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Antineoplastic and Antibacterial Activity of some Mononuclear Ru(II) Complexes

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These ligands (L) show a bidentate behavior, forming octahedral ruthenium complexes. The title complexes were subjected to *in-vivo* anticancer activity tests against a transplantable murine tumor cell line, Ehrlich's Ascitic Carcinoma (EAC) and *in-vitro* antibacterial activity against several Gram positive and Gram negative bacterial strains. $[Ru(bpy)_2(ihqs)]Cl_2$ and $[Ru(bpy)_2(hc)]Cl_2$ (where bpy = 2,2′-bipyridine, ihqs = 7-iodo-8hydroxy quinoline-5-sulphonic acid and hc = 3-hydroxy coumarin) showed promising antitumor activity. Treatment with these complexes prolonged the life span of EAC bearing mice as well as decreased their tumor volume and viable ascitic cell count. All the tested complexes exhibited mild to moderate antibacterial activity.

Keywords: Ru(II) complexes; Anticancer; Antibacterials

INTRODUCTION

The organic chemistry of the transition metal ruthenium is well developed, particularly with amine and imine rings, and provides for many approaches to innovative new metallopharmaceuticals.[1,2,5–9] Advantages of utilizing ruthenium complexes in drug development include i) reliable methods of synthesizing stable complexes with predictable structures, ii) the ability to fine tune ligand affinities, electron transfer and substitution rates and reduction potentials and iii) an increasing knowledge of the biological effects of ruthenium complexes.[10]

Since then complexes such as *cis*-$[Ru(DMSO)_4Cl_2]$,[1,2] (DMSO = dimethyl sulfoxide) have been prepared and showed modest antitumor activity. $Ru(DMSO)(arene)Cl_2$ (where, arene = C_6H_6) was shown to inhibit topoisomerase II (DNA gyrase) activity.[3] Other arene complexes such as $[X(\acute{\eta}^6\text{-arene})(en)Ru(II)]^+$(where, arene = C_6H_6 or substituted C_6H_6, en = ethylenediamine and X = halide) were found to inhibit the growth of the human ovarian cancer cell line A 2780, but did not inhibit topoisomerase I or II.[4] Complexes such as *trans*-$(IndH)[Ru(Ind)_2Cl_4]$ (where, Ind = indazole), *mer*-$[Ru(terpy)Cl_3]$ (where, terpy = 2,2′-6′-terpyridine) and $[Ru(chd\text{-}H_2)Cl_2]$ (chd = 1,2-cyclohexanediamine tetraacetate) have been reported to be highly active as antitumor agents.[5–7] $(ImH)[Ru(Im)Cl_5]$,[8] $(ImH)[Ru(Im)_2Cl_4]$[9] (where, Im = imidazole) and $(ImH)[Ru(Im)(DMSO)Cl_4]$[11] (NAMI-A) are also well known antitumor agents. According to the "activation by reduction" hypothesis, the Ru(III) complexes may serve as prodrugs that are activated by reduction *in-vivo* to coordinate more rapidly to biomolecules.[12–14]

Ruthenium (III) complexes bearing 2-hydroxy-1-naphthaldehyde thiosemicarbazone as a ligand have been studied for antimicrobial and antifungal activity.[15] Sulu *et al.*,[16] have evaluated ruthenium complexes for their *in vitro* antifungal activity with a range of MIC values between 16 and 250 μg/ml. Chohan *et al.*[17–19] have reported the antibacterial properties of zinc (II), cobalt (II), nickel (II) and copper (II) complexes with various bidentate ligands containing oxygen, nitrogen and sulfur as donor atoms. A previous investigation[20] in our group has dealt with the antitumor and antibacterial activity of the type $[Ru(bpy)_2(R\text{-}TSC)]^{2+}(ClO_4)_2$, (where bpy = 2,2′-bipyridine, R-TSC = alkyl/aryl

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substituted thiosemicarbazide). The synthesis and characterization of ruthenium complexes of the type $[Ru(R)_2(L)]^{2+}$, (where R = 1, 10-phenanthroline/2,2′-bipyridine and L = 5,7-disubstituted-8-OH-quinoline, picolinic acid and hydroxy coumarin) is described.

