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Synthesis and anticancer activity of certain mononuclear Ru (II) Complexes

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Abstract

Bis(1,10-phenanthroline/2,2′-bipyridine) ruthenium(II)complexes containing TCP, TTZ OPBI, and BTSC ligands (where, TCP = 1-thiocarbamoyl-3,5-diphenyl-2-pyrazoline,TTZ = 2-(3,5-diphenyl-4,5-dihydropyrazol-1-yl)-4-phenylthiazole, OPBI = 2-hydroxyphenyl benzimidazole and BTSC = benzoin thiosemicarbazone) have been prepared and characterized. The spectral data suggested that the ligands were coordinated with the metal through nitrogen, sulfur and oxygen atoms. The target complexes were tested *in vivo* for anticancer activity against transplantable murine tumor cell line, Ehrlich's Ascitic Carcinoma (EAC). All these complexes increased the life span of the EAC-bearing mice, decreased their tumor volume and viable ascitic cell count as well as improved Hb, RBC and WBC counts. These results suggest that the Ru(II) complexes exhibit significant antitumor activity in EAC-bearing mice. It was also observed that the ruthenium complexes protected red blood cells from 2,2′-azo-bis(2-methylpropionamidine) dihydrochloride (AAPH)- induced hemolysis. The inhibitory effect was dose-dependent at a concentration of 20–120 μg/ml.

Keywords: *Ru (II) complexes, anticancer activity, hematological profile, AAPH*

Introduction

The success of platinum based anticancer drugs has focussed the screening of new metal-based anticancer complexes towards looking for damage caused to DNA. Due to the side effect and resistance of cisplatin in malignant disease this lead to a change in drug discovery strategies towards development of non-platinum metal complexes as anticancer agents. Several ruthenium complexes possess anticancer activity in various cancer cell lines [1,2] and several researchers are trying to synthesize less toxic Ru complexes with molecules found in the biological systems [3,4]. Various studies are involved with their focus on ruthenium complexes to inhibit cancer progression [5].

There are three main reasons that make ruthenium complexes well suited for medicinal applications: (i) the rate of ligand exchange, (ii) the range of accessible oxidation states, and (iii) the ability of ruthenium to mimic iron in binding to certain biological molecules. A certain novel class of Ru (terpy) $(NN)Cl^+$ complex (where terpy = terpyridine and NN = bidentate nitrogen ligand) was prepared and studied for it's reactivity towards the formation of Ru-DNA adduct as revealed by gel mobility shift assay [6]. Ru(III)

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complexes may act as prodrugs that are activated by reduction *in vivo* to coordinate more rapidly by utilizing oxygen and other nutrients. In this context the development of blood vessels often fails to keep pace with tumor growth and there is usually a lower oxygen content in tumor cells [7].

The molecular interactions in isolated mammalian nuclei of three ruthenium complexes. Na_2[*trans*{$RuCl_4$(DMSO)(imidazole)}] NAMI, Na_2 [*trans*{$RuCl_4$(DMSO)(oxazole)}]NAOX and Na_2[*trans*{$RuCl_4$(TMSO) (isoquinoline)}] TEQU, which are putative antineoplastic chemotherapeutic agents effective in reducing metastatic tumors *in vivo*, have been investigated and compared with the well-known antitumor drug cisplatin [8].

A previous investigation by our group has described the antitumor and antibacterial activities of the type $[Ru(R)_2(L)]^{2+}$ (R = 1,10-phenanthroline/2,2′-bipyridine and L = tpl,4-Cl-tpl,4-CH_3-tpl,4-OCH_3-tpl, 4-NO_2-tpl,pai, where tpl = thiopicolinanilide and pai = 2-phenyl azo imidazole) [9]. The present investigation, is concerned with investigating novel ruthenium complexes and their anticancer activity against EAC cell lines. AAPH-induced RBC hemolysis was also studied for the determination of the protective effect of ruthenium complexes, which also has an important role in hemorrheology.

Materials and methods

Chemistry

All chemicals used in this study were of AR grade (SD fine chemicals, Mumbai and E. Merck, Mumbai). Hydrated ruthenium trichloride was purchased from SRL, Mumbai and used as received. AAPH was purchased from Sigma-Aldrich, Germany. FTIR spectra were recorded in KBr discs on a Jasco V410/Schimadzu IR spectrometer. ^{1}H-NMR spectra were recorded on a Bruker Ultraspec (500 MHz/ AMX400 MHz/300 MHz) spectrometer and the reported chemical shifts were against TMS. FAB mass spectra were recorded on a JEOL JMS600 spectrum with mNBA matrix. Benzoin thiosemicarbazone (BTSC) was prepared according to the literature method [10].

