SYNTHESIS, CHARACTERIZATION, DNA BINDING AND CLEAVAGE ACTIVITY OF RUTHENIUM(II) COMPLEXES WITH HETEROCYCLIC SUBSTITUTED THIOSEMICARBAZONES

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ABSTRACT

A new Schiff base ligands ($H_2L^1-H_2L^3$) was synthesized by the condensation reaction of 4-acyl pyrazolones and 2-benzothiazolyl thiosemicarbazide. The ruthenium(II) Schiff base complexes were prepared by the interaction of the ligands with $[RuHCl(CO)(EPh_3)_2(B)]$ ($E=P/As$; $B=PPh_3/AsPh_3/py$). They were characterized by elemental analysis, IR, UV-vis, 1H , ^{13}C , and ^{31}P NMR spectral data. The interaction of the complexes with CT-DNA has been investigated by electronic absorption spectroscopy technique and the binding mode of the complexes with CT-DNA has been explored. Furthermore, the DNA cleavage activities of the complexes were performed by agarose gel electrophoresis on CT-DNA.

Keywords: Ruthenium(II) complexes, heterocyclic thiosemicarbazide, acyl pyrazolone, DNA-interaction.

INTRODUCTION

Multidendate ligands are extensively used in coordination chemistry, since they can be applied in the construction of new frame works with interesting properties.¹ Among these ligands, the linear or cyclic schiff bases have attracted much attention since most of their compounds prepared to date exhibit noteworthy bioactivity and desirable or predictable physicochemical, stereochemical, electrochemical, structural properties, etc.^{2,3} These properties are due not only to the diverse condensation products of the amine-aldehyde reaction, but also to the participation of the specific metal atom and ligands. Their use as tools for the analysis of pharmacological,^{4,5} substances and as analgesic, anti-inflammatory, antibiotic, antimicrobial,⁶ and especially as anticancer,⁷ agents is well known.

Pyrazolone-5 derivatives, especially 4-acyl pyrazolone, form an important class of organic compounds and represent a big scientific and applied interest in biological, analytical application, catalysis, dye and extraction metallurgy, etc.⁸⁻¹³ Furthermore, 4-acylpyrazolone derivatives have the potential to form different types of coordination compounds due to the several electron-rich donor centers,¹⁴⁻¹⁶ and tautomeric effect of the enol form and keto form.¹⁷⁻¹⁹ Thiosemicarbazones are of considerable pharmacological interest since a number of chemotherapeutic properties. The wide range of biological activities possessed by substituted thiosemicarbazones and their metal complexes include cytotoxic, antitumor, antibacterial and antiviral properties. This is particularly true for the heterocyclic thiosemicarbazones.²⁰ It has been suggested that the antitumor activity of heterocyclic thiosemicarbazones is due to the compounds modifying the reductive conversion of ribonucleotides to deoxyribonucleotides as a result of the inhibition of the ribonucleotide reductase enzyme which inhibits DNA synthesis.²¹ The ligands coordinate to metal ions inside the cell, forming complexes which supposedly act as the true active species. The biological properties of the ligands can be modified and in fact enhanced, by the linkage to metal ions.^{22,23}

Many metal-containing compounds have been shown to exhibit anticancer activity. In particular the platinum based drugs developed over the last 30 years have been remarkably successful in the treatment of testicular and ovarian cancer.^{24,25} More recently, several ruthenium compounds have been under investigation for their antitumor activity.^{26,27} With these considerations, in this paper we report the synthesis, characterization, DNA binding and DNA cleavage studies of ruthenium(II) complexes containing Schiff base derived from 4-acyl pyrazolone and benzothiazolyl thiosemicarbazide (Scheme 1)

Where $R = H/CH_3/C_6H_5$;

Scheme 1. Thio-enol form of Schiff base ligands.