MATERIALS AND METHODS

The solvents of AR grade were obtained from SD Fine Chem., Mumbai and E. Merck, Mumbai. The reagents (puriss grade) were obtained from Fluka and E. Merck. Hydrated ruthenium trichloride was purchased from Loba Chemie, Mumbai and used as received. UV/visible spectra were run on a Beckmann DU 64 spectrophotometer. FTIR spectra were recorded in KBr discs on a Jasco V410/Schimadzu IR spectrometer. ^{1}H-NMR spectra were measured in $CDCl_3$ and d_6-DMSO on a Bruker Ultraspec 500 MHz/300 MHz AMX spectrometer. The reported chemical shifts were against TMS. FAB Mass spectra were recorded on a JEOL JMS600 mass spectrometer in mNBA matrix. 7-Iodo-8-hydroxy-quinoline-5-sulfonic acid (IHQS) was obtained from the Central Drug Laboratory, Kolkata, India. Picolinic acid[22] (PA) and 3-hydroxy-coumarin[21] (HC) were prepared according to literature methods.

Preparation of Various 5,7 Substituted-8-hydroxy-quinolines

Preparation of 8-hydroxy-5-nitroso-quinoline

To a mixture of 36.5 g of 8-hydroxyquinoline in 105 ml of concentrated HCl cooled to 0°C, was added 18 g of $NaNO_2$ in 30 ml of water in portions of 5 ml. The yellow lumpy mass precipitated was filtered and washed with excess cold water to give, 8-hydroxy-5-nitroso quinoline which was dried under vacuum.

Preparation of 8-hydroxy-5-nitro-quinoline[23]

Finely powdered 8-hydroxy-5-nitroso-quinoline (3 g) was added with vigorous stirring to a mixture of concentrated HNO_3 (9 ml) and water (6 ml) at 17°C. Oxides of nitrogen were evolved and the nitroso-quinoline was rapidly converted to the insoluble nitro compound. After 75 min with occasional shaking the mixture was diluted with water, cooled to 0°C and made alkaline with sodium acetate. The product was collected washed with water and recrystallized from ethanol. Yield 73% mp 176°C (Lit. 180°C).

General Procedure for Preparing 7-halogeno-5-nitro-8-hydroxy Quinolines[24]

1 g of 5-nitro-8-hydroxy quinoline was finely powdered and dissolved in (300 ml) water containing KOH (900 mg) by stirring at room temperature. A mixture of iodine or bromine in the appropriate potassium salt was prepared and added to the mixture. Stirring was continued at room temperature for 2 h and the product was precipitated by acidification, filtered and dried in air.

7-Bromo-5-nitro-8-hydroxy Quinoline (BNQ)

Yield 68%, mp. 202°C (lit. 200°C). IR (KBr) cm^{-1}: 3100–2700 (O—H), 1623 (C=C), 1563–1516 (N—O), 1402–1311 (C—OH, str), 1254 (O—H def. coupled). Calcd. for $C_9H_5BrN_2O_3$: C, 40.18; H, 1.87; N, 10.41. Found C, 40.12; H, 1.80; N, 10.47.

7-Iodo-5-nitro-8-hydroxy Quinoline (INQ)

Yield 65%, m. p. 236°C (lit. 237°C). IR (KBr) cm^{-1}: 3103–2697 (O—H), 1626 (C=C), 1560–1516 (N—O), 1408–1309 (C—OH, str), 1250 (O—H def. coupled). Calcd. for $C_9H_5IN_2O_3$: C, 34.17; H, 1.59; N, 8.86. Found C, 33.97; H, 1.80; N, 9.02.

Preparation of *Cis*-bis(R)dichlororuthenium(II)[25] *Cis*-$[Ru(R)_2Cl_2]^{+2}$ where R = 2,2′-bipyridine/1,10-phenanthroline

$RuCl_3.3H_2O$ (1.15 g, 2.5 mmol) and R (5 mmol) were refluxed in 50 ml DMF for 3 h under a nitrogen atmosphere. The reddish brown solution slowly turned purple and the product precipitated. The solution was cooled overnight at 0°C. A fine microcrystalline mass was filtered off. The residue was repeatedly washed with 30% LiCl solution and finally recrystallised from the same. The product was dried and stored in a vacuum dessicator over P_2O_5 for further use. (Yield 75%).

Preparation of the $[Ru(R)_2(L)](ClO_4)_2$ Complexes (Ru1–Ru5) [where R = 2,2′-bipyridine/1,10-phenanthroline and L = BNQ, INQ, IHQS, PA, HC]

To the black microcrystalline *cis*-$Ru(R)_2Cl_2$ (106 mg, 2 mmol) excess of ligand (L) (2.5 mmol) was added and the mixture refluxed in ethanol under a nitrogen atmosphere. The reaction was monitored by TLC. The reaction mixture was cooled to room temperature and the complex separated by column chromatography on efficient supports separately chosen for each complex. Excess of saturated sodium perchlorate solution was added to the pure complexes dissolved in ethanol to precipitate out the complex.

