 169.62–168.01 ppm is assigned to C–O carbons. The multiplets appeared around 120.14–139.76 ppm region are assigned to aromatic carbons. A sharp singlet at 23.09–25.28.42 ppm is assigned to methyl carbon.

^{31}P NMR spectral studies

^{31}P NMR spectra of some of the complexes were recorded to confirm the presence of triphenylphosphine group in the complexes. A sharp singlet was observed around 31.49–34.74 ppm due to presence of triphenylphosphine ligand in the complexes.

From the above all spectral data, an octahedral geometry was proposed tentatively for all the complexes in which the two azomethine nitrogens and two phenoxy groups of the ligands are in coordination with ruthenium(II) metal ion (Fig. 2).

Antioxidant activity

Free radicals contain one or more unpaired electrons, produced in normal or pathological cell metabolism. Reactive oxygen species (ROS) react easily with these

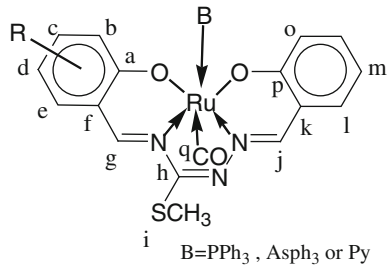


Fig. 2 Structure of new ruthenium(II) complexes

free radicals to become radicals themselves. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals ($O_2^{-\cdot}$) and hydroxyl radicals ($OH^{\cdot}$) as well as non-free radical species (H_2O_2) and the singlet oxygen ($^1O_2$) (Gulcin et al. 2004; Rice-Evans and Diplock 1993). They are formed in living organisms in different ways, including normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages and peroxisomes. They are natural by-products of our body's metabolism. The free radical can induce DNA damage in humans. The damage to DNA has been suggested to contribute to aging and various diseases including cancer and chronic inflammation (Tsai et al. 2001).

Hence, we carried out experiments to explore the free radical scavenging ability of the ligands and complexes, with the hope of developing potential antioxidants and therapeutic reagents for respiratory diseases such as asthma emphysema and asbestosis. The antioxidant potential of free ligands and corresponding ruthenium(II) complexes against DPPH radical, OH 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 were shown in Table 1 (Fig. 3). It was observed that, the 50% inhibitory concentration (IC_{50}) value of ligands and complexes varies from 11.86 to 22.56 μM and from 5.58 to 8.30 μM respectively, against OH radical. The ligands showed their IC_{50} values against NO and DPPH radicals up to 27.75–35.27 and 61.80–67.87 μM respectively, whereas the complexes showed their IC_{50} values up to 11.78–18.33 and 35.11–50.25 μM respectively. From the above results, it can be concluded that less scavenging activity was exhibited by the free ligands when compared to that of their corresponding ruthenium complexes which is due to the chelation of them with the ruthenium ions. Among all free radicals, the hydroxyl radical ($OH^{\cdot}$) is by far the most potent and therefore the most dangerous oxygen metabolite, elimination of this radical is one of the major aims of antioxidant administration (Udilova et al. 2003). Further, it is significant to mention that the metal complexes synthesized herein possess superior antioxidant activity against the above said radicals than that of the standard antioxidant Vitamin C and butylated hydroxyl toluene (Sathiya Kamatchi et al. 2012). Moreover, the ruthenium(II) complexes showed higher antioxidant activity when compared to that of other metal complexes (Udilova et al. 2003).

Table 1 Antioxidant activity of ligands and ruthenium(II) complexes against various radicals

Compound	IC_{50}(μM)		
	$DPPH^{\cdot}$	$OH^{\cdot}$	$NO^{\cdot}$
H_2L^1	65.17	22.56	27.75
H_2L^2	61.80	11.86	35.27
H_2L^4	67.87	21.49	32.79
$[Ru(CO)(PPh_3)L^1]$	42.88	5.58	12.63
$[Ru(CO)(PPh_3)L^2]$	48.55	6.31	18.33
$[Ru(CO)(PPh_3)L^3]$	50.23	8.30	11.78
$[Ru(CO)(PPh_3)L^4]$	50.25	6.17	13.96
$[Ru(CO)(AsPh_3)L^1]$	35.11	5.89	13.53
$[Ru(CO)(Py)L^1]$	42.92	5.72	13.51
Vitamin C	145.80	234.21	216.85
BHT	86.92	162.35	152.82

