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Synthesis, spectral, DNA binding and cleavage properties of ruthenium(II) Schiff base complexes containing PPh_3/$AsPh_3$ as co-ligands

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Abstract: Dihydroxybenzaldehyde-based Schiff base ligands (L^1–L^3) and their ruthenium(II) complexes were synthesized and characterized by elemental analysis, ^{1}H-, ^{13}C- and ^{31}P-NMR, UV–Vis, IR and mass spectroscopy. The DNA binding of the ruthenium(II) complexes was investigated by UV–Vis absorption spectroscopy. The experiments revealed that all the compounds could bind to DNA through electrostatic interactions and the intrinsic binding constants (K_b) were estimated under similar sets of experimental conditions. The absorption spectral study indicated that the ruthenium(II) complexes had intrinsic binding constants in the range of $(1.6–8.6)\times10^4$ mol^{-1} dm^3. The complex $[Ru(CO)(PPh_3)_2(L^3)]$ bound more strongly than the other complexes. In addition, the DNA cleavage properties for all ruthenium(II) complexes were tested.

Keywords: Schiff base; ruthenium(II) complexes; CT-DNA; nuclease activity.

INTRODUCTION

It is well known that deoxyribonucleic acid (DNA) plays an important role in the life process since it contains all the genetic information for cellular function. However, DNA is the primary intracellular target of anticancer drugs due to the interaction between small molecules and DNA, which cause DNA damage in cancer cells, blocking the division of cancer cells and resulting in cell death. This is due to their possible application as new therapeutic agents and their photochemical properties that make them potential probes of DNA structure and conformation.[1–3] The binding interaction of transition metal complexes with DNA is of interest for both therapeutic and scientific reasons.[4] Many transition metal complexes are known to bind to DNA *via* both covalent and non-covalent interac-

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tions. In covalent binding, the labile ligand of the complexes is replaced by a nitrogen base of DNA. On the other hand, the non-covalent DNA interactions include intercalative, electrostatic and groove (surface) binding of cationic metal complexes along the outside of the DNA helix, and the major or minor groove. Schiff bases are potential anticancer drugs and when administered as their metal complexes, the anticancer activity of these complexes is enhanced in comparison to that of the free ligand. Schiff base complexes are considered the most important stereochemical models in transition metal coordination chemistry due to their preparative accessibility and structural variety. It was suggested that the azomethine linkage in Schiff bases is responsible for their biological activities, such as antitumour, antibacterial, antifungal and herbicidal activities.[5]

Metal complexes are employed in many fields of drug discovery. Platinum coordination complexes are widely used as antitumour drugs. The first platinum antitumour drug introduced into clinical practice was *cis*-diamminedichloroplatinum(II) (cisplatin), which became the most widely used anticancer drug in the world. However, intrinsic and acquired tumour resistance diminishes the clinical efficacy of cisplatin and other platinum drugs. In addition cisplatin is of high toxicity, leading to side effects that limit the administered dose.[6] These limiting issues have led to an intense effort to design new transition metal-based compounds that are capable of overcoming problems associated with cisplatin while maintaining the same level of activity and broadening the spectrum of the therapeutic effect. In attempts to find a new, metal-based anticancer drug with activity complementary to cisplatin, several ruthenium complexes have recently been investigated for their antitumour activity.[7]

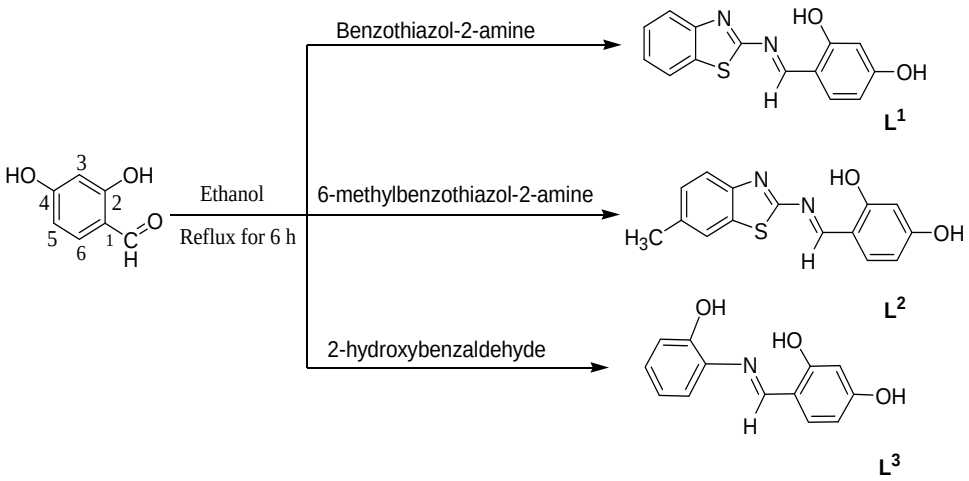
Bearing these facts in mind, our interest was focused on the synthesis of ruthenium(II) complexes containing tridentate Schiff base ligands and triphenylphosphine or triphenylarsine as co-ligands. The Schiff base ligands were derived by the condensation of 2,4-dihydroxybenzaldehyde with 2-aminobenzothiazole, 2-amino-6-methylbenzothiazole or *o*-aminophenol (Scheme 1). Then the new Schiff base ligands and their ruthenium(II) complexes were characterized by elemental analysis, FT-IR, electronic, mass spectra, and ^{1}H-, ^{13}C- and ^{31}P-NMR spectroscopy. Additionally, a comparative study of the interaction of the ruthenium complexes with CT-DNA has been employed in order to investigate the potential mechanism of their biological properties using UV–Vis spectroscopic and gel electrophoresis techniques.