Ru1 *(where R = 1,10-phenanthroline, L = BNQ)*

The complex was purified by column chromatography on a neutral alumina support using dichloromethane and isopropanol (10%) as eluent. The excess of solvent was distilled off. To the pure complex in ethanol a saturated solution of sodium

perchlorate was added and the mixture left overnight at 0°C when microcrystalline precipitate was obtained. The crystals were filtered off and dried over $CaCl_2$ in a vacuum desiccator. Yield 43%, Black crystals. IR (KBr) cm^{-1}: 3010 (C—H), 1625 (C=C), 1550 (N—O), 1396 (C—O). λ_{max} nm (methanol): 230, 260, 375, 470. Calcd. for $RuC_{33}H_{21}BrN_6O_{11}Cl_2$: C, 42.63; H, 2.26; N, 9.04. Found C, 42.61; H, 2.30; N, 9.10%. ^{1}H-NMR (DMSO-d_6) δ ppm: 9.18 (d, $J = 8.05$ Hz, 1H), 8.85–8.81 (m, 3H), 8.74 (s, 1H), 8.65–8.59 (m, 2H), 8.43–8.31 (m, 4H), 8.12 (dd, $J = 8.22, 5.22$ Hz, 1H), 8.06 (d, $J = 4.56$ Hz, 1H), 7.99–7.93 (m, 2H), 7.68–7.61 (m, 4H), 7.55 (dd, $J = 8.99, 5.00$ Hz, 1H). FAB-MS (mNBA): 830, $[Ru(phen)_2(BNQ)]^{2+}(ClO_4)^-$; 731, $[Ru(phen)_2(BNQ)]^{2+}$; 549, $[Ru(phen)(BNQ)]^{2+}$; 462, $[Ru(phen)_2]^{2+}$; 281, $[Ru(phen)]^{2+}$.

Ru2 ***(where R = 1,10-phenanthroline, L = INQ)***

Yield 41%, Black crystals. IR (KBr) cm^{-1}: 3005 (C—H), 1623 (C=C), 1555 (N—O), 1388 (C—O). λ_{max} nm (methanol): 230, 255, 372, 473. Calcd. for $RuC_{33}H_{21}IN_6O_{11}Cl_2$: C, 40.61; H, 2.05; N, 8.62: Found C, 40.03; H, 2.35; N, 9.01%. ^{1}H-NMR (DMSO-d_6) δ ppm: 9.16 (d, J=8.04 Hz, 1H), 8.87 (s, 1H), 8.84–8.81 (m, 3H), 8.65–8.59 (m, 2H), 8.43–8.31 (m, 4H), 8.14 (dd, $J = 7.63, 5.84$ Hz, 1H), 8.05 (d, J=4.67 Hz, 1H), 7.99 (d, $J = 4.73$ Hz, 1H), 7.96 (dd, $J = 8.26, 5.20$ Hz, 1H), 7.66–7.60 (m, 4H), 7.55 (dd, $J = 8.98, 5.02$ Hz, 1H). FAB-MS (mNBA): 876, $[Ru(phen)_2(INQ)]^{2+}(ClO_4)^-$; 776, $[Ru(phen)_2(INQ)]^{2+}$; 596, $[Ru(phen)(INQ)]^{2+}$; 462, $[Ru(phen)_2]^{2+}$; 281, $[Ru(phen)]^{2+}$.

Ru3 (where R = 2,2′-bipyridine, L = IHQS)

The complex was prepared in a similar manner to Ru1. The complex was separated by column chromatography on silica gel (230–400 mesh size) with chloroform and methanol as eluent. The perchlorate salt was not prepared as the chloride salt gave good crystals suitable for spectral analysis. Yield 48%, Black crystals. IR (KBr) cm^{-1}: 3425 (O—H), 3100 (C—H), 1623 (C=C), 1548 (N—O), 1385 (C—O). λ_{max} nm (methanol): 230, 260, 375, 470. Calcd. for $RuC_{29}H_{21}N_5O_4\ SCl_2I$: C, 43.83; H, 2.64; N, 8.82: Found C, 43.09; H, 2.35; N, 9.25%.

^{1}H-NMR (DMSO-d_6) δ ppm: 8.84 (d, $J = 0.75$ Hz, 1H), 8.80 (d, $J = 8.12$ Hz, 1H), 8.75 (dd, $J = 8.01, 3.77$ Hz, 2H), 8.70 (d, $J = 8.11$ Hz, 1H), 8.49 (d, $J = 5.35$ Hz, 1H), 8.20–8.05 (m, 3H), 8.01–7.94 (m, 2H), 7.85 (d, $J = 5.49$ Hz, 1H), 7.80 (d, $J = 5.52$ Hz, 1H), 7.69 (d, $J = 5.42$ Hz, 1H), 7.67 (t, $J = 13.06$ Hz, 1H), 7.52 (t, $J = 10.50$ Hz, 1H), 7.40–7.30 (m, 4H).