EXPERIMENTAL

Materials and Instrumentation

Reagent grade chemicals were used without further purification in all the synthetic work. All the solvents were purified by standard methods. $RuCl_3 \cdot 3H_2O$, triphenylphosphine/arsine were purchased from Himedia. Calf-thymus (CT-DNA) was purchased from Bangalore Genei, Bangalore, India. Infrared spectra were recorded on a FT-IR Perkin Elmer spectrophotometer RXI model as KBr pellets in the range $4000-400\text{ cm}^{-1}$. Elemental analyses were performed with a model Vario ELIII CHNS at Sophisticated Test and Instrumentation Centre (STIC), Cochin University, Kerala. Electronic spectra were recorded in DMSO solution in a Systronics 2202 Double beam spectrophotometer in $800-200\text{ nm}$ range. 1H , ^{13}C and ^{31}P NMR spectra were recorded on Bruker WM DCX 500 MHz instrument using TMS and ortho phosphoric acid as an internal standard at SAIF, Indian Institute of Technology, Chennai. Melting points were recorded on Veego VMP-DS model heating table and were uncorrected. Benzothiazolyl thiosemicarbazide,²⁸ and the metal precursors $[RuHCl(CO)(PPh_3)_3]$,²⁹ $[RuHCl(CO)(AsPh_3)_3]$,³⁰ $[RuHCl(CO)(PPh_3)_2(py)]$ ³¹ were prepared according to the reported procedures.

Preparation of dibasic tridentate acylpyrazolone of benzothiazolyl thiosemicarbazone ligands

To a methanolic solution (40 mL) of 2-benzothiazolyl thiosemicarbazide (10 mmol), 4-acyl pyrazolones (10 mmol) was added and stirred along with a few drops of glacial acetic acid. The mixture was then reflux for about 8 h. After cooling the reaction mixture to room temperature, the solid product formed was filtered, washed with methanol and dried under vacuum (Scheme 1), and the purity of the ligands were checked by TLC and subjected to purification by column chromatography.

Preparation of ruthenium(II) complexes

All the reaction was carried out under anhydrous condition. The dibasic tridentate acylpyrazolone of benzothiazolyl thiosemicarbazone (0.2 mmol) were added to a solution of $[\text{RuHCl}(\text{CO})(\text{EPh}_3)_2(\text{B})]$, ($\text{E}=\text{P}/\text{As}$; $\text{B}=\text{PPh}_3/\text{AsPh}_3/\text{py}$) (0.2 mmol) in 1:1 molar ratio in benzene-methanol mixture, and they reflux for 12 h. The resulting solution was concentrated to about 3 mL and the complexes were precipitated by the addition of small quantity of petroleum ether (60-80 °C). The complexes were then filtered off, washed with petroleum ether. The purity was checked by TLC and subjected to purification by column chromatography. This solid was recrystallized from $\text{CH}_2\text{Cl}_2/\text{n-hexane}$ mixture. Our sincere efforts to obtain single crystal of the complexes were gone unsuccessful.

DNA-binding and Cleavage assay

Electronic absorption spectroscopy

Experiments involving the interaction of the ruthenium(II) complexes with CT-DNA were carried out in double distilled water with tris(hydroxymethyl)-aminomethane (Tris, 5 mM) and sodium chloride (50 mM) and adjusted to pH 7.2 with hydrochloric acid. A solution of CT-DNA in the buffer gave a ratio of UV absorbance of about 1.9 at 260 and 280 nm, 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 $6600 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ at 260 nm. Electronic absorption titration experiments were performed by maintaining the concentration of the complexes as constant (25 μM) but with variable nucleotide concentration from 0 to 25 μM . While measuring the absorption spectra, equal amounts of DNA were added to both complex and reference solutions to eliminate the absorbance of DNA itself. The data were then fit into the following equation and the intrinsic binding constant K_b was calculated in each case.¹⁵

$$[\text{DNA}] / (\epsilon_a - \epsilon_b) = [\text{DNA}] / (\epsilon_b - \epsilon_f) + 1/K_b(\epsilon_b - \epsilon_f)$$

Where $[\text{DNA}]$ is the concentration of DNA in base pairs, the apparent absorption coefficient ϵ_a , ϵ_b and ϵ_f correspond to $A_{\text{obsd}}/[\text{complex}]$, the extinction coefficient of the free compound and the extinction coefficient of the compound when fully bound to DNA respectively. In plots of $[\text{DNA}] / (\epsilon_a - \epsilon_b)$ versus $[\text{DNA}]$, K_b is given by the ratio of 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/NaCl buffer (pH 7.1). The reaction was incubated at 37 °C for 2 h. After incubation, 1 μL of loading buffer (0.25% bromophenol blue, 0.25% xylene cynol and 60% glycerol) was added to the reaction mixture and loaded onto a 1% agarose gel containing 1.0 $\mu\text{g/mL}$ of ethidium bromide. The electrophoresis was carried out for 2 h at 50 V in Tris-acetic acid EDTA buffer. The bands were visualized under UV light and photographed.

