Synthesis, spectroscopic, electrochemical and antibacterial studies of new Ru(II) 1,10-phenanthroline complexes containing aryldiazopentane-2,4-dione as co-ligand

Lallan Mishra,*[a] Ajay Kumar Yadaw,[a] Sudha Srivastava[b] and Anant Bahadur Patel[b]

[a] *Faculty of Science, Chemistry Department, Banaras Hindu University, Varanasi 221 005, India. E-mail: lmishra@banaras.ernet.in; Fax: +91 542 317 074*

[b] *Division of Chemical Physics, Tata Institute of Fundamental Research (TIFR), Mumbai, India*

Received (in Strasbourg, France) 9th February 2000, Accepted 10th April 2000
Published on the Web 19th June 2000

Mononuclear and dinuclear Ru(II) complexes of 1,10-phenanthroline containing aryldiazopentane-2,4-diones (L^1H–L^3H_2) as co-ligands were prepared and characterised using IR, ^1H NMR, UV/Vis spectra in addition to their elemental analysis and FAB mass spectral data. A representative ligand L^2H_2 and its complex $[Ru_2L^2(phen)_4]^{2+}$ (phen = 1,10-phenanthroline) were characterised also by ^1H–^1H COSY, TOCSY and ^1H–^{13}C HMBC spectral data. Electrochemical behaviour of the complexes was studied in acetonitrile solution and showed irreversible Ru^{II}/Ru^{III} redox couples. Luminescence and UV/Vis spectral properties of the complexes in the presence and absence of buffered solutions of calf thymus DNA were also compared. Antibacterial activity of the complexes has been evaluated against *Pseudomonas aeruginosa.*

Ruthenium polypyridyl complexes have been the subject of considerable research interest owing to their unique photophysical, photochemical and redox properties.[1,2] Among the polypyridyl groups, 1,10-phenanthroline (phen) is[3] more rigid and planar than 2,2′-bipyridine and natural products containing 1,10-phenanthroline rings have been found to show interesting bio-activities.[3] Additionally, aza compounds have been studied in the context of many important biological processes[4] such as protein synthesis inhibition, carcinogenesis and nitrogen fixation, and their antitumour properties have been understood in view of the observation[5] that they act as DNA cross-linking agents. Furthermore, the luminescence of Ru(II) complexes bearing 1,10-phenanthroline as terminal ligand has been exploited to recognize and react with nucleic acids, which again allows them to act as diagnostic and therapeutic agents.[6] Thus, in view of the above facts and in continuation to our earlier interest[7] on Ru(II) polypyridyl complexes, we found it worthwhile to synthesise new mono- and dinuclear Ru(II) 1,10-phenanthroline complexes containing aryldiazopentane-2,4-dione as co-ligand for spectroscopic and antibacterial studies.

Experimental

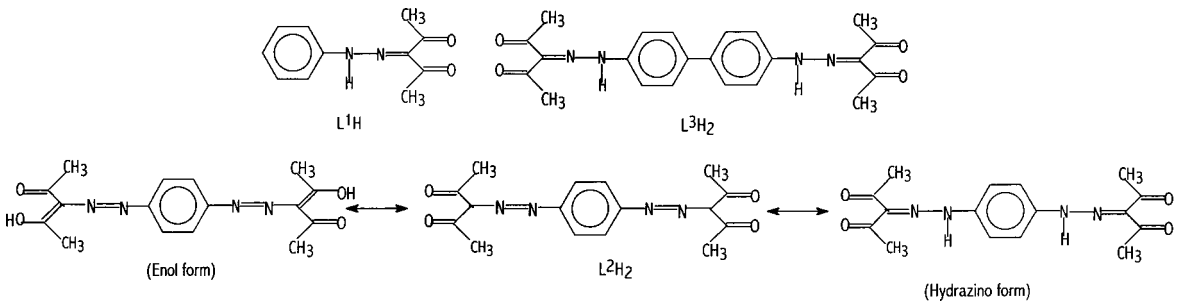
Solvents, obtained from Merck, were distilled prior to use. Pentane-2,4-dione, aniline, *p*-phenylenediamine, benzidine and $RuCl_3 \cdot 3H_2O$, purchased from Aldrich, were used as supplied whereas $Ru(phen)_2Cl_2$ was prepared using a literature procedure.[8] Calf thymus DNA and sodium citrate, obtained from Sigma Chemical Co., were used as supplied. Complexes, prepared under N_2 atmosphere, were purified by column chromatography using neutral alumina.