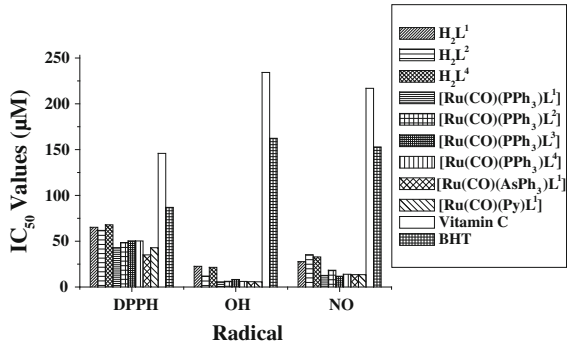


Fig. 3 Scavenging effect of the ligands and ruthenium(II) complexes on various radicals compared with standard vitamin C and BHT

In vitro cytotoxic activity evaluation by MTT assay

MTT assay was performed on human breast cancer cell line (MCF-7) and human skin carcinoma cell line (A431) to check the anticancer activity of the complexes. The cytotoxicity of the tested complexes was expressed by median growth inhibitory concentration (IC_{50}), which required producing 50% cytotoxic effect against MCF-7 and A431 cells after 48 h exposure to the tested complexes. The cytotoxicities of the complexes were found to be concentration dependent. The cell viability decreased with increasing concentrations of complexes (Figs. 4, 5, 6, 7). The

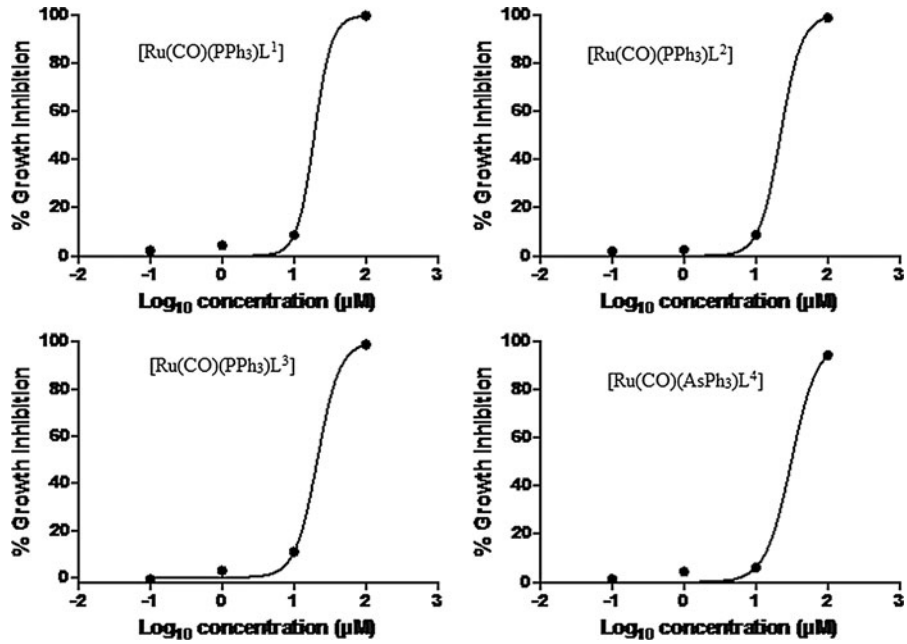


Fig. 4 The % growth inhibition against $\log_{10}$ concentrations of different complexes on breast cancer cell line (MCF-7)