RESULT AND DISCUSSION

A new series of ruthenium(II) acylpyrazolone of benzothiazolyl thiosemicarbazone complexes were synthesized. These are stable in air at room temperature, non-hygroscopic in nature and soluble in common solvents such as dichloromethane, dimethylformamide, dimethylsulphoxide, etc. The analytical data (Table 1) of the ligands and complexes are in good agreement with the calculated values thus confirming the proposed molecular formulae (Scheme 2).

Where $\text{R} = \text{H}/\text{CH}_3/\text{C}_6\text{H}_5$; $\text{E} = \text{P}/\text{As}$; $\text{B} = \text{PPh}_3/\text{AsPh}_3/\text{py}$

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

Spectroscopic data

IR Spectroscopy

There are conspicuous differences between the IR spectrum of the complexes and that of the free ligands are shown in Table 2. In the free ligands, a medium intensity band around $3207\text{-}3219 \text{ cm}^{-1}$ is assigned to $\nu(\text{NH})$ ³³ and the strong band around $1619\text{-}1623 \text{ cm}^{-1}$ is allocated as $\nu(\text{C}=\text{O})$ of pyrazolone ring.⁸ The free ligands display absorption for $\nu(\text{C}=\text{S})$ in the region $832\text{-}837 \text{ cm}^{-1}$. These bands are all absent in the complexes but, two new bands at $1308\text{-}1329 \text{ cm}^{-1}$ for $\nu(\text{C}-\text{O})$ and $737\text{-}752 \text{ cm}^{-1}$ for $\nu(\text{C}-\text{S})$ are observed. From these observations, it is concluded that the ligands reacts in the thioenol form and the enolic protons are replaced by ruthenium(II) ion in the complexes. This shift confirms the participation of oxygen and sulphur in the C-O-M and C-S-M bond. In the low frequency region $430\text{-}470 \text{ cm}^{-1}$ and $438\text{-}458 \text{ cm}^{-1}$ are attributed to (M-O) and (M-S).^{34,35} The band at $(1609\text{-}1612 \text{ cm}^{-1})$ due to $\nu(\text{C}=\text{N})$ azomethine group of the Schiff base underwent a shift to lower frequency ($1574\text{-}1589 \text{ cm}^{-1}$) after complexation, indicating the bonding of unsaturated nitrogen of the azomethine group to the metal ion.³⁶ IR spectrum of the ligands and complexes revealed a medium intensity band around $1507\text{-}1513 \text{ cm}^{-1}$ and $683\text{-}685 \text{ cm}^{-1}$ is assigned to $\nu(\text{C}=\text{N})$ and $\nu(\text{C}-\text{S}-\text{C})$ of the thiazole ring. These bands remains unchanged for all the complexes which demonstrated that the thiazole group of nitrogen and sulphur does not coordinate to the ruthenium metal.³⁷ For all the complexes, a strong band in the region $1942\text{-}1967 \text{ cm}^{-1}$ is due to terminally coordinated carbonyl group. For the complexes $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^1]$, $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^2]$ and $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^3]$, the IR spectrum showed a medium intensity band at $1093\text{-}1119 \text{ cm}^{-1}$ which is characteristics of coordinated nitrogen base. Characteristic bands for triphenylphosphine/arsine are also present in the expected region.³⁸