Preparation of 1-Thiocarbamoyl-3,5-diphenyl-2-pyrazoline (TCP) [*11*]. A mixture of chalcone (2.08 g, 10 mmol) and thiosemicarbazide (0.91 g, 10 mmol) in 50 ml ethanol and a solution of KOH (1.0 g, 1.8 mmol) in 5 ml ethanol was refluxed for 2 h. After cooling, the precipitate was filtered off, washed with ethanol and ether. Yield 66%, m.p. 203–204°C (lit 206°C). IR (KBr) cm^{-1}: 3425(N–H), 3036(C–H), 1591(C=N), 1340 (C=S). Calcd. for $C_{16}H_{15}N_3S$: C, 68.30; H, 5.37; N, 14.93. Found C, 67.52; H, 4.27; N, 15.90%.

Preparation of 2-(3,5-Diphenyl-4,5-dihydropyrazol-1-yl)-4-phenylthiazole(TTZ) [11]. In a flask fitted with a reflux condenser TCP (0.56 g) and 2-bromoacetophenone (0.2 g) were added and the mixture was refluxed in alcohol (5 ml) for 1 h, diluted with water (20 ml) and neutralized with 5% $NaHCO_3$ solution. The precipitate was filtered off and washed with water and ether. Yield 75%, m.p. 213°C. IR (KBr) cm^{-1}: 3052 (C–H), 1609, 1582(C=N), 728(C–S). Calcd. for $C_{24}H_{19}N_3S$: C, 75.56; H, 5.02; N, 11.01. Found C, 74.86; H, 4.07; N, 11.98%.

Preparation of 2-hydroxyphenyl benzimidazole (OPBI) [12]. A mixture of 5.9 g (0.055 mol) of *o*-phenylenediamine, 7.1 g (0.052 mol) of salicylic acid and polyphosphoric acid (PPA) (40 g) was stirred 160°C for 4 h under nitrogen. The mixture was poured into 400 ml of water and the resulting solution adjusted to pH = 7 with Na_2CO_3. The precipitated solid was filtered off, recrystalised from ethyl acetate to yield the product as a colorless solid. Yield 65%, m.p. 235°C. IR (KBr) cm^{-1}: 3100–2700(O–H), 1623(C=C), 1612(C=N), 3055(C–H). Calcd. for $C_{13}H_{10}N_2O$: C, 74.27; H, 4.79; N, 13.32. Found C, 73.98; H, 4.05; N, 14.08%.

Preparation of Cis-$[Ru(R)_2Cl_2]^{+2}$(where, R = 1, 10-phenanthroline/2,2′-bipyridine). A mixture of $RuCl_3 \cdot 3H_2O$ (1.15 g, 2.5 mmol) and R (5 mmol) in DMF was refluxed for 3 h when a purple coloured product slowly precipitated. The solution was left overnight at 0°C, which gave a microcrystalline product which was filtered off, washed with 30% LiCl solution and finally recrystallised from the same. The product (yield 75%) was dried and stored in a vacuum dessicator over P_2O_5 for future use.

Preparation of the $[Ru(phen)_2(L)](ClO_4)$ complexes (Ru1-Ru6)[(where phen = 1,10-phenanthroline and L = OPBI, TTZ, BTSC and TCP). $Ru(phen)_2Cl_2$ (106 mg, 2 mmol) and excess of ligand (L) (2.5 mmol) was mixed in dry ethanol and the resulting solution was refluxed under a nitrogen atmosphere. The reaction was monitored by TLC. After completion of the reaction, the mixture was subjected to column chromatography using silica gel (230–400 mesh). The column was eluted with chloroform and methanol in an ascending order of polarity. After collecting the product, the excess solvent was evaporated under reduced pressure and a saturated aqueous solution of sodium perchlorate was added. The resultant solution was kept at 0°C overnight to give a microcrystalline precipitate.

Ru1. 48%, black crystals, IR (KBr) cm^{-1}: 1601(C=N), 3041 (C–H), 1625 (C=C). Calcd. for

$C_{37}H_{25}ClN_6O_5Ru$: C, 57.70; H, 3.27; N, 10.91. Found C, 56.98; H, 4.04; N, 10.50%. ^{1}H NMR (DMSO-d_6): δ ppm: 13.17 (s, 1H), 9.20 (d, J = 4.81, 1H), 8.70 (d, J = 2.91, 3H), 8.51 (dd, J = 8.09, 13.79, 2H), 8.35 (m,5H), 8.05 (t, J = 13.38, 1H), 7.90 (t, J = 13.42, 1H), 7.80 (d, J = 7.81,1H), 7.62 (d, J = 4.13, 1H), 7.51 (t, J = 5.06, 2H), 7.42 (d, J = 7.51,1H), 7.01 (m,2H),6.51 (t, J = 14.54, 2H), 6.34 (d, J = 8.35, 1H), 5.39 (d, J = 8.4, 1H). FAB-MS (mNBA): 770 $[Ru(phen)_2(OPBI)]^{2+}(ClO_4)^-$; 671 $[Ru(phen)_2(OPBI)]^{2+}$; 490 $[Ru(phen)(OPBI)]^{2+}$; 462$[Ru(phen)_2]$.