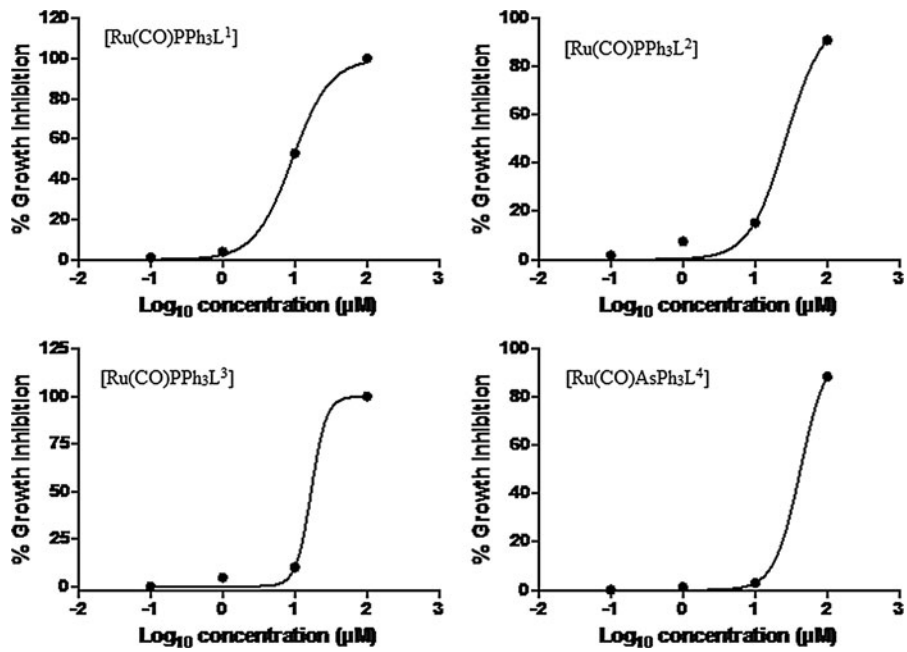


Fig. 5 The % growth inhibition against $\log_{10}$ concentrations of four different complexes on skin carcinoma cell line (A431)

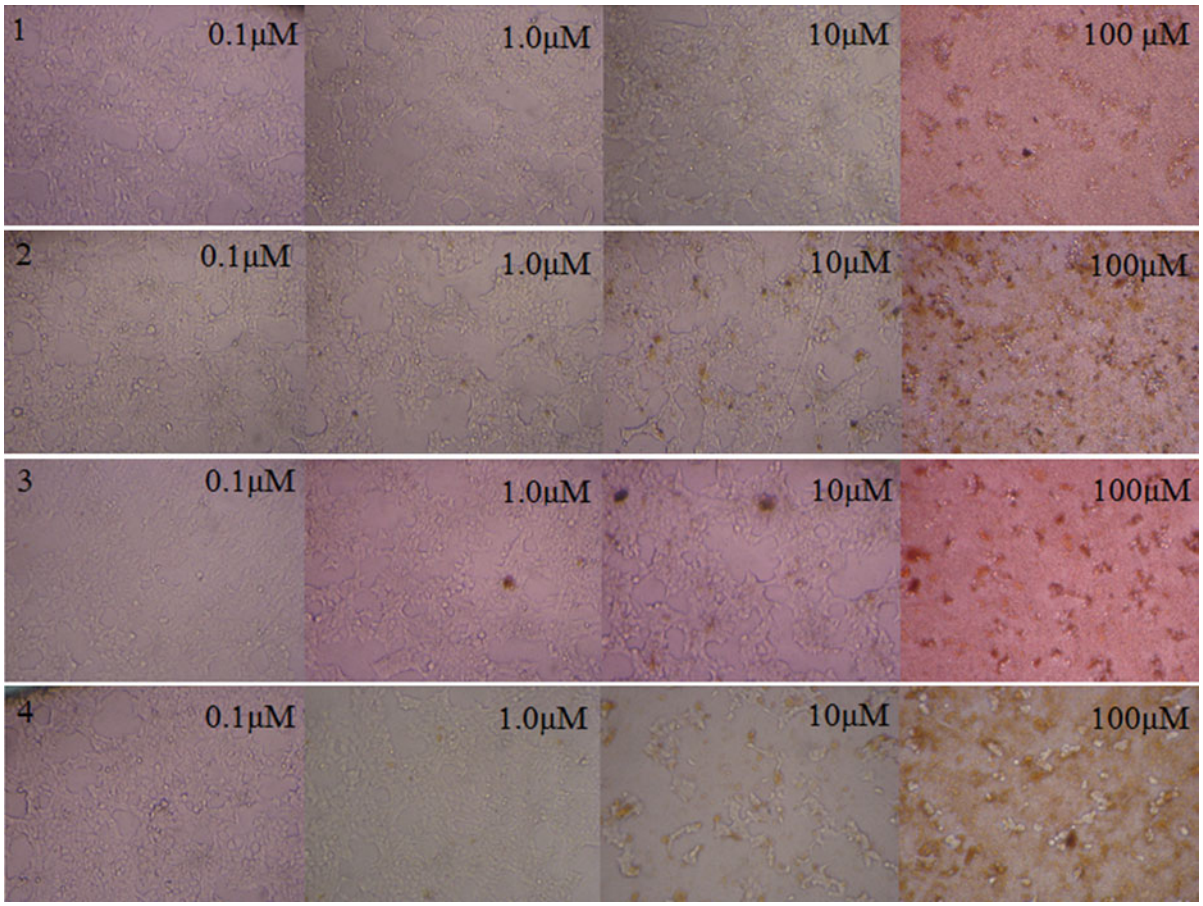


Fig. 6 Cytotoxic effect of ruthenium(II) complexes against MCF-7 at different concentrations (0.1, 1.0, 10, 100 μM). Cell viability decreased with increasing concentrations of complexes. (1-[$Ru(CO)(PPh_3)L^1$]; 2-[$Ru(CO)(PPh_3)L^2$]; 3-[$Ru(CO)(PPh_3)L^3$]; 4-[$Ru(CO)(AsPh_3)L^4$])