Electronic spectra

The electronic spectra of all the ligands and its complexes were taken in DMSO and display several bands which were assigned to various transitions on the basis of their absorption wavelength and molar absorption coefficient were given in Table 2. The electronic spectra of the free ligands show two types of transitions, the first one appears at $303\text{-}368 \text{ nm}$, which can be assigned to $\pi - \pi^*$ transition of the phenolic chromophore.³⁹ The bands corresponding to the $\pi - \pi^*$ transitions occur in the same position with slight shift as that of their complexes $306\text{-}370 \text{ nm}$ which reflects that the ligands are involved in the coordination with ruthenium ion. The second type of transition appears at $397\text{-}400 \text{ nm}$, which can be assigned to $n - \pi^*$ transition of acylpyrazolone of benzothiazolyl thiosemicarbazone hetero atoms. There is a bathochromic shift ($16\text{-}24 \text{ nm}$) in the position of the bands corresponding to $n - \pi^*$ transitions. This suggests that the hetero atoms (N, O and S) in the ligands are involved in coordination with ruthenium ion.⁴⁰ The ground state of ruthenium(II) in an octahedral environment is $^1\text{A}_1$, arising from the t_{2g}^6 configuration. The excited state terms are $^3\text{T}_1$, $^3\text{T}_2$, $^1\text{T}_1$ and $^1\text{T}_2$. Hence four bands corresponding to the transition $^1\text{A}_1 \rightarrow ^3\text{T}_1$, $^1\text{A}_1 \rightarrow ^3\text{T}_2$, $^1\text{A}_1 \rightarrow ^1\text{T}_1$ and $^1\text{A}_1 \rightarrow ^1\text{T}_2$ are possible in order of increasing energy. Apart from these intra ligand transitions, three other sets of bands were present in the spectra of all the complexes. The transitions

observed at 447-524 nm in the spectra of the complexes can be assigned to charge transfer transitions.⁴¹⁻⁴³ These bands obscure the weak d-d transitions occurring in this region. 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.⁴⁴

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

Ligands and Complexes	Molecular formula	Molecular weight	Colour	Melting point °C	Elemental Analysis Calculated (found)			
					C %	H%	N%	S%
H ₂ L ¹	C ₁₉ H ₁₆ N ₆ OS ₂	408.08	Yellow	186	55.86 (55.63)	3.95 (4.13)	20.57 (20.92)	15.70 (15.62)
H ₂ L ²	C ₂₀ H ₁₈ N ₆ OS ₂	422.53	Brown	195	56.85 (56.57)	4.29 (4.49)	19.89 (19.75)	15.18 (15.09)
H ₂ L ³	C ₂₅ H ₂₀ N ₆ OS ₂	484.11	Brown	226	61.96 (62.09)	4.16 (4.21)	17.34 (17.59)	13.23 (13.39)
[Ru(CO)(PPh ₃) ₂ L ¹]	C ₅₆ H ₄₄ N ₆ O ₂ P ₂ RuS ₂	1060.15	Pink	275	63.44 (63.27)	4.18 (4.32)	7.92 (8.02)	6.04 (6.19)
[Ru(CO)(AsPh ₃) ₂ L ¹]	C ₅₆ H ₄₄ N ₆ O ₂ As ₂ RuS ₂	1148.03	Pink	282	58.59 (58.46)	3.86 (3.74)	7.32 (7.09)	5.59 (5.44)
[Ru(CO)(py)(PPh ₃)L ¹]	C ₄₄ H ₃₈ N ₇ O ₂ PRuS ₂	892.99	Pink	286	58.89 (58.71)	3.91 (3.76)	11.18 (11.03)	7.31 (7.19)
[Ru(CO)(PPh ₃) ₂ L ²]	C ₅₇ H ₄₆ N ₆ O ₂ P ₂ RuS ₂	1074.16	Pink	294	63.73 (63.88)	4.32 (4.58)	7.82 (7.89)	5.97 (6.12)
[Ru(CO)(AsPh ₃) ₂ L ²]	C ₅₇ H ₄₆ N ₆ O ₂ As ₂ RuS ₂	1162.06	Pink	>300	58.91 (58.73)	3.99 (4.11)	7.23 (7.49)	5.52 (5.38)
[Ru(CO)(py)(PPh ₃)L ²]	C ₄₅ H ₄₀ N ₇ O ₂ PRuS ₂	907.02	Pink	>300	59.31 (59.18)	4.07 (4.27)	11.00 (10.89)	7.20 (7.39)
[Ru(CO)(PPh ₃) ₂ L ³]	C ₆₂ H ₄₈ N ₆ O ₂ P ₂ RuS ₂	1136.23	Pink	288	65.54 (65.38)	4.26 (4.38)	7.40 (7.52)	5.64 (5.51)
[Ru(CO)(AsPh ₃) ₂ L ³]	C ₆₂ H ₄₈ N ₆ O ₂ As ₂ RuS ₂	1224.13	Pink	>300	60.83 (60.76)	3.95 (3.73)	6.87 (6.73)	5.24 (5.06)
[Ru(CO)(py)(PPh ₃)L ³]	C ₅₀ H ₄₂ N ₇ O ₂ PRuS ₂	969.09	Pink	291	61.75 (61.86)	4.02 (4.23)	10.29 (10.54)	6.73 (6.67)

Table 2. IR and electronic spectroscopic data of the ligands and ruthenium(II) complexes.