IR spectra of the ligands and their Ru(II) complexes (KBr pellets) were recorded in the 4000–400 cm^{-1} range using a Perkin–Elmer 783 spectrophotometer. One dimensional ^1H NMR, ^1H–^1H COSY, TOCSY and ^1H–^{13}C COSY spectra were recorded on a 300 MHz Bruker Avance DRx FT NMR spectrometer. FAB mass spectral data were obtained from CDRI (Lucknow, India). Microanalytical, absorption, luminescent and electrochemical studies were carried out at the University of Tokyo (Japan). Luminescence measurements were carried out at Tata Institute of Fundamental Research (TIFR, Mumbai, India) whereas UV/Vis absorption spectral studies on a Unicam UV2-100 UV/Vis spectrophotometer and antibacterial activity of the Ru(II) complexes were carried out at the bio-technology centre of BHU (Varanasi, India).

Syntheses

The ligands (L^1H, L^2H_2 and L^3H_2) shown in Scheme 1 were synthesised and characterised using IR, $^1H/^{13}C$ NMR and FAB mass spectral data as reported earlier by us.[7]

The Ru(II) complexes were synthesised by our earlier reported[7] procedure.



Scheme 1 Structure of ligands L^1H–L^3H_2.

$[RuL^1(phen)_2](PF_6)$ (1). To prepare this complex $Ru(phen)_2Cl_2$ (532 mg, 1 mmol) in ethanol (10 mL) was added dropwise to an ethanolic solution (10 mL) of L^1H (204 mg, 1 mmol) under N_2 atmosphere. The resulting contents were then refluxed for 20 h while monitoring the progress of the reaction using TLC. The solution was filtered and cooled at room temperature for 24 h. The concentrated filtrate was precipitated by the addition of a saturated aqueous solution of NH_4PF_6. The crystalline solid thus obtained by centrifugation was washed with H_2O, followed by EtOH and Et_2O, and it was purified by column chromatography performed on neutral alumina as the support using CCl_4–CH_3CN (1 : 3) as the eluent. The eluate, obtained after evaporation, was dissolved in acetone and reprecipitated by adding a saturated aqueous solution of NH_4PF_6. After repeated washings with H_2O, followed by EtOH and Et_2O, the precipitate was dried *in vacuo* and analysed (Table 1).

$[Ru_2L^2(phen)_4](PF_6)_2\cdot 3H_2O$ (2) and $[Ru_2L^3H(phen)_4](PF_6)_3$ (3). The complexes $[Ru_2L^2(phen)_4](PF_6)_2\cdot 3H_2O$ (**2**) and $[Ru_2L^3H(phen)_4](PF_6)_3$ (**3**) were also prepared by refluxing a 1 : 2 molar ratio of the ligand (L^2H_2, 155 mg and L^3H_2, 203 mg, 0.5 mmol each) and $Ru(phen)_2Cl_2$ (532 mg in each case) in ethanol (~25 mL) for 20–24 h. The complexes were precipitated and purified by a similar procedure as that described for complex **1**. The analytical data along with other physical properties of the complexes are reported in Table 1.

Results and discussion

Based on the elemental (C,H,N) analyses and FAB mass spectral data, the molecular compositions assigned for the complexes are shown in Table 1. The complexes were found to be thermally stable and soluble in acetone, acetonitrile, DMF and DMSO. Their specific conductances (Table 1) recorded in acetonitrile solution (10^{-3} M) were consistent with the reported values.[9]

In the complexes the deprotonation of the enolic pentane-2,4-dione portion is supported by the number of counter anions present in the formula obtained on the basis of the elemental analysis. Further evidence is provided by the disappearance of NH proton(s) in the ^1H NMR spectra upon complexation, except for complex **3** in which only one NH group is found to be deprotonated from the peak integration.