screening results are summarized in Tables 2 and 3. It is evident that all of the tested complexes showed anticancer activity, with IC_{50} values ranging from 19.18 to 31.10 μM for MCF-7 and 9.30-42.64 for A431 cell lines. From the IC_{50} values of complexes, it is observed that the cytotoxic activity of complexes decreasing in the order [$Ru(CO)(PPh_3)L^1$] > [$Ru(CO)(PPh_3)L^3$] > [$Ru(CO)(PPh_3)L^2$] > [$Ru(CO)(AsPh_3)L^4$] against MCF-7 and A431 cell lines. The lower toxicity of ruthenium complexes compared to platinum drugs has been attributed to the ability of ruthenium compounds to specifically accumulate in cancer tissues. Cisplatin and their derivatives mainly target DNA where as ruthenium pharmaceuticals bind to multiple biological targets (Beckford et al. 2009; Kalaivani et al. 2012; Sathyadevi et al. 2012).

DNA cleavage studies by gel electrophoresis

The cleavage reaction on plasmid DNA with ruthenium(II) complexes 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 (Xie et al. 2013). Figure 8 showed the gel electrophoresis separation of pBR 322 DNA after incubation with the ruthenium(II) complexes and irradiation at 365 nm for 30 min. No obvious DNA cleavage was observed for controls in