Ligands/Complexes	FT-IR cm ⁻¹						UV-vis λ _{max} , nm (ε, M ⁻¹ cm ⁻¹)
	ν _(C=N)	ν _(C=S)	ν _(C-S)	ν _(C=O)	ν _(C-O)	ν _(C=O)	
H ₂ L ¹	1609	835	-	1620	-	-	303 (1100), 368 (1410), 399 (1748)
H ₂ L ²	1603	832	-	1619	-	-	308 (1108), 368 (1408), 397 (1738)
H ₂ L ³	1612	837	-	1623	-	-	307 (1104), 368 (1409), 400 (1738)
[Ru(CO)(PPh ₃) ₂ L ¹]	1583	-	745	-	1315	1946	308 (1135), 368 (1442), 421 (1938), 452 (2065), 481 (2072)
[Ru(CO)(AsPh ₃) ₂ L ¹]	1585	-	749	-	1329	1957	307 (1137), 370 (1446), 421 (1948), 454 (2063)
[Ru(CO)(py)(PPh ₃)L ¹]	1583	-	747	-	1319	1952	309 (1147), 368 (1480), 416 (1884), 448 (2010)
[Ru(CO)(PPh ₃) ₂ L ²]	1578	-	739	-	1308	1957	306 (1108), 368 (1400), 430 (1978), 464 (2091), 490 (2138)
[Ru(CO)(AsPh ₃) ₂ L ²]	1582	-	752	-	1321	1942	308 (1147), 368 (1475), 416 (1913), 447 (2024), 483 (2068), 522 (1932)
[Ru(CO)(py)(PPh ₃)L ²]	1574	-	746	-	1317	1954	308 (1154), 368 (1484), 418 (1945), 450 (2054), 486 (2116)
[Ru(CO)(PPh ₃) ₂ L ³]	1589	-	737	-	1323	1967	308 (1156), 368 (1488), 418 (1941), 449 (2055), 481 (2101)
[Ru(CO)(AsPh ₃) ₂ L ³]	1586	-	744	-	1326	1958	308 (1151), 368 (1484), 416 (1923), 447 (2044), 481 (2094), 524 (1885)
[Ru(CO)(py)(PPh ₃)L ³]	1584	-	749	-	1318	1942	308 (1151), 368 (1478), 397 (1777), 495 (1083)

NMR Spectra

The ¹H NMR spectra of all the ligands and complexes were recorded in DMSO-d₆ solution for confirming the binding mode of the Schiff base ligand to ruthenium ion shown in Table 3 and Fig. 1 and 2. The aromatic protons for all the ligands appeared as a multiplet at 6.90-7.80 ppm. On complexation, the protons on the phenyl ring remains 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 ring system,⁴⁵ and these signals in the complexes cannot be distinguished from the aromatic signals of PPh₃/AsPh₃ due to their extensive overlap appeared at 6.61-7.94 ppm.⁴⁶ The signal due to the -N(4)H proton for all the ligands at 8.62-8.78 ppm,⁴⁷ which disappears in the case of metal complexes, showing the bonding of thiolic sulphur to the metal after the deprotonation of the functional group. The signals observed at 8.14-8.25 ppm,⁴⁸ are due -N(5)H proton for the free ligands. On complexation, -N(5)H signal usually shifts and appears at 8.21-8.32 ppm.⁴⁹ The ligands and complexes showed signal at 2.12-2.75 ppm due to methyl group. The ligand H₂L¹ and its complexes showed signal at 9.80-9.87 ppm corresponds to azomethine proton (-CH=N-).

Fig. 1. ¹H NMR spectrum of Ligand H₂L¹

Fig. 2. ^1H NMR spectrum of $[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^1]$. Fig. 3. ^{13}C NMR spectrum of $[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^1]$.