FAB-MS (mNBA): 799, $[Ru(bpy)_2(IHQS)]^{2+}Cl^-$; 764, $[Ru(bpy)_2(IHQS)]^{2+}$; 609, $[Ru(bpy)(IHQS)]^{2+}$; 413, $[Ru(bpy)_2]^{2+}$; 257, $[Ru(bpy)]^{2+}$.

Ru4 (where R = 2,2′-bipyridine, L = PA)

The complex was prepared in a similar manner to Ru1. The complex was separated by column chromatography on silica gel (230–400 mesh size) with chloroform and methanol as eluent. Yield 41.5%, Brown crystals. IR (KBr) cm^{-1}: 3150 (C—H), 1623 (C=O), 1360 (C=N). λ_{max} nm (methanol): 240, 290, 325 and 470 nm. Calcd. for $RuC_{26}H_{20}N_5O_{10}Cl_2$: C, 42.51; H, 2.72; N, 9.54: Found C, 42.23; H, 2.55; N, 10.23%. ^{1}H-NMR (d_6-DMSO) δ ppm: 8.85–8.72 (ddm, 5H), 8.21–8.16 (m, 2H), 8.05–7.98 (m, 4H), 7.93 (d, $J = 5.38$ Hz, 1H), 7.86 (d, $J = 5.53$ Hz, 1H), 7.81 (t, $J = 13.02$ Hz, 1H), 7.66–7.62 (m, 2H), 7.51–7.47 (m, 2H), 7.42 (q, $J = 14.12, 7.5$ Hz, 2H). ^{13}C-NMR (d_6-DMSO): 171.38 (s), 158.56 (s), 157.53 (d), 157.02 (s), 153.18 (s), 152.97 (s), 151.58 (s), 150.86 (s), 150.25 (s), 149.90 (s), 137.47 (s), 136.88 (s), 136.52 (s), 135.65 (s), 128.78 (s), 127.49 (d), 127.06 (s), 126.74 (s), 124.27 (s), 123.87 (s), 123.57 (s).

FAB-MS (mNBA): 635, $[Ru(bpy)_2(PA)]^{2+}(ClO_4)^-$; 536, $[Ru(bpy)_2(PA)]^{2+}$; 413, $[Ru(bpy)_2]^{2+}$; 379, $[Ru(bpy)(PA)]^{2+}$; 257, $[Ru(bpy)]^{2+}$.

Ru5 (where R = 2,2′-bipyridine, L = HC)

The complex was prepared in a similar manner to Ru1. The complex was separated by column chromatography on silica gel (230–400 mesh size) with chloroform and methanol as eluent. Yield 53%, Black crystals. IR (KBr) cm^{-1}: 3491 (O—H), 3068 (C—H), 1604 (C=O). λ_{max} nm (methanol): 245, 293, 340, 504. Calcd. for $RuC_{29}H_{22}N_4O_{11}Cl_2$: C, 44.96; H, 2.84; N, 7.24: Found C, 44.24; H, 2.50; N, 8.03%.

Evaluation of Therapeutic Effect *In Vivo*

Albino Swiss mice (18–20 g body weight) were maintained in identical laboratory conditions and given standard food pellets (Hindustan Lever Ltd, Bombay, India) and water *ad libitum*. LD_{50} values of the synthesized complexes were measured according to the literature.[26] The animals were divided into eight groups each containing 12 mice. To group I was administered vehicle (5 ml/kg body weight i.p.) and group II kept as Ehrlich Ascites Carcinoma control (EAC; 2×10^6 EAC cells/mouse i.p.). Group III was treated with standard drug Cisplatin (2 mg/kg body weight). All the complexes were administered (i.p.) at a dose of 2 mg/kg body weight in groups IV–VIII respectively. All the complexes (Ru1–Ru5) and cisplatin were administered daily for 9 days starting 24 h after tumor transplantation. Six animals from each group were sacrificed 18 h after the last dose. The ascitic fluid volume, ascitic cell counts and hematological parameters were noted. Mean survival time (MST) for remaining 6 mice of each group was noted.