EXPERIMENTAL

Materials and instrumentation

Reagent grade chemicals were used without further purification in all the synthetic work. All solvents were purified by standard methods. $RuCl_3 \cdot 3H_2O$, triphenylphosphine / arsine were purchased from Himedia. Calf thymus DNA (CT-DNA) was purchased from Bangalore Genei, Bangalore, India. Infrared spectra were recorded on a Perkin Elmer FT-IR spectro-

photometer (model RXI) as KBr pellets in the range 4000–400 cm^{-1}. Elemental analyses were performed with a Vario ELIII CHNS instrument at the Sophisticated Test and Instrumentation Centre (STIC), Cochin University, Kerala, India. Electronic spectra were recorded in DMSO solution in a Systronics 2202 double beam spectrophotometer in the 800–200 nm range. The ^{1}H-, ^{13}C- and ^{31}P-NMR spectra were recorded on Bruker WM DCX 500 MHz instrument using TMS and orthophosphoric acid as internal standards at SAIF, Indian Institute of Technology, Chennai. The mass spectra were recorded using a JEOL GC mate instrument at SAIF, Indian Institute of Technology, Chennai, India. The DNA cleavage studies were performed using the gel documentation system, Gelstan. Melting points were recorded on a Veego VMP-DS model heating table and are uncorrected. The metal precursors $[RuHCl(CO)(PPh_3)_3]$,[8] $[RuHCl(CO)(AsPh_3)_3]$,[9] were prepared according to reported procedures.



Scheme 1. Preparation of the Schiff base ligands.

Preparation of the Schiff base ligands

A solution of 2,4-dihydroxybenzaldehyde (1.38 g, 10 mmol) in ethanol (15 ml) was added to a stirred solution of 2-aminobenzothiazole/2-amino-6-methylbenzothiazole/*o*-amino phenol (1.09–1.64 g, 10 mmol) in ethanol (20 mL). The mixture was refluxed for 6 h. Upon cooling, a precipitate was formed, which was filtered off, dried and recrystallized from ethanol (Scheme 1).

Preparation of the ruthenium(II) Schiff base complexes

All the new ruthenium(II) complexes were prepared by the following general procedure (Scheme 2). A solution of $[RuHCl(CO)(B)_3]$ (0.190–0.216 g, 0.20 mmol) in benzene (20 mL, B = PPh_3/$AsPh_3$), was added to a stirred solution of Schiff base ligands (0.045–0.056 g, 0.20 mmol) in methanol (15 mL). The mixture was refluxed for 12 h. The solvent was then evaporated under reduced pressure and the solid mass filtered and washed with petroleum ether. The purity of the complexes was checked by thin layer chromatography and was further purified by column chromatography using 1:10 CH_2Cl_2:*n*-hexane as an eluent. This solid was recrystallized from CH_2Cl_2/*n*-hexane mixture. All attempts to obtain single crystals of the complexes were unsuccessful.

DNA-binding and cleavage assay

Electronic absorption spectroscopy. Experiments involving the interaction of the ruthenium(II) complexes with CT-DNA were realized in double distilled water containing tris(hydroxymethyl)aminomethane (Tris, 5 mM) and sodium chloride (50 mM), with the pH adjusted to 7.2 using hydrochloric acid. A solution of CT-DNA in the buffer gave a UV absorbance ratio at 260 to 280 nm of about 1.9, indicating that the DNA was sufficiently free of protein. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar extinction coefficient value of 6600 dm^3 mol^{-1} cm^{-1} at 260 nm. Electronic absorption titration experiments were performed by maintaining the concentration of the complexes constant (25 μM) but with variable nucleotide concentrations from 0 to 25 μM. While measuring the absorption spectra, equal amounts of DNA were added to both compounds and reference solutions to eliminate the absorbance of DNA itself. The data were then fitted into the following equation and the intrinsic binding constant K_b was calculated in each case:[10]

$$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_b-\varepsilon_f) + (1/K_b)(\varepsilon_b-\varepsilon_f) \quad (1)$$

where [DNA] is the concentration of DNA in base pairs. The apparent absorption coefficients ε_a, ε_f and ε_b correspond to A_{obsd}/[complex], the extinction coefficient of the free compound and the extinction coefficient of the compound when fully bound to DNA, respectively. In plots of $[DNA]/(\varepsilon_a-\varepsilon_f)$ *versus* [DNA], K_b is given by the ratio of the slope to the intercept.

DNA cleavage studies. The DNA cleavage activity of the ruthenium(II) complexes was monitored by agarose gel electrophoresis on CT-DNA. Each reaction mixture contained 30 μM of CT-DNA, 30 and 60 μM of each complex in 50 mM Tris–HCl, (pH 7.1). The reaction was incubated at 37 °C for 2 h. After incubation, 1μL of loading buffer (0.25 % bromophenol blue, 0.25 % xylene cyanol and 60 % glycerol) was added to the reaction mixture and loaded onto a 1 % agarose gel containing 1.0 μg mL^{-1} of ethidium bromide (3,8-diamino-5-ethyl-6--phenylphenanthridinium bromide). The electrophoresis was performed for 2 h at 50 V in Tris–acetic acid–EDTA (ethylenediaminetetraacetic acid) buffer, pH 7.1. The bands were visualized under UV light and photographed.