The ^{13}C NMR spectra of the ligands H_2L^1 , H_2L^3 and the complexes, $[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^1]$, $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^1]$, $[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}^2]$ and $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^3]$ were recorded at room temperature in $\text{DMSO}-d_6$ solution and the assignments are listed in the Table 3 and shown in Fig. 3. ^{13}C NMR spectra for the ligands H_2L^1 and H_2L^3 displaced a single resonance at 148-149 ppm,⁵⁰ due to the azomethine carbon atom. The downfield shift of this signal in the complexes at 148-152 ppm clearly indicates the $\text{C}=\text{N}$ carbons are affected by coordination.⁵¹ The thiolic carbon of the thiosemicarbazone ligands appeared at 139 ppm. Upon coordination, these signals are shifted to upfield and appeared at 131-132 ppm for the complexes.⁵² The signal due to methyl carbon for the ligands and its complexes appear at 16-21 ppm. The aromatic carbons of free ligands and its complexes show signal in the region 104-135 ppm. For all the complexes the terminal carbonyl group appeared in the range 189-193 ppm.

Table 3. ^1H and ^{13}C NMR data of ligands and ruthenium(II) complexes

Ligands/Complexes	^1H NMR data (ppm)					^{13}C NMR data (ppm)				
	$\text{HC}=\text{N}$ (s)	Aromatic (m)	$\text{N}(4)\text{H}$ (s)	$\text{N}(5)\text{H}$ (s)	CH_3 (s)	Aromatic Carbon	CH_3	$\text{C}-\text{S}$	$\text{C}=\text{N}$	$\text{C}\equiv\text{O}$
H_2L^1	9.80		8.71	8.25	2.49	119-129	16	139	149	-
H_2L^2	-	6.90-7.80	8.62	8.24	2.36	-	-	-	-	-
H_2L^3	-	6.94-7.78	8.78	8.14	2.62	118-135	21	139	148	-
$[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^1]$	9.82	7.12-7.94	-	8.22	2.12	104-128	17	132	148	189
$[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}^1]$	9.87	6.92-7.66	-	8.27	2.23	-	-	-	-	-
$[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^1]$	9.85	6.68-7.76	-	8.24	2.62	117-129	18	132	151	192
$[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^2]$	-	6.92-7.57	-	8.27	2.62	-	-	-	-	-
$[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}^2]$	-	7.05-7.67	-	8.32	2.75	117-127	16	131	148	193
$[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^2]$	-	7.20-7.70	-	8.22	2.48	-	-	-	-	-
$[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^3]$	-	6.93-7.80	-	8.32	2.35	-	-	-	-	-
$[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}^3]$	-	6.61-7.66	-	8.21	2.62	-	-	-	-	-
$[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^3]$	-	6.63-7.80	-	8.27	2.37	117-129	18	132	152	191

DNA Binding and Cleavage

Electronic absorption spectroscopy

The interactions of metal complexes with DNA have been the subject of interest for the development of effective chemotherapeutic agents. Transition metal centers are particularly attractive moieties for such research since they exhibit well-defined coordination geometries and also often possess distinctive electrochemical or photophysical properties, thus enhancing the functionality of the binding agent.⁵³ The electronic spectra of the complexes upon the addition of DNA are shown in Fig 3. The titrations results showed clearly that with increasing concentration of DNA added to the complexes, significant hyperchromism with red shift was observed for the $[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^1]$, $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^1]$, $[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}^2]$, $[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^3]$ and

In order to confirm the presence of triphenylphosphine group and to determine the geometry of the complexes ^{31}P NMR spectra were recorded. ^{31}P NMR spectra of the complex $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^1]$ and $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^2]$ were recorded in $\text{DMSO}-d_6$ solution. The observation of a sharp singlet at 28.2 and 27.8 ppm for the complexes confirms the presence of only one phosphine group.