IR spectral studies

The spectra of the Ru(II) complexes show a strong peak in the region of 1674–1680 cm^{-1} assigned to $\nu_{C=O}$, which is similar to that found[7] in the spectra of the respective free ligands, indicating that at least one uncoordinated C=O group is present in the complexes. Furthermore, it was noticed in the spectra of the metal complexes that $\nu_{N=N}$, observed at 1460–1440 cm^{-1}, disappeared and a new peak assigned[10] as $\nu_{C=N}$ appeared at 1630–1625 cm^{-1}. This shows that the ligands coordinate as the hydrazino form in their metal complexes. Peaks observed at ~840, 2850 and 3000 cm^{-1} were assigned[7,10] as $\nu_{PF_6^-}$, ν_{CH} aliphatic and ν_{CH} aromatic, respectively. To gain further structural information the ^1H NMR spectra of the complexes were recorded in DMSO-d_6.

^1H NMR spectral studies

The spectra of the free ligands (L^1H and L^3H_2) showed the absence of the CH proton of the pentane-2,4-dione part whereas a peak at δ 14.76–15.00, assigned as an NH proton, appeared. This observation was corroborated by the ^1H–^{13}C heteronuclear multiple bond correlation (HMBC) spectrum recorded for the representative ligand L^2H_2 (Fig. 1). It was noticed from this spectrum that the peak observed at δ 14.92 could be correlated with the aromatic protons (6.5–7.5 ppm) while the peak at δ 12.92 correlated with the methyl protons, indicating that the former peak arises from NH while the latter peak comes from an OH proton. This confirms that the ligands coordinate in their hydrazino form.

Additionally, ^1H NMR spectra of the complexes showed that two singlets at δ 2.05–2.63, assigned[11] as methyl protons, were shifted to low field relative to the free ligands at δ 2.00–2.30. Furthermore, on the basis of ^1H–^1H COSY and TOCSY NMR studies (Figs. 2 and 3) carried out for a representative complex (**2**), peaks observed at δ 7.90, 8.20, 8.50 and 8.98 were assigned[12] as H_3/H_8, H_2/H_9, H_5/H_6, H_4/H_7 protons of the phenanthroline part. The singlet observed for the H_5/H_6 protons could arise due to the magnetic equivalence of these protons.[12]

In the ^1H–^1H COSY NMR spectrum of complex **2** it is also seen that intense peaks are accompanied by weak peaks that could not be correlated with the intense ones but were found

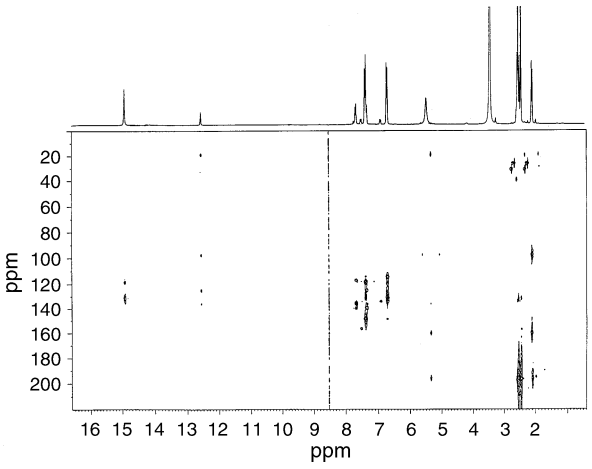


Fig. 1 ^1H–^{13}C HMBC NMR spectrum of ligand L^2H_2.

Table 1 Physical properties and analytical data of Ru(II) complexes **1–3**

	Molecular formula, % yield, colour and FAB MS data	% Composition required (found)			Ω_M/ ohm^{-1} cm^2 mol^{-1}
		C	H	N	
1	$[RuL^1(phen)_2](PF_6)$ 56.0, light brown 809 $[M + PF_6]^+$	51.92 (52.56)	3.34 (3.63)	10.38 (10.13)	205
2	$[Ru_2L^2(phen)_4](PF_6)_2\cdot 3H_2O$ 58.0, dark brown 1395 $[M + PF_6]^+$	48.18 (48.62)	3.38 (3.24)	10.53 (9.96)	425
3	$[Ru_2L^3H(phen)_4](PF_6)_3\cdot 0.67NH_4PF_6$ 60.0, yellowish red 1327 $[M]^{3+}$	44.90 (44.51)	2.97 (2.91)	9.48 (9.59)	800