RESULTS AND DISCUSSION

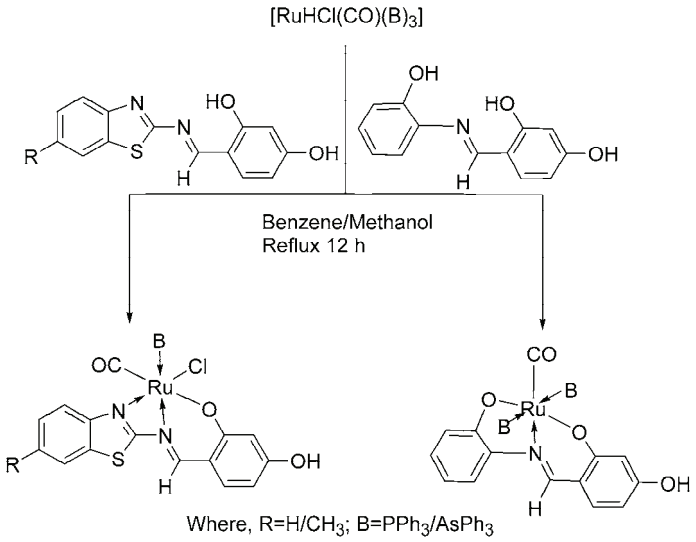
A new series of ruthenium(II) dihydroxybenzaldehyde Schiff base complexes were synthesized, stable in air at room temperature, non-hygroscopic in nature and soluble in common solvents such as dichloromethane, dimethylformamide and dimethyl sulphoxide. The analytical data (Table I) of the ligands and complexes are in good agreement with the calculated values, thus confirming the proposed molecular formulae (Scheme 2).

IR spectra

The IR spectra of the complexes, in comparison with those of the free ligands, display certain changes, which gives an indication about the mode of coordination and their structure. The significant IR spectral bands of the ligands and the complexes are listed in Table II. The free Schiff bases show a very strong absorption around 1636–1626 cm^{-1}, which is the characteristic of the azomethine (>C=N) group.[11] Coordination of the Schiff bases to the ruthenium ion through the azomethine nitrogen atom is expected to reduce the electron density in the azo

TABLE I. Physicochemical analysis data of the ligands and ruthenium(II) Schiff base complexes

Ligands and complexes	Empirical formula	Colour	Yield, %	M.p. °C	Found (Calcd.), %			
					C	H	N	S
L^1	$C_{14}H_{10}N_2O_2S$	Yellow	82	221	62.56 (62.21)	3.81 (3.73)	10.11 (10.36)	12.14 (11.86)
L^2	$C_{15}H_{12}N_2O_2S$	Yellow	78	212	63.42 (63.34)	4.48 (4.24)	9.44 (9.85)	11.49 (11.28)
L^3	$C_{13}H_{11}NO_3$	Orange	72	245	67.80 (68.11)	4.51 (4.84)	6.37 (6.11)	–
[RuCl(CO)(PPh_3)(L^1)] (**1**)	$C_{33}H_{24}ClN_2O_3PRuS$	Pink	66	285	56.62 (56.94)	4.03 (3.69)	4.28 (3.94)	4.73 (4.52)
[RuCl(CO)(PPh_3)(L^2)] (**2**)	$C_{34}H_{26}ClN_2O_3PRuS$	Pink	62	275	57.12 (57.50)	4.01 (4.15)	4.04 (4.17)	4.42 (4.55)
[Ru(CO)(PPh_3)$_2$(L^3)] (**3**)	$C_{51}H_{41}NO_4P_2Ru$	Green	61	293	68.12 (68.45)	4.51 (4.62)	1.05 (1.57)	–
[RuCl(CO)($AsPh_3$)(L^1)] (**4**)	$C_{33}H_{24}ClN_2O_3AsRuS$	Brown	68	267	53.21 (53.56)	3.70 (3.27)	4.02 (3.79)	4.54 (4.33)
[RuCl(CO)($AsPh_3$)(L^2)] (**5**)	$C_{34}H_{26}ClN_2O_3AsRuS$	Brown	64	271	53.40 (54.15)	3.01 (3.48)	3.92 (3.71)	4.30 (4.25)
[Ru(CO)($AsPh_3$)$_2$(L^3)] (**6**)	$C_{51}H_{41}NO_4As_2Ru$	Black	63	288	62.67 (62.33)	4.42 (4.20)	1.67 (1.43)	–



Scheme 2. Formation of the new ruthenium(II) Schiff base complexes.

methine link and thus lower the ν(C=N) absorption frequency. Hence these bands undergo shift to lower frequencies (1621–1594 cm^{-1}) after complexation, indicating coordination of the azomethine nitrogen to ruthenium.[12,13] The IR spectrum of the ligands revealed a medium intensity band at 1599–1598 cm^{-1}