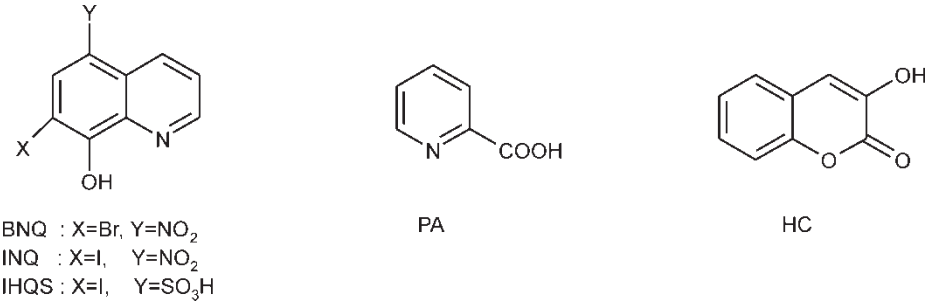


FIGURE 1 Structures of ligands (L).

Tumour Volume and Viable Count

Ascites volume was noted by taking it in a graduated centrifuge tube and packed cell volume determined by centrifuging at 1000 g for 5 min. Viability of ascitic cells were checked by the trypan blue (0.4% in normal saline) dye exclusion test and the count was taken in a Neubauer counting chamber. The effect of the ruthenium complexes on tumor growth was monitored by recording the mortality daily and percentage increase in life span (% ILS) was calculated by the following formula: ILS (%) = [(Mean survival of treated group)/Mean survival of control group) − 1] × 100.

Hematological Studies[27]

Blood was obtained from the tail vein, 24 h after the last dose. For the total count blood was drawn into RBC or WBC pipettes, diluted and counted in a Neubauer counting chamber. The hemoglobin concentration was determined by Sahli's Hemoglobinometer method. Differential count of leukocytes was done on a freshly drawn blood film using Leishman's stain.

Evaluation of Antibacterial Activity[28]

Solutions of the ruthenium complexes: Ru1 0.125 mM, Ru2 0.204 mM, Ru3 0.222 mM, Ru4 0.272 mM and Ru5 0.258 mM respectively were made in sterile water containing 5% DMF under aseptic conditions. The solutions were sterilized by membrane filtration. Solid agar and liquid broth culture media No. 1 were used for all the test organisms and the pH was adjusted to 7.2. Antimicrobial activity of the ruthenium complexes against different strains of bacteria was determined by the cup-plate method, and activity was expressed in terms of diameters of zone of inhibition. The innoculum was prepared by washing a fresh 5 ml medium slant of test organisms with 5 ml sterile water and further diluting the 1 ml washing to 10 ml. This suspension (0.15 ml) was added to 15 ml melted medium at a temperature 45–50°C and plates were prepared. Holes of diameter 6 mm were dug into the agar plates with a sterile borer and filled with the drug concentration. The plates were incubated for at 35°C for 24 h. The results were compared with that of the standard chloramphenicol.

RESULTS AND DISCUSSION

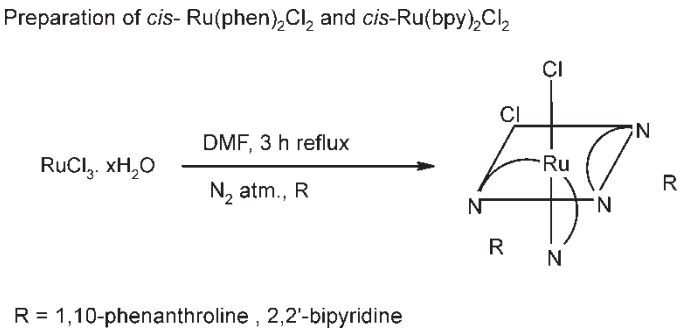
Chemistry

The ligands L (BNQ, INQ, IHQS, PA and HC) used are capable of exhibiting bidentate behavior, as shown in Figure 1.

The starting material for the synthesis of the complexes were *cis*-bis(1,10-phenanthroline)dichlororuthenium(II) and *cis*-bis(2,2′-bipyridine)dichlororuthenium(II). In these homoleptic chelates the first two ligands to enter the complex in a stepwise assembly were the 1,10-phenanthroline and 2,2′-bipyridine molecules. Since both the ligands are identical a single step method was adopted for its synthesis. Ruthenium trichloride was refluxed in DMF in the presence of 1,10-phenanthroline or 2,2′-bipyridine, in excess of the stoichiometric amount, which afforded the final product *cis*-bis(1,10-phenanthroline)dichlororuthenium(II) and *cis*-bis(2,2′-bipyridine)dichlororuthenium(II)[11] (Scheme 1). The introduction of the third ligand (L) was carried out in the presence of anhydrous alcohol. The final chelate formed had ionic chloride in the molecule and hence a polar solvent was used to complete the aforesaid reaction (Scheme 2).

The IR spectra of the ligands BNQ, INQ and IHQS showed the strong and broad band at 3100–2700 cm^{-1} region, corresponding to O—H stretching (bonded), and 1254 cm^{-1} region corresponding to O—H(def) and 1402–1311 cm^{-1} region for (C—OH).