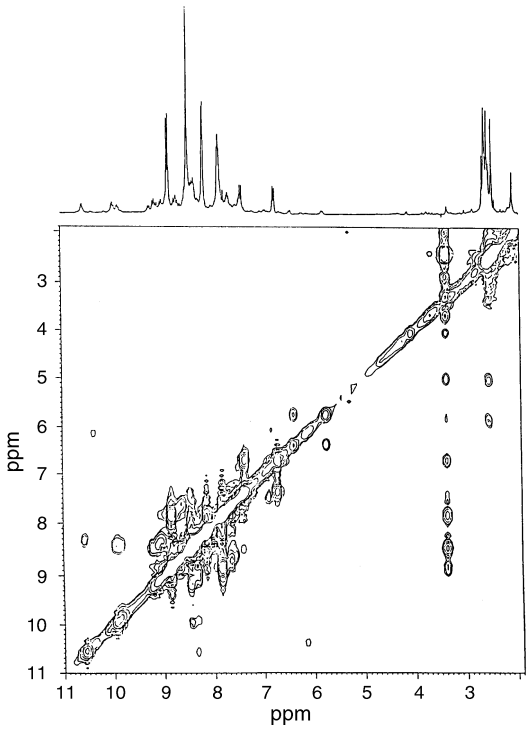


Fig. 2 ^1H–^1H COSY NMR spectrum of the Ru(II) complex **2** with L^2H_2.

to be correlated with each other. Although the reason for this is not yet understood, it is consistent with an earlier report.[13]

Thus, on the basis of elemental analyses, spectroscopic data and in view of earlier reports[7,10] the structures proposed for the complexes are shown in Scheme 2.

Electrochemistry and luminescence properties

A cyclic voltammetric (CV) study of the complexes was performed in CH_3CN (10^{-3} M) solution using 0.1 mol dm^{-3} of $[Bu_4N]^+(PF_6^-)$ as supporting electrolyte, Ag/Ag^+ as reference and platinum as working electrodes. The two ruthenium centres in the dinuclear Ru(II) complexes could not be differentiated by the CV study due to similar electronic environments around the Ru(II) centres. However, the metal-based oxidations were observed at 1.27, 1.29 and 1.71 V (Table 2) in complexes **1**–**3** respectively. The positive shift of the oxidation potential for complex **3** as compared to the reported[14] oxidation potential at 1.4 V for the standard complex $[Ru(phen)_3]^{2+}$ could be understood either in terms of less $\pi^* \leftarrow$ metal back-bonding provided by the L^3H_2 ligand or in terms of an electrostatic effect as reported earlier.[15] Since the terminal ligand showed only one reduction peak between -1.47 and -1.82 V no definite conclusions could be drawn regarding the ligand-based reduction.

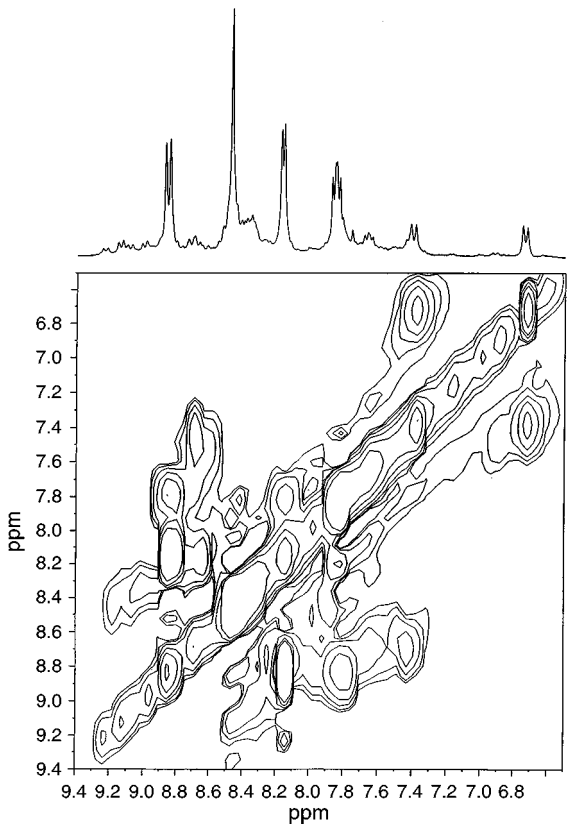


Fig. 3 ^1H–^1H TOCSY NMR spectrum of the Ru(II) complex **2** with L^2H_2.