(C=N) of the thiazole ring, which were shifted to lower frequencies (1586–1580 cm^{-1}) after complexation in the spectra of complexes **1**, **2**, **4** and **5**, which also indicated that the thiazole ring was affected upon coordination to the ruthenium metal ion.[14] The free ligands exhibit a broad band at 3426–3370 cm^{-1}, which may be assigned to the phenolic ν(OH) and this band was absent in the spectra of all the complexes, implying deprotonation of the Schiff bases prior to coordination. The hydroxy protons were displaced by the metal leading to higher ν(C–O) values (1285–1252 cm^{-1}) compared to those of the free ligands (1274–1242 cm^{-1}), suggesting that the other coordinating atom was the phenolic oxygen.[15] The binding of the metal to the ligand through nitrogen and oxygen atoms was further supported by the appearance of new bands in the 460–400 cm^{-1} and 540–510 cm^{-1} ranges due to ν(M–N) and ν(M–O),[16] respectively, in the spectra of all the complexes. A strong band for all the complexes in the region 1957–1931 cm^{-1} is due to terminally coordinated carbonyl groups. The ν(C–S–C) at 743 cm^{-1} of the thiazole ring remained unchanged, which demonstrated that the sulphur atom of the thiazole group does not coordinate to the ruthenium metal. In addition, the Schiff base complexes showed strong vibrations near 520, 695, 740 and 1430 cm^{-1}, which are attributed to the triphenylphosphine or triphenylarsine fragments.[17]

TABLE II. FT-IR and electronic spectral data for the ligands and ruthenium(II) Schiff base complexes

Ligands and complexes	FT-IR, ν / cm^{-1}					UV–Vis
	C=N	Ph-OH	Ph-CO	C≡O	C=N thiazole	λ_{max} / nm
L^1	1649	3390	1254	–	1598	309, 368, 416, 448
L^2	1626	3426	1242	–	1599	307, 368, 420, 442
L^3	1651	3370	1274	–	–	310, 366, 392, 435
$[RuCl(CO)(PPh_3)(L^1)]$ (**1**)	1597	–	1285	1941	1573	308, 365, 421, 450, 486
$[RuCl(CO)(PPh_3)(L^2)]$ (**2**)	1597	–	1258	1931	1575	308, 362, 425, 452, 481
$[Ru(CO)(PPh_3)_2(L^3)]$ (**3**)	1611	–	1313	1943	–	308, 368, 420, 452, 480
$[RuCl(CO)(AsPh_3)(L^1)]$ (**4**)	1594	–	1284	1939	1568	306, 368, 416, 441, 481, 524
$[RuCl(CO)(AsPh_3)(L^2)]$ (**5**)	1594	–	1252	1957	1582	304, 363, 412, 442, 483, 521
$[Ru(CO)(AsPh_3)_2(L^3)]$ (**6**)	1621	–	1309	1943	–	307, 369, 420, 454

Electronic spectra

The electronic spectra of all the ligands and complexes in DMSO showed four to six bands in the 306–524 nm regions as given in Table II. The electronic spectra of all the free ligands showed two types of transitions, the first one appeared in the range 309–368 nm that could be assigned to π–π* transitions,

which were due to transitions involving molecular orbitals located on the phenolic chromophore. These peaks were shifted in the spectra of the complexes. This may be due to the donation of a lone pair of electrons by the oxygen of the phenoxy group to the central metal atom.[18] The second type of transitions appeared at the range 309–448 nm that could be assigned to n–π* transitions, which were due to transitions involving the molecular orbitals of the C=N chromophore. These bands were also shifted upon complexation, indicating that the imine group nitrogen atom could be coordinated to the metal ion.[19]

The ground state of ruthenium(II) in an octahedral environment is $^1A_{1g}$, arising from the t_{2g}^6 configuration. The excited state terms are $^3T_{1g}$, $^3T_{2g}$, $^1T_{1g}$ and $^1T_{2g}$. Hence four bands corresponding to the transition $^1A_{1g}\rightarrow{}^3T_{1g}$, $^1A_{1g}\rightarrow{}^3T_{2g}$, $^1A_{1g}\rightarrow{}^1T_{1g}$ and $^1A_{1g}\rightarrow{}^1T_{2g}$ in order of increasing energy are possible. The bands around 521–524 nm and 392–486 nm are assigned to $^1A_{1g}\rightarrow{}^1T_{1g}$ and charge transfer (CT) transitions, respectively.[12,20] The charge transfer bands observed in all the complexes due to M→L transitions are possible in the visible region. Moreover, the presences of carbonyl, triphenylphosphine/arsine and heterocyclic bases as ligands, which are capable of producing strong ligand field e_g^* levels place these levels relatively high in energy. Therefore, the lowest charge bands due to excitation of an electron from the metal t_{2g} level to an unfilled molecular orbital derived from the π* level of the ligands should appear in the relatively high energy region compared to those due to $t_{2g}\rightarrow e_g^*$ transitions.[20–22] The other high intensity bands in the region 306–369 nm region were assignable to ligand centred (LC) transitions and have been designated as π–π* and n–π* transition. The pattern of the electronic spectra of all the complexes indicated the presence of an octahedral environment around the ruthenium(II) ion, similar to that of other ruthenium(II) octahedral complexes.[23]

Mass spectra

The EI mass spectra of the ligands and complexes were recorded. The maximum peaks are observed at *m*/*z*, 284, 230, 696, 755 for the ligands L^2 and L^3 and for complexes [RuCl(CO)(PPh_3)(L^1)] and [RuCl(CO)($AsPh_3$)(L^2), respectively, which match well with the corresponding calculated masses.