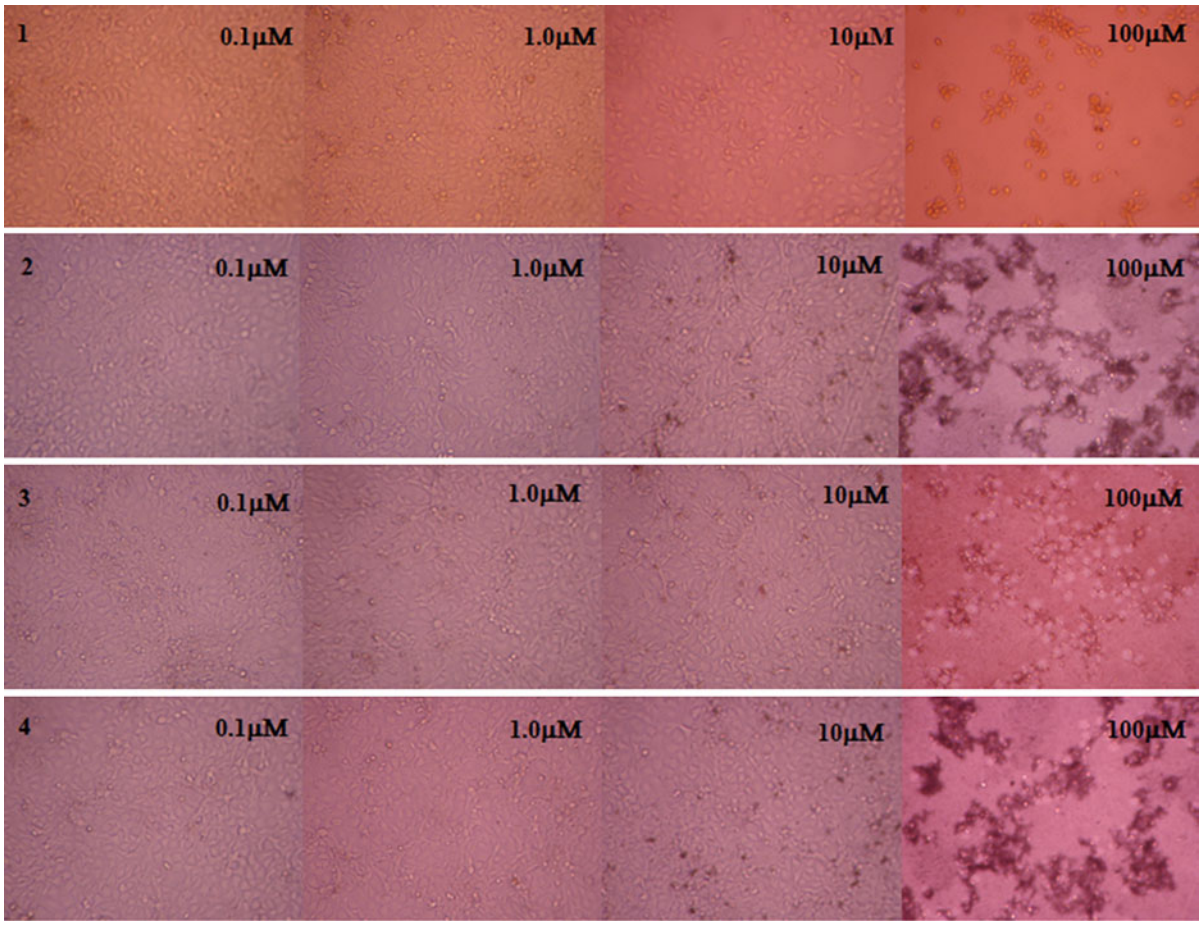


Fig. 7 Cytotoxic effect of ruthenium(II) complexes against A431 at different concentrations (0.1, 1.0, 10, 100 μM). Cell viability decreased with increasing concentrations of complexes. (1-[$Ru(CO)(PPh_3)L^1$]; 2-[$Ru(CO)(PPh_3)L^2$]; 3-[$Ru(CO)(PPh_3)L^3$]; 4-[$Ru(CO)(AsPh_3)L^4$])

Table 2 IC_{50} (μM) value of ruthenium(II) complexes against breast cancer cell line (MCF-7)

Complex	% Cell inhibition				IC_{50}
	0.1 μM	1 μM	10 μM	100 μM	
[$Ru(CO)(PPh_3)L^1$]	2.24	4.37	8.64	99.79	19.18
[$Ru(CO)(PPh_3)L^2$]	1.92	2.56	8.75	98.93	21.98
[$Ru(CO)(PPh_3)L^3$]	0.89	2.91	10.85	98.77	21.19
[$Ru(CO)(AsPh_3)L^4$]	1.28	4.37	6.08	94.34	31.10

Table 3 IC_{50} (μM) value of ruthenium(II) complexes against human skin carcinoma cell line (A431)

Complex	% Cell inhibition				IC_{50}
	0.1 μM	1 μM	10 μM	100 μM	
[$Ru(CO)(PPh_3)L^1$]	1.05	3.89	52.89	100	9.30
[$Ru(CO)(PPh_3)L^2$]	1.70	7.56	15.20	90.94	26.70
[$Ru(CO)(PPh_3)L^3$]	−0.03	4.61	10.04	100	16.87
[$Ru(CO)(AsPh_3)L^4$]	0.03	1.27	2.98	88.54	42.64

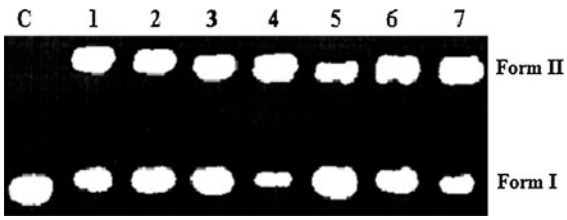


Fig. 8 Agarose gel electrophoresis diagram showing the cleavage of pBR 322 DNA by ruthenium(II) Schiff base complex. Lane C, control DNA (untreated complex); lane 1–4, DNA treated with $[Ru(CO)(PPh_3)L^1]$ at different concentrations (5, 10, 15, 20 μg/ml) respectively; lane 5–7, DNA treated with $[Ru(CO)(PPh_3)L^4]$ at different concentrations(5, 10, 20 μg/ml) respectively

which the complex was absent. With increasing concentration of the ruthenium(II) complexes ($[Ru(CO)(PPh_3)L^1]$ and $[Ru(CO)(PPh_3)L^4]$), the amount of form-I of pBR 322 DNA diminishes gradually, whereas that of form-II increases slowly. These results indicated that the scission occurs on one strand (nicked).

Conclusion

New ruthenium(II) complexes were synthesized and characterized using spectral and elemental analyses. An octahedral geometry was tentatively proposed for all the complexes from the spectral data. Radical scavenging ability of the complexes was found to be higher when compared to their corresponding ligands. This is due to the chelation of ligands with ruthenium metal. *In vitro* cytotoxicity of few complexes has been evaluated by MTT assay. The results showed that the complexes displayed good anticancer activity against human breast cancer (MCF-7) and human skin carcinoma cell line (A431). In addition, the ruthenium(II) complexes are efficiently cleaved the pBR 322 DNA.

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