When BNQ, INQ and IHQS were complexed, the band due to O—H stretching vanished and the ruthenium metal formed the coordinate covalent bond with its endocyclic N1 and covalent bond with exocyclic hydroxylated O8 atoms. But in the Ru3 complex, the broad band at 3425 cm^{-1} corresponded to O—H of SO_3H.

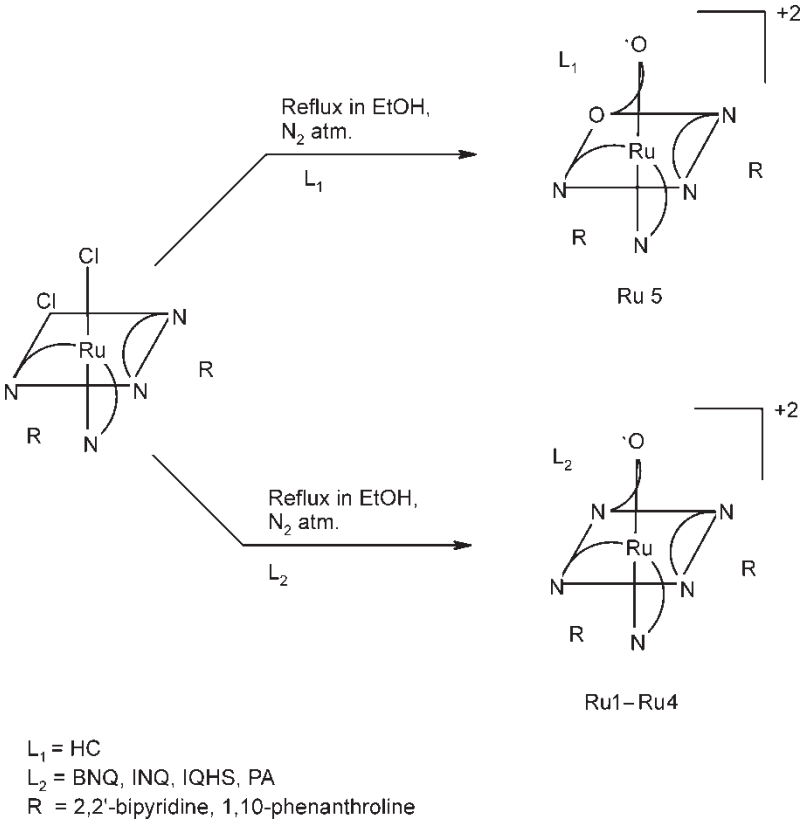


SCHEME 1 Preparation of *cis*-Ru(phen)$_2$Cl$_2$ and *cis*-Ru(bpy)$_2$Cl$_2$.

Picolinic acid (PA) shows IR bands at 1621 cm^{-1} for the carbonyl group and 3426 cm^{-1} for the hydroxyl group. On complexation the peak at 3426 cm^{-1} is no longer visible but the C = O stretching peaks are unaltered. From this it can be concluded that ruthenium ion bonded with a ring nitrogen and the oxygen of the hydroxyl portion of the carboxylic acid.

In the IR spectra of the ligand, 3-hydroxy coumarin (HC), the band observed at 3371 cm^{-1} corresponded to O—H stretching and the band at 1691 cm^{-1} to C=O stretching. On complexation, the band due to the hydroxyl group was shifted to 3491 cm^{-1} and that of C=O stretching was shifted to 1604 cm^{-1}; from this observation it can be concluded that the ruthenium ion is coordinated via the carbonyl oxygen and hydroxyl oxygen of the ligand.

Complexes such as Ru(bpy)$_2$Cl$_2$ or Ru(phen)$_2$Cl$_2$ exhibit a C_2 element of symmetry and hence in their NMR spectra the eight hydrogens of the bipyridine or the phenanthroline are unique and the two bipyridine or phenanthroline moieties are equivalent. The coordination of ligands BNQ, INQ, IHQS, PA and HC to Ru(R)$_2$Cl$_2$ results in compounds of the type Ru1–Ru5. The resultant complexes then loose the C_2 element of symmetry as has been



SCHEME 2 Preparation of tris-chelates from *cis*-Ru(phen)$_2$Cl$_2$ and *cis*-Ru(bpy)$_2$Cl$_2$.