From the emission data shown in Table 2 it can be noticed that the complexes emit at a wavelength shorter than that reported for the standard $[Ru(phen)_3]^{2+}$ ($\lambda_{em} = 596$ nm)[14] when excited at 440 nm. Weaker emission observed from complex **3** as compared to other complexes corroborates the electrochemical finding. The better quenching of emission observed from the dinuclear complexes **2** and **3** as compared to the mononuclear complex **1** is also consistent with the report by Meyer *et al.*[16] and the greater quenching observed from complex **3** as compared to complex **2** could be due to the increased conjugation[17] provided by the L^3H_2 ligand.

Since Ru(II) polypyridyl complexes are reported to recognise and react with DNA a preliminary investigation on the spectral changes observed when the complexes are in the presence of DNA has also been carried out.

Luminescence, ^1H NMR and UV/Vis spectral studies in the presence of calf thymus DNA

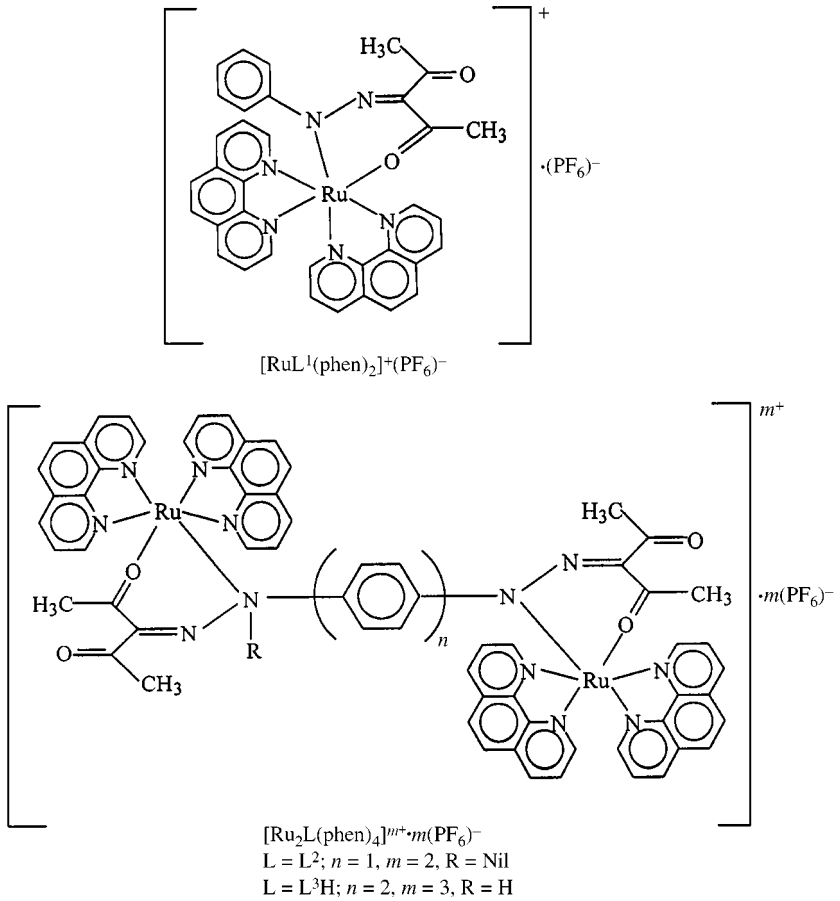
Photophysical[18] and ^1H NMR studies[12] have shown that the binding of $[Ru(phen)_3]^{2+}$ with DNA occurs mainly through electrostatic interactions, hydrophobic interactions against the minor groove and base-stacking intercalation from the major groove side. In this context, it has also been reported[18] that the B-form of calf thymus DNA binds ruthenium polypyridyl complexes with a preference for the A–T (A = adenine, T = thymine) regions and shows changes in photophysical properties upon binding.