NMR spectra

The ^{1}H-NMR spectra of ligands and complexes were recorded in DMSO-d_6 solution for confirming the binding mode of the Schiff base to ruthenium ion and the values are given in Table III and the spectra are shown in Figs. 1 and 2. The aromatic proton for the ligands appears as a multiplet at δ 6.24–8.03 ppm. On complexation, the protons on the phenyl ring remain more or less unchanged in the complexes, even if there are slight variation in their resonances due to the delocalization of electron density in the system,[24] and these signals in the com-

plexes cannot be distinguished from the aromatic signals of PPh_3/$AsPh_3$ due to their extensive overlap appearing at δ 6.22–8.24 ppm.[25] The protons of the hydroxyl groups appear as broad singlets at δ 11.64–11.98 (2-OH), δ 9.79–9.92 ppm (4-OH) and 14.27 ppm (Ph–OH) for the free Schiff base ligands. In the spectra of the complexes, the resonances arising from the hydroxyl (2-OH) and (Ph–OH) proton were not observed, indicating the coordination of the hydroxyl oxygens to the metal ion.[14] The signal due to the azomethine proton (–HC=N) was found to be considerably deshielded at δ 9.12–9.35 ppm relatively to that of the free Schiff base ligand δ 8.77–9.24 ppm as a consequence of electron donation to the metal centre. The methyl proton for L^2 and its complexes appeared as a singlet at δ 2.90–2.34 ppm.

TABLE III. ^{1}H-NMR data for the ligands and ruthenium(II) Schiff base complexes

Ligands and complexes	^{1}H-NMR data, δ / ppm
L^1	6.39–8.03 (*m*, Ar), 9.24 (*s*, HC=N), 11.98 (*s*, 2-OH), 9.92 (*s*, 4-OH)
L^2	6.20–7.65 (*m*, Ar), 9.07 (*s*, HC=N), 11.90 (*s*, 2-OH), 9.79 (*s*, 4-OH), 2.29 (*s*, CH_3)
L^3	6.24–7.43 (*m*, Ar), 8.77 (*s*, HC=N), 11.60 (*s*, 2-OH), 9.92 (*s*, 4-OH), 14.27 (*s*, OH)
[RuCl(CO)(PPh_3)L^1] (**1**)	7.15–7.67 (*m*, Ar), 9.32 (*s*, HC=N), 9.94 (*s*, 4-OH)
[RuCl(CO)(PPh_3)L^2] (**2**)	7.26–7.70 (*m*, Ar), 9.12 (*s*, HC=N), 9.84 (*s*, 4-OH), 2.34 (*s*, CH_3)
[Ru(CO)(PPh_3)$_2L^3$] (**3**)	6.62–8.23 (*m*, Ar), 9.14 (*s*, HC=N), 9.92 (*s*, 4-OH)
[RuCl(CO)($AsPh_3$)L^1] (**4**)	6.92–7.76 (*m*, Ar), 9.35 (*s*, HC=N), 10.14 (*s*, 4-OH),
[RuCl(CO)($AsPh_3$)L^2] (**5**)	6.26–7.83 (*m*, Ar), 9.32 (*s*, HC=N), 9.91 (*s*, 4-OH), 2.28 (*s*, CH_3)
[Ru(CO)($AsPh_3$)$_2L^3$] (**6**)	6.82–7.66 (*m*, Ar), 9.16 (*s*, HC=N), 9.85 (*s*, 4-OH)

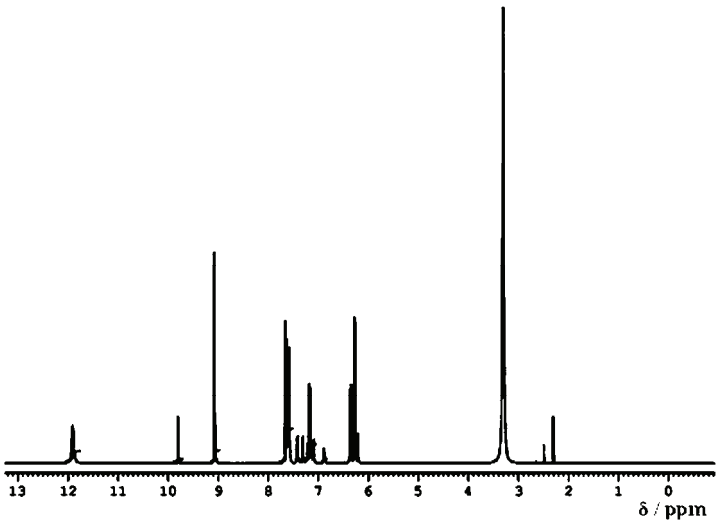


Fig.1. ^{1}H-NMR spectrum of ligand L^2.