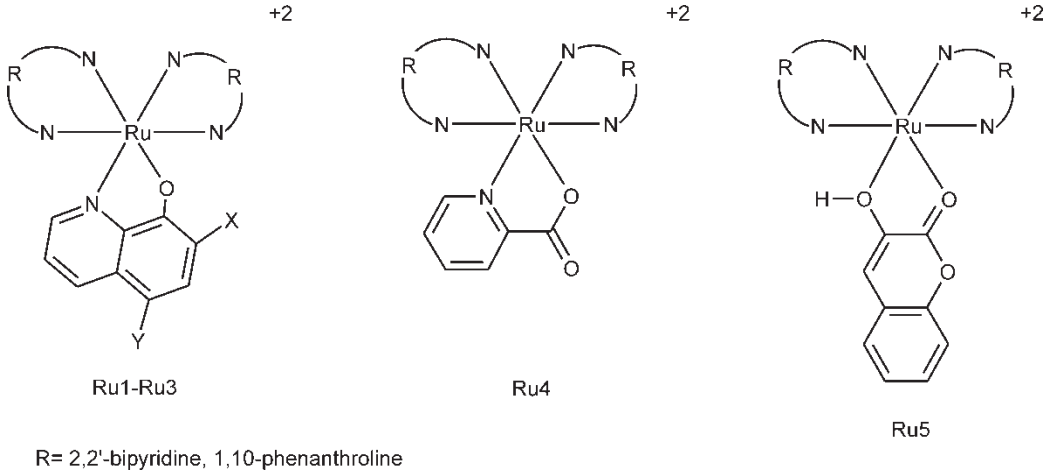


FIGURE 2 Proposed structures of Ru(II) complexes.

observed for the complexes $Ru(azpy)_2$(9-Me-adenine) and $Ru(bpy)_2$(9-Me-adenine).[29] In such a situation the two bipyridine or phenanthroline moieties become nonequivalent. Thus in the case of Ru1 and Ru2 there are twenty well-resolved resonance peaks (δ 9.178–7.5166, 20H, aromatic), 16 well-resolved resonance peaks (δ 8.84–7.30, 20H, aromatic) for Ru3. In Ru4 there are 9 well resolved resonance peaks.

These complexes show broad and intense visible bands between 350 to 450 nm due to metal to ligand charge transfer transition. In the UV region the bands at 290 nm and 310 nm are assigned to bipyridine ligand π–π* charge transfer transitions. The same transition is found in free 2,2′-bipyridine/1,10-phenanthroline at 280 nm thus the coordination of the ligand results in a red shift in the transition energy. There are also two shoulders at 390 and 500 nm which are, tentatively, attributed to a metal to ligand charge transfer transitions. The synthesized complexes were stable up to 270°C.

The FAB-MS of the prepared complexes has shown the following fragmentation pattern. The first fragment was the ion pair $[Ru(R)_2(L)]^{2+}Cl^-/ClO_4^-$ (often very weak or absent) followed by cation $[Ru(R)_2(L)]^{2+}$ (usually distinct) and others [Ru (R)(L)]$^{2+}$; $[Ru(R)_2]^{2+}$; $[Ru(R)]^{2+}$and $[Ru(L)]^{2+}$- respectively [where R = 2,2′-bipyridine/1,10-phenanthroline and L = BNQ/INQ/IHQS/PA/HC]. This type of fragmentation was also reported for $[Ru(phen)_2(phi)]Cl_2$ and $[Ru(bpy)_2(phi)]Cl_2$[30] (bpy = 2,2′-bipyridine; phen = 1,10-phenanthroline; phi = 9,10-phenanthrenequinonediimine). Thus the FAB-MS of the ruthenium complexes confirms their composition.

Based on the above observations, it is tentatively suggested that the prepared Ru(II) complexes showed an octahedral geometry (Figure 2).

Biological Activity and Discussion

The results are summarized in Tables I–III and the pharmacological data were analyzed statistically by ANOVA followed by Dunnett's test as a post hoc test of significance. Statistical significance was considered only when $p < 0.05$ and $F > F_{critical}$. All the complexes were tested for their anticancer activity against EAC bearing mice. Ru3 and Ru5 were found to increase the life span of the tumor hosts by 62 and 66% respectively. Ru3 and Ru5 were also found to bring the altered hemoglobin and RBC values of the EAC-bearing mice to near normal

TABLE I Antineoplastic activity (mean ± SEM) of ruthenium complexes against EAC bearing mice

Treatment	Total body weight (g)	Mean survival time (days)	ILS*(%)	Tumor volume (ml)	Viable cells in ascitic fluid (%)
Group I	22.2 ± 0.5	–	–	–	–
Group II	28.3 ± 0.6	21	–	3.4 ± 0.3	95.2 ± 3.5
Group III	18.2 ± 0.7	22	5	–	–
Group IV	23.1 ± 1.1	35	66	0.7 ± 0.01	42.3 ± 1.3
Group V	22.9 ± 0.8	34	62	0.9 ± 0.03	45.5 ± 1.3
Group VI	23.5 ± 0.4	33	57	0.6 ± 0.01	48.7 ± 2.1
Group VII	23.2 ± 0.5	34	62	0.9 ± 0.03	47.2 ± 2.5
Group VIII	25.1 ± 0.6	27	29	1.7 ± 0.04	69.1 ± 2.6

Group I: Vehicle (5 ml/kg); Group II: EAC (2×10^6 cells/mouse); Group III: Cisplatin (2 mg/kg) + EAC; Group IV–Group VIII: Ruthenium complexes (2 mg/kg) + EAC. *ILS = increase in life span.