Luminescence anisotropy[17] plotted against DNA base pairs per complex (Fig. 4) for the representative complex **2** shows that it reaches a maximum for two DNA base pairs per complex, indicating that the complex intercalates between two base pairs, preferentially in the A–T regions in view of the above report.[18] Additionally, quenching of the luminescence observed from the complexes (**1**, 10%; **2**, **3**, 15–20%) in the presence of DNA solution is also taken as a comparative parameter for their interaction[19,20] with DNA. The quenching in luminescence could be due to DNA-mediated electron transfer as has been reported[18,20] earlier.

Table 2 Luminescence and electrochemical data of Ru(II) complexes

	Luminescence data[a]			Electrochemical data[c]	
Complex	λ_{max}/nm	$I_{rel}^{em\,b}$	$\phi \times 10^{-3}$	$E_{1/2}^{ox}$/V	$E_{1/2}^{red}$/V
1	595	0.195	7.8	1.27	−1.47
2	595	0.169	6.7	1.29	−1.69
3	592	0.114	4.5	1.71	−1.82

[a] Recorded in acetonitrile at 20 °C. Solutions were 10^{-6} mol dm^{-3} for luminescence data. [b] Relative to $[Ru(phen)_3]^{2+}$ in acetonitrile ($\lambda_{ex} = 440$ nm). [c] Obtained in acetonitrile solution containing 0.1 mol dm^{-3} $[Bu_4N]^+(PF_6)^-$ as supporting electrolyte. Solutions were 10^{-3} mol dm^{-3} and potentials were determined with reference to an Ag/Ag^+ electrode at room temperature at 200 mV s^{-1} scan rate.



Scheme 2 Proposed structures for the Ru(II) complexes.

To gain further support for this interaction, the ^{1}H NMR spectrum of complex **2** was recorded in the presence of DNA. The broadening and upfield shift in the peak position of phenanthroline and phenyl protons by 0.16 and 0.10 ppm, respectively, indicate their interaction with DNA. Since the DNA solution used in this titration was very dilute as compared to the complex solution, DNA peaks were not observed in the ^{1}H NMR spectrum. Thus, on the basis of anisotropic luminescence and ^{1}H NMR studies it could be considered that there is base-pair intercalation while bonding of the complex with DNA is weaker as the decrease in the luminescent intensity was not very significant.

Furthermore, strong electrostatic interactions of the complexes with DNA were considered in view of the UV/Vis spectral changes observed in the presence of DNA. In the UV/Vis spectral data of the complexes recorded in Table 3, the red shifts and hyperchromic effects observed in the presence of calf thymus DNA solution show, in view of earlier reports,[21,22] that interaction of the complexes with DNA had occurred. However, this effect was found to be more significant in the 204–216 nm region, indicating that the anionic part of the DNA might be interacting[21,22] more efficiently. Furthermore, absorption spectral data observed for complex **3** upon stepwise addition of different amounts of calf thymus DNA, presented in Table 4, also showed significant red shifts and hyperchromism, especially in the intra-ligand and MLCT regions (Fig. 5). Again, the absorption peak in the 204 nm

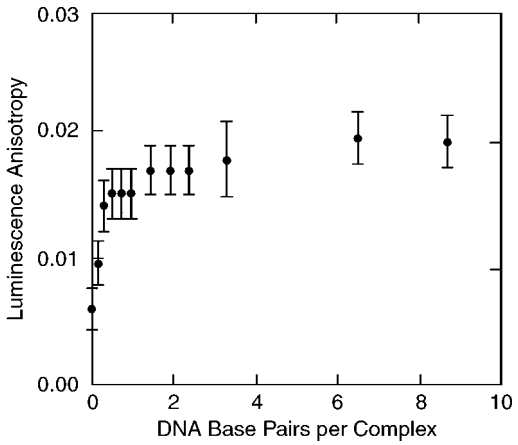


Fig. 4 Variation of fluorescence anisotropy *vs.* DNA base pairs per complex **2**.

Table 3 UV/Vis absorption data of Ru(II) complexes in presence and absence of calf thymus DNA solution[a]

	−DNA		+DNA (100 μL) soon after mixing	
Complex	λ_{max}/nm	$\varepsilon_{max} \times 10^3$	λ_{max}/nm	$\varepsilon_{max} \times 10^3$
1			212	74.30
	225	41.40	227	78.00
	262	57.00	263	105.10
	362	104.60	265	119.60
	440	9.90	442	19.70
2			212	185.40
	223	120.20	223	140.60
	264	195.40	264	226.00
	401	89.10	408	95.50
	444	34.80	446	40.00
3			216	204.30
	222	146.00	222	195.50
	260	229.00	264	273.00
	391	56.80	408	84.20
	440	35.00	445	40.00

[a] Solutions of the complexes and DNA were 10^{-5} M in acetonitrile and 0.30 mg mL^{-1} in citrate buffer (pH 8.6), respectively.