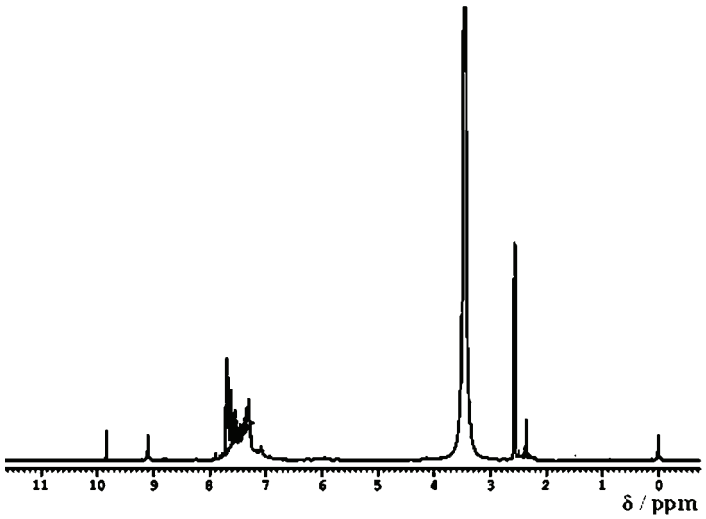


Fig. 2. ^{1}H-NMR spectrum of [RuCl(CO)(PPh_3)L^2].

The ^{13}C-NMR data were recorded in DMSO-d_6 solution and the assignments for the ligands and the complexes are listed in Table IV and a respective spectrum is shown in Fig. 3. The ^{13}C-NMR spectra of all the Schiff base ligands displayed a single resonance at δ 150–152 ppm,[26] showing that the azomethine carbon atoms were equivalent, which also confirms the structure of the ligands. The signal at δ 164–165 ppm corresponds to thiazolic C=N carbon.[27] The downfield shift of these two signals at δ 164–165 and 169–172 ppm clearly indicates that both the C=N carbons were affected by coordination.[28] The aromatic carbons of the free ligands and the corresponding complexes show signals in the region δ 102–139 ppm. The signal due to the methyl carbon of L^2 and the corresponding complexes appeared at δ 21–22 ppm. For all the complexes, the terminal carbonyl carbon appeared in the range δ 191–194 ppm.[29]

TABLE IV. ^{13}C-NMR data for the ligands and ruthenium(II) Schiff base complexes

Ligands and complexes	^{13}C-NMR data, δ / ppm
L^1	117–137 (Ar C), 152 (C=N), 164 (thiazole, C=N)
L^2	102–134 (Ar C), 151 (C=N), 165 (thiazole, C=N), 21 (CH_3)
L^3	107–134 (Ar C), 150 (C=N),
[RuCl(CO)(PPh_3)L^1] (**1**)	128–133 (Ar C), 164 (C=N), 170 (thiazole, C=N), 194 (C≡O)
[RuCl(CO)(PPh_3)L^2] (**2**)	122–134 (Ar C), 165 (C=N), 169 (thiazole, C=N), 191 (C≡O), 21 (CH_3)
[Ru(CO)(PPh_3)$_2L^3$] (**3**)	122–139 (Ar C), 165 (C=N), 191 (C≡O)
[RuCl(CO)($AsPh_3$)L^1] (**4**)	112–134 (Ar C), 165 (C=N), 172 (thiazole, C=N), 191 (C≡O)
[RuCl(CO)($AsPh_3$)L^2] (**5**)	127–133 (Ar C), 164 (C=N), 171 (thiazole, C=N), 192 (C≡O), 22 (CH_3)
[Ru(CO)($AsPh_3$)$_2L^3$] (**6**)	128–139 (Ar C), 164 (C=N), 193 (C≡O)

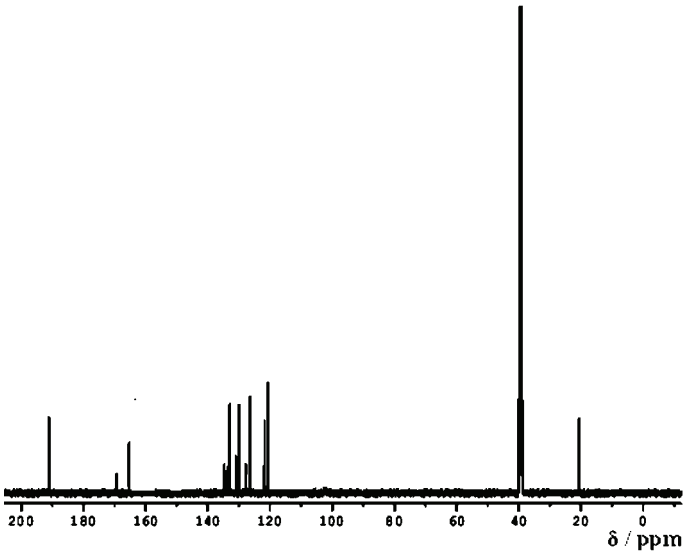


Fig. 3. ^{13}C-NMR spectrum of [RuCl(CO)(PPh_3)L^2].

In order to confirm the presence of triphenylphosphine groups and to determine the geometry of the complexes ^{31}P-NMR spectra were recorded. The ^{31}P--NMR spectra of the complexes [Ru(CO)Cl(PPh_3)L^1] and [Ru(CO)(PPh_3)$_2$L^3] were recorded in DMSO-d_6 solution. The observation of a sharp singlet at δ 28.2 and 32.2 ppm for the complexes [Ru(CO)Cl(PPh_3)L^1] and [Ru(CO)(PPh_3)$_2$L^3], respectively, confirmed the presence of only one triphenylphosphine group. The appearance of only one signal for [Ru(CO)(PPh_3)$_2$L^3] suggests that the two triphenylphosphine groups are magnetically equivalent and hence, they must be *trans* to each other in the complex.