TABLE II Effect of ruthenium complexes against EAC bearing mice on hematological profiles (mean ± SEM)

Treatment	Hb (g/dl)	RBC (count $\times 10^8$)	WBC (count $\times 10^6$)	Lymphocyte (%)	Granulocyte (%)	Monocyte (%)
Group I	12.8 ± 0.3	12.9 ± 1.1	6.9 ± 1.1	71.3 ± 2.4	2.1 ± 0.2	26.6 ± 0.4
Group II	9.1 ± 0.6	5.8 ± 0.8	15.9 ± 2.9	63.3 ± 2.7	32.5 ± 0.8	4.2 ± 1.2
Group III	9.9 ± 0.4	6.2 ± 0.3	9.9 ± 1.8	66.5 ± 3.1	28.1 ± 2.1	5.4 ± 0.3
Group IV	10.2 ± 0.4	6.5 ± 0.5	8.9 ± 0.8	79.7 ± 1.9	5.2 ± 0.3	15.1 ± 0.8
Group V	10.1 ± 0.2	6.4 ± 0.5	8.8 ± 0.6	79.8 ± 2.8	5.4 ± 0.4	14.8 ± 0.3
Group VI	10.5 ± 0.3	6.9 ± 0.2	9.3 ± 0.5	77.3 ± 2.1	5.2 ± 0.7	17.5 ± 0.7
Group VII	8.9 ± 0.4	5.3 ± 0.4	10.1 ± 0.4	77.7 ± 2.2	4.8 ± 0.6	17.5 ± 0.4
Group VIII	10.9 ± 0.1	7.2 ± 0.3	9.5 ± 1.1	80.9 ± 3.1	8.9 ± 0.9	11.2 ± 0.5

Group I: Vehicle (5 ml/kg); Group II: EAC (2×10^6 cells/mouse); Group III: Cisplatin (2 mg/kg) + EAC; Group IV–Group VIII: Ruthenium complexes (2 mg/kg) + EAC.

TABLE III Antibacterial activity of ruthenium complexes

Complex	*Vibrio cholerae* 865	*Vibrio cholerae* 14033	*Staph. aureus* 6571	*Staph. aureus* 8530	*Shigella flexneri*	*Shigella sonnai*
Ru 1 (0.215 mM)	14 (++)	12 (++)	14 (++)	12 (++)	11 (++)	13 (++)
Ru 2 (0.204 mM)	13 (++)	08 (+)	12 (++)	11 (++)	10 (+)	13 (++)
Ru 3 (0.222 mM)	12 (++)	09 (+)	12 (++)	10 (+)	11 (++)	11 (++)
Ru 4 (0.272 mM)	NA	NA	NA	NA	NA	NA
Ru 5 (0.258 mM)	NA	NA	NA	NA	NA	NA
STD	30 (+++++)	20 (+++)	26 (+++++)	26 (+++++)	20 (+++)	19 (+++)

STD = Chloramphenicol 10 μg/ml (0.031 mM). The figures represent diameter of zone of inhibition in mm (including bore size of 6 mm). Ru 1–Ru 5 = Ruthenium complexes. ' + ' Signs in parenthesis indicate scale of activity based on diameter of inhibition zone: < = 10 m (+), < = 15 mm (++), < = 20 mm (+++), < = 25 mm (++++) and < = 30 mm (++++ +). NA = No activity.

values. The results of the present study clearly demonstrated the tumor inhibitory activity of the ruthenium chelates against transplantable murine tumor cell line (Table I). The improvement in hematological profile (Table II) of the tumor bearing mice following treatment with ruthenium complexes could be secondary to tumor regression or due to the action of the compounds themselves.

The complexes were also evaluated for their antibacterial activity by the cup-plate method. A moderate antibacterial activity was observed (Table III) for Ru1–Ru3 against microorganisms such as *Vibrio cholerae* 865, *Staphyllococcus aureus* 6571 and *Shigella flexneri.* However Ru4 and Ru5 failed to show antibacterial activity against any of the tested organisms.

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