Table 4 UV/Vis absorption spectral data of complex **3** upon addition of different amounts of calf thymus DNA solution[a]

−DNA		+DNA (50 μL)		++DNA (50 μL)[b]		+++DNA (50 μL)[b]	
λ_{max}/nm	$\varepsilon_{max} \times 10^3$	λ_{max}/nm	$\varepsilon_{max} \times 10^3$	λ_{max}/nm	$\varepsilon_{max} \times 10^3$	λ_{max}/nm	$\varepsilon_{max} \times 10^3$
		204	525.00	208	570.00	212	600.00
220	146.00	220	390.00	222	410.00	224	465.00
260	229.00	260	325.00	264	330.00	264	350.00
391	56.80	412	63.40	425	69.20	232	82.80
440	35.00	443	50.00	452	65.00	452	75.00

[a] Same concentrations as given in Table 3. [b] ++ and +++ indicate stepwise addition to the previously used DNA solution.

region became more and more intense and red shifted as the amount of DNA solution was increased from 50 to 150 μL.

Thus, the DNA binding behaviour of the complexes makes them prospective candidates to be evaluated for their bioactivities in view of earlier and current reports.[7,18] Therefore, an attempt has been made to correlate the antibacterial activity of the complexes with their DNA binding capability.

Antibacterial activity

The growth inhibition of the highly resistant bacteria *Pseudomonas aeruginosa* in the presence of the complexes in acetonitrile solution (10^{-5} and 10^{-6} M) was evaluated using susceptibility testing methods reported in the literature.[23] Though the present study is carried out in the presence of calf thymus DNA due to its easy availability, microbial DNA may not bring about too much structural difference. For disk assays, Whatman paper disks containing the compounds to be tested were placed on the surface of nutrient agar plates (Himedia, Pvt. Ltd., Bombay, India) previously spread with 0.1 mL of overnight cultures of *P. aeruginosa*. After 36 h of incubation at 37 °C the diameters of the measured inhibition zones are reported in Table 5. Inhibitory effects by the free ligands on the same bacterium have been discussed earlier by us.[7]

In view of the interaction of free ligands and their Ru(II) complexes with DNA, we compared the antibacterial activity in the presence and absence of DNA solution. Since free ligands containing DNA solution have already been reported[7] to show poorer inhibition against *P. aeruginosa*, only Ru(II) complexes were evaluated for their antibacterial activity in the presence and absence of DNA solution. The data shown in Table 5 show that the complexes containing DNA solution have a smaller zone of inhibition as compared to that observed in the absence of DNA solution.

Thus, on the basis of the above observation it can be stated that (*i*) a system that has already been bound with DNA shows poorer activity as compared to the unbound system and (*ii*) complexes **1**–**3** bearing 1,10-phenanthroline as terminal ligand show better bacterial inhibition as compared to complexes[7] bearing 2,2′-bipyridine as terminal ligand.

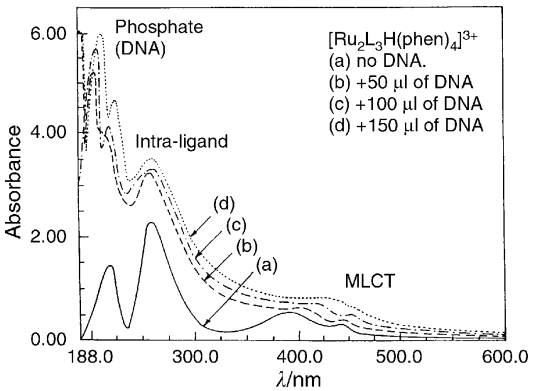


Fig. 5 UV/Vis absorption spectra of complex **3** (10^{-5} M in acetonitrile) in the absence and presence of different amounts of calf thymus DNA solution. (a) No DNA, (b) 50 μl, (c) 100 μL and (d) 150 μL of DNA solution (0.30 mg mL^{-1} in pH 8.60 citrate buffer).