DNA binding study

The interactions of metal complexes with DNA are of interest for the development of effective chemotherapeutic agents. Transition metal centres are particularly attractive moieties for such research since they exhibit well-defined coordination geometries and often possess distinctive electrochemical or photophysical properties, thus enhancing the functionality of the binding agent.[2] Electronic absorption spectroscopy is one of the most useful techniques for DNA-binding studies of metal complexes. The interactions of the ruthenium complexes with CT-DNA were investigated by UV–Vis absorption titrations. The binding of the ruthenium(II) complexes to DNA helices were characterized by following the changes in the absorbance and shift in wavelength on each addition of DNA solution to the complex. Upon addition of increasing amounts of CT-DNA from 0 to 25 μM, a significant “hyperchromic” effect of the intraligand bands at 252–

–323 nm was observed accompanied by a moderate red shift of 2–3 nm, indicative of the breakage of the DNA helix. However, there were no appreciable wavelength shifts in the charge transfer band. These spectral characteristics suggest that the complexes and ligand bind either to the external contact (electrostatic binding) or to the major and minor grooves of DNA. As the concentration of the DNA was increased, the absorption bands of the [RuCl(CO)(PPh_3)L^2] complex initially showed hyperchromism but on further increasing, hypochromism with a blue shift of 5 nm was observed. An isosbestic point was observed at 295 nm. This behaviour reveals an electrostatic association of the complex with the helix surface.[30,31] Generally, hypochromism and hyperchromism are the two spectral features which are closely connected with the double helix structure of DNA. The observation of hypochromism is indicative of intercalative mode of binding of DNA to the complexes along with the stabilization of the DNA double helix structure.[32] On the other hand, the observation of hyperchromism is indicative of breakage of the secondary structure of DNA.[33] The binding constant of the complex [RuCl(CO)(PPh_3)L^2] could not be evaluated due to the random changes in the absorption on the addition of DNA. Hence, the observation of hyperchromism with a red shift for the ligand and complexes showed that they interact with the secondary structure of CT-DNA by breaking its double helix structure.

In order to compare the DNA-binding affinity of the ruthenium(II) complexes quantitatively, their intrinsic binding constants were calculated by monitoring the changes in absorption of the higher energy band with increasing concentration of DNA(Eq. (1) and Fig. 4). The intrinsic binding constants K_b were calculated and were found to be (1.6–8.6)×10^4 mol^{-1} dm^3, for the ruthenium(II) complexes (Fig. 4 and Table V). The magnitude of the binding constant clearly showed that complex [Ru(CO)(PPh_3)$_2$(L^3)] bound more strongly with CT-DNA than the other complexes. The significant difference in DNA-binding affinity of the ruthenium(II) complexes may be a result of the fact that complex with different co-ligands shows different binding affinity with DNA. Interestingly, the K_b values obtained for the above ruthenium(II) complexes are comparable with those for another known complex [Ru(dmp)$_2$(APIP)]$^{2+}$, APIP = 2-(2-aminophenyl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline, (2.3–3.3)×10^4 mol^{-1} dm^3.[34]

DNA cleavage activity

To assess the DNA cleavage ability of the new ruthenium(II) complexes, calf thymus DNA was incubated with two different concentrations of the complexes in 5 mM Tris–HCl/50 mM NaCl buffer at pH 7.2 for 2 h without the addition of a reductant. Upon gel electrophoresis of the reaction mixture, concentration-dependent DNA cleavage was observed. When the concentration of

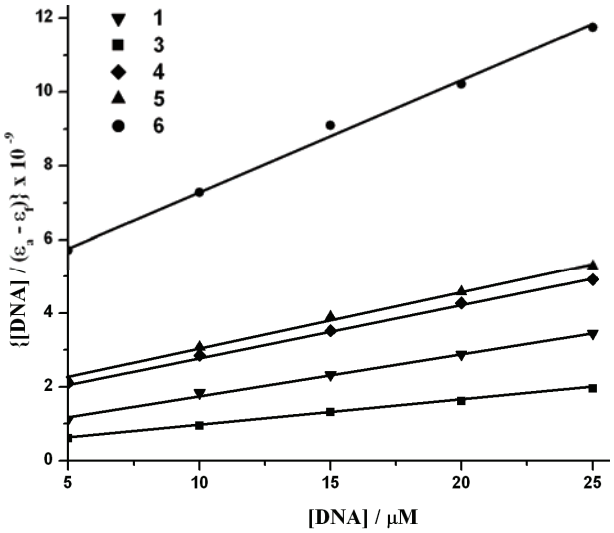


Fig. 4. Plots of [DNA]/(ε_a–ε_f) *versus* [DNA] for the titration of the prepared compounds with CT-DNA.