$[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^3]$ complexes. This can be attributed to a strong interaction between DNA and complexes. However, there were no appreciable wavelength shifts in the charge transfer band. Based on the results obtained from the UV-vis titration, it is inferred that the complexes underwent a non-intercalative mode of binding with DNA. 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. On the other hand, the observation of hyperchromism is indicative of the break age of the secondary structure of DNA. Hence, the observation of hyperchromism with red shift for our complexes showed that the new complexes interact with the secondary structure of CT-DNA by breaking

its double helix structure. A similar hyperchromism has been observed for a ruthenium(II) complexes bearing Schiff base ligand.⁵⁴

The intrinsic binding constant (K_b) were calculated for ruthenium(II) complexes are varies in the range of $6.2 \times 10^4 \text{ M}^{-1}$ ($[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^1]$), $7.8 \times 10^4 \text{ M}^{-1}$ ($[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}^1]$), $1.6 \times 10^4 \text{ M}^{-1}$ ($[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}^2]$), $4.8 \times 10^4 \text{ M}^{-1}$ ($[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^3]$), $8.2 \times 10^4 \text{ M}^{-1}$ ($[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}^3]$) respectively. The significant difference in DNA-binding affinity of the ruthenium(II) complexes can be understood as a result of the fact that the complex with different ligands shows stronger binding affinity with DNA. On comparing the results, $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}^3]$ shows more binding affinity than the other complexes. Interestingly, the K_b values obtained for the above ruthenium(II) complexes are comparable than those for the other known polypyridyl Ru(II) complexes $1.0\text{--}4.8 \times 10^4 \text{ M}^{-1}$.^{55,56}

Fig. 4. Absorption spectral traces of the ruthenium(II) complexes (a) $[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^1]$, (b) $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}^1]$, (c) $[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}^2]$, (d) $[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^3]$ and (e) $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}^3]$ with increasing concentration of CT-DNA in a Tris HCl - NaCl buffer (pH 7.2).

DNA cleavage activity

The cleavage efficiency of ruthenium(II) complexes, $[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^1]$, $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}^1]$, $[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}^2]$ and $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}^3]$ were compared to that of the control is due to their efficient DNA binding ability. The cleavage ability of the ruthenium(II) complexes to CT-DNA was investigated by gel electrophoresis in Tris HCl/NaCl buffer (pH 7.2) without any reductant and incubated for 2 h at 37 °C. Control experiment using DNA alone does not show any significant cleavage of CT-DNA even on longer exposure time. All the ruthenium(II) complexes cleave DNA at different concentrations as compared with control DNA Fig. 5. The amount of cleavage was enhanced with increasing concentration of the complexes, showing their potential chemical nuclease activity. The DNA cleavage results showed that $[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^1]$ has more cleavage activity than the other complexes.

Fig. 5. The gel electrophoresis showing the chemical nuclease activity of the CT-DNA incubated at 37 °C for 2 h with different concentration of complex $[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^1]$, $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}^1]$, $[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}^2]$ and $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}^3]$; Lane 1: DNA control; Lane 2: 30 μM $[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^1]$ + DNA; Lane 3: 60 μM $[\text{Ru}(\text{CO})(\text{PPh}_3)_2\text{L}^1]$ + DNA; Lane 4: 30 μM $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}^1]$ + DNA; Lane 5: 60 μM $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}^1]$ + DNA; Lane 6: 30 μM $[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}^2]$ + DNA; Lane 7: 60 μM $[\text{Ru}(\text{CO})(\text{AsPh}_3)_2\text{L}^2]$ + DNA; Lane 8: 30 μM $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}^3]$ + DNA; Lane 9: 60 μM $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)\text{L}^3]$ + DNA.

CONCLUSION

Ruthenium(II) complexes with heterocyclic substituted thiosemicarbazone were synthesized and characterized. Based on various physico-chemical and spectroscopic methods, the complexes are tentatively assigned an octahedral geometry. Further the newly synthesized complexes were evaluated for DNA-binding and DNA cleavage studies. All the complexes bind to DNA through an electrostatic mode which shows that the molecular ellipticity is less. $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^+]$ shows highest binding affinity than other complexes. From the binding constant values, it is inferred that the pyridine/triphenylphosphine complexes bind more with CT-DNA than the other complexes. The DNA cleavage study reveals that all ruthenium complexes have the ability to cleavage nucleic acids and the extent of the cleavage was found to be dose dependent. $[\text{Ru}(\text{CO})(\text{py})(\text{PPh}_3)_2\text{L}^+]$ has higher cleavage activity than other complexes. DNA-complex binding is believed to be the key reaction responsible for the anticancer activity of the compounds.

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