Table 5 Antibacterial activity (zone of inhibition) of Ru(II) complexes against *Pseudomonas aeruginosa* in the presence and absence of calf thymus DNA solution[a]

	Zone of inhibition/cm			
	10^{-5} M Complex		10^{-6} M Complex	
Complex	no DNA	+DNA	no DNA	+DNA
1	1.20	0.80	1.00	0.70
2	1.40	0.55	1.20	0.50
3	1.30	0.60	1.10	0.60

[a] The concentration and pH of the citrate buffer solution of calf thymus DNA were 25 μg mL^{-1} and 8.60, respectively. Inhibition (~0.2 cm) due to pure CH_3CN was subtracted.

Acknowledgements

The authors are thankful to the authorities of the School of Biotechnology, B.H.U., India, Dr. Raja Roy, CDRI, Lucknow, India and to Prof. K. Araki, Tokyo University, Japan, for providing instrumental facilities to make this study possible. Financial support received from B.H.U., India, is gratefully acknowledged.

Notes and references

1 B. J. Coe, D. A. Friessen, D. W. Thompson and T. J. Meyer, *Inorg. Chem.*, 1996, **35**, 4575.
2 G. A. Lawrance, *Polyhedron*, 1985, **4**, 817.
3 P. G. Sammes and G. Yahioglu, *Chem. Soc. Rev.*, 1994, **23**, 327.
4 S. Patai, *The Chemistry of the Hydrazo, Azo and Azoxy Groups*, Wiley and Sons, New York, 1975, part 1, ch. 13.
5 S. R. Rajski and R. M. Williams, *Chem. Rev.*, 1998, **98**, 2723 and references therein.
6 R. M. Hartshorn and J. K. Barton, *J. Am. Chem. Soc.*, 1992, **114**, 5919.
7 L. Mishra, A. K. Yadaw, C. S. Choi and K. Araki, *Indian J. Chem., Sect. A*, 1999, **38**, 339 and references therein.
8 B. P. Sullivan, D. J. Salman and T. J. Meyer, *Inorg. Chem.*, 1978, **17**, 3334.
9 R. A. Krause, *Inorg. Chim. Acta.*, 1977, **22**, 209.
10 K. Krishnankutty and J. Michael, *J. Coord. Chem.*, 1991, **22**, 327.
11 D. C. Nonbebel and A. Mitchell, *Tetrahedron*, 1979, **35**, 2031.
12 J. G. Collins, A. D. Slecman, J. R. Aldrich–Wright, I. Greguric and T. W. Hambley, *Inorg. Chem.*, 1998, **37**, 3133.
13 N. C. Fletcher and F. R. Keene, *J. Chem. Soc., Dalton Trans.*, 1998, 2293.
14 P. D. Beer, S. W. Dent and N. C. Fletcher, *Polyhedron*, 1996, **15**, 2983.
15 G. Giuffrida, V. Ricevuto, G. Guglielmo, S. Campagna and M. Ciano, *Inorg. Chim. Acta.*, 1992, **194**, 22.
16 S. Boyde, G. F. Strouse, W. E. Jones and T. J. Meyer, *J. Am. Chem. Soc.*, 1990, **112**, 7395.
17 S. G. Schulman, *Fluoresc. Phosphoresc. Spectrosc.*, 1977, **59**, 102 and references therein.

18 K. E. Erkkila, D. T. Odom and J. K. Barton, *Chem. Rev.*, 1999, **99**, 2777.
19 W. Trettnak, *Fluorescence Spectroscopy*, G. S. Wolfbeis, Springer Verlag, New York, 1993, p. 79.
20 J. A. Cowan, *Inorganic Biochemistry*, Wiley, New York, 1997, p. 363.
21 M. Rodriguez and A. J. Bard, *Inorg. Chem.*, 1992, **31**, 7129.
22 P. Umapathy, R. A. Harnesswala and C. S. Dorai, *Polyhedron*, 1985, **4**, 1595.
23 C. V. Cervantes, J. Chavez, N. A. Cordova, P. Delamora and J. A. Velasco, *J. Microbias*, 1986, **48**, 159.