TABLE V. Binding constant value for interaction of the ruthenium(II) complexes with CT-DNA

Complex	$K_b \times 10^4$ / mol^{-1} dm^3
[RuCl(CO)(PPh_3)(L^1)] (**1**)	8.2
[RuCl(CO)(PPh_3)(L^2)] (**2**)	–
[Ru(CO)(PPh_3)$_2$(L^3)] (**3**)	8.6
[RuCl(CO)($AsPh_3$)(L^1)] (**4**)	5.6
[RuCl(CO)($AsPh_3$)(L^2)] (**5**)	3.3
[Ru(CO)($AsPh_3$)$_2$(L^3)] (**6**)	1.6

the complexes **1**–**6** was increased from 30 to 60 μM, the production of Form II of DNA increased, (Fig. 5 for complexes **1**–**3**, and Fig. 6 for complexes **4**–**6**). No DNA cleavage was observed for the control in which metal complex was absent (Fig. 5, lane 1, and Fig 6, lane 8). With increasing concentration of the ruthenium(II) complexes (Fig. 5, lanes 2 and 3 for complex **1**; lanes 4 and 5 for complex **2**; lanes 6 and 7 for complex **3** and Fig. 6, lanes 9 and 10 for complex **4**; lanes 11 and 12 for complex **5**; lanes 13 and 14 for complex **6**) the amount of Form I of CT-DNA diminished gradually and the amount of the nicked circular DNA (Form II) increased remarkably. When the concentration was increased to 60 μM for all the complexes, the DNA was completely converted from Form I to Form II, showing the potential chemical nuclease activity of the complexes. Moreover, complex [RuCl(CO)(PPh_3)(L^1)] (**1**) exhibited greater cleavage efficiency than other complexes, which could be attributed to the longer metal-to-ligand charge transfer (MLCT) excited state lifetime of [RuCl(CO)(PPh_3)(L^1)].

Fig. 5. Gel electrophoresis showing the chemical nuclease activity of CT-DNA incubated at 37 °C for 2 h with different concentrations of complexes **1**–**3**; lane 1, DNA control; lane 2, DNA + complex **1** (30 μM); lane 3, DNA + complex **1** (60 μM); lane 4, DNA + complex **2** (30 μM); lane 5, DNA + complex **2** (60 μM); lane 6, DNA + complex **3** (30 μM); lane 7, DNA + complex **3** (60 μM).

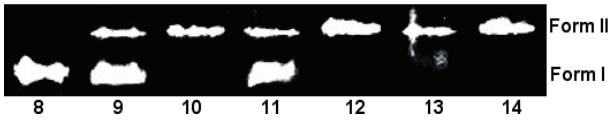


Fig. 6. Gel electrophoresis showing the chemical nuclease activity of the CT-DNA incubated at 37 °C for 2 h with different concentrations of complexes **4**–**6**; lane 8, DNA control; lane 9, DNA + complex **4** (30 μM); lane 10, DNA + complex **4** (60 μM); lane 11, DNA + complex **5** (30 μM); lane 12, DNA + complex **5** (60 μM); lane13, DNA + complex **6** (30 μM); lane 14, DNA + complex **6** (60 μM).

CONCLUSION

Three novel Schiff base ligands and their ruthenium(II) complexes were designed, synthesized and characterized by elemental analyses, stoichiometric and spectroscopic studies. Based on the characterization, an octahedral geometry was tentatively proposed for all the new ruthenium(II) complexes. Furthermore, *in vitro* DNA binding studies were performed for complexes **1**–**6** using the absorption titration technique. These complexes are unique and act synergistically at the molecular level but with different binding modes. This study revealed that the complexes bind electrostatically to the DNA double helix surface. The binding constants were found to be $(1.6–8.6)\times10^4$ mol^{-1} dm^3 for complexes **1** and **3**–**6**. Interestingly, the K_b values obtained for the above ruthenium(II) complexes were comparable to those for other known $[Ru(dmp)_2(APIP)]^{2+}$ complexes (APIP = 2-(2-aminophenyl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline), $(2.3–3.3)\times10^4$ mol^{-1} dm^3. From the values of the binding constant, it was inferred that the triphenylphosphine complexes bind more with CT-DNA than the corresponding triphenylarsine complexes. The DNA cleavage study revealed that all ruthenium complexes had the ability to cleave nucleic acids and the extent of the cleavage was found to be dose dependent. The information obtained in this study could be helpful in the understanding of the mechanism of interactions of ruthenium(II) complexes with nucleic acids and should be useful in the development of potential probes for investigation of the structure and conformation of DNA, or new therapeutic agents for some diseases.

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ИЗВОД

СИНТЕЗА И СПЕКТРАЛНА КАРАКТЕРИЗАЦИЈА КОМПЛЕКСА РУТЕНИЈУМА(II) СА ШИФОВИМ БАЗАМА КОЈИ САДРЖЕ PPh_3/$AsPh_3$ ЛИГАНДЕ И ИСПИТИВАЊЕ ЊИХОВИХ ИНТЕРАКЦИЈА СА ДНК

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У овом раду су синтетизоване Шифове базе полазећи од дихидроксибензалдехида (L^1–L^3), као и одговарајући рутенијум(II) комплекси. Сва једињења су окарактерисана применом елементалне микроанализе, ^{1}H-, ^{13}C- и ^{31}P-NMR, и IR спектроскопије, UV–Vis спектрофотометрије и масене спектрометрије. Применом UV–Vis спектрофотометрије испитиване су интеракције комплекса рутенијума(II) и ДНК. На основу добијених резултата, закључено је да се сви комплекси електростатички везују за ДНК, при чему су одговарајуће константе везивања (K_b) у опсегу $(1{,}6–8{,}6)\times10^4$ mol^{-1} dm^3. Додатно је испитивано раскидање веза у молекулу ДНК помоћу комплекса рутенијума(II).

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