Ru2. 40%, Black crystals. IR (KBr) cm^{-1}: 3041 (C–H), 1614, 1586 (C=N), 728 (C–S). Calcd. for $C_{48}H_{35}ClN_7O_4RuS$: C, 61.17; H, 3.74; N, 10.40. Found C, 60.65; H, 3.18; N, 11.22%. FAB-MS (mNBA): 942$[Ru(phen)_2(TTZ)]^{2+}(ClO_4)^-$; 843 $[Ru(phen)_2\ (TTZ)]^{2+}$; 663 $[Ru(phen)(TTZ)]^{2+}$; 462 $[Ru(phen)_2]$.

Ru3. 46%, Black crystals. IR (KBr) cm^{-1}: 3412 (O–H), 3260, 3164 (NH & NH_2), 1601 (C=N), 1392 (C=S). Calcd. for $C_{39}H_{31}ClN_7O_5RuS$: C, 55.35; H, 3.69; N, 11.59. Found C, 55.01; H, 3.19; N, 12.39%. FAB-MS (mNBA): 846 $[Ru(phen)_2(BTSC)]^{2+}(ClO_4)^-$; 747 $[Ru(phen)_2(BTSC)]^{2+}$; 566 $[Ru(phen)(BTSC)]^{2+}$; 462 $[Ru(phen)_2]$.

Ru4. 43%, Black crystal. IR (KBr) cm^{-1}: 3425 (N–H), 1583 (C=N), 3032 (C–H), 1326 (C=S). Calcd for $C_{40}H_{31}ClN_7O_4RuS$: C, 57.04; H, 3.71; N, 11.64. Found C, 56.65; H, 3.24; N, 12.02%. ^{1}H-NMR (DMSO-d_6): δ ppm: 10.15 (d, J = 4.12, 1H), 10.05 (d, J = 4.04, 1H), 9.75 (s,1H), 9.59 (s, 1H), 8.82 (d, J = 6.52, 1H), 8.75 (t, J = 10.78), 8.46 (dd, J = 3.49, 6.35, 1H), 8.39 (d, J = 6.32, 1H), 8.35 (dd, J = 3.19,7.14, 2H), 8.26 (d, J = 6.92, 1H), 8.20 (d, J = 6.85,2H), 8.08 (d, J = 7.11,1H), 7.49-7.41 (m,5H), 7.86 (d, J = 5.62, 1H), 7.81 (d, J = 5.84, 2H), 7.67 (d, J = 4.13, 1H), 7.54 (t, J = 4.01,10.32, 1H), 7.49–7.41 (m, 5H), 7.32 (dd, J = 4.08,6.38, 1H), 7.12 (d, J = 5.48, 1H), 6.55 (s, 1H), 6.32(s,1H),3.12(dd, J = 7.8,14.2,1H),3.90(dd, J = 4.8,10.8,1H).

FAB-MS(mNBA):842 $[Ru(phen)_2(TCP)]^{2+}(ClO_4)^-$; 743 $[Ru(phen)_2\ (TCP)]^{2+}$; 562 $[Ru(phen)(TCP)]^{2+}$; 462 $[Ru(phen)_2]$.

General procedure for preparing cis-[Ru(bpy)$_2$(L)]Cl$_2$ (where bpy = 2,2′-bipyridine, L = TCP,BTSC). The complexes was prepared in a similar manner to that for **Ru1** above with microcrystalline *cis*-$Ru(bpy)_2Cl_2$ and ligand (TCP,BTSC). Finally, they were purified on column chromatography using silica gel as stationary phase and chloroform-methanol as eluent. In order to prepare **Ru5**, the final product was poured into a saturated solution of sodium perchlorate and kept at 0°C overnight when a microcrystalline precipitate was obtained. The crystals were filtered off and dried over $CaCl_2$ in vacuum.

Ru5:- 46%, Black crystal. IR (KBr) cm^{-1}: 3409 (N–H), 1576 (C=N), 3029 (C–H), 1321 (C=S). Calcd. for $C_{36}H_{31}ClN_7O_4RuS$: C, 54.44; H, 3.93; N, 12.34. Found C, 54.91; H, 3.33; N, 12.97%. FAB-MS (mNBA): 794 $[Ru(bpy)_2(TCP)]^{2+}(ClO_4)^-$; 695 $[Ru(bpy)_2(TCP)]^{2+}$; 538 $[Ru(bpy)\ (TCP)]^{2+}$; 413 $[Ru(bpy)]$.

Ru6. 46%, Black crystals. IR (KBr) cm^{-1}: 3408 (O–H), 3260, 3158 (NH & NH_2), 1596 (C=N), 1396 (C=S). Calcd. for $C_{35}H_{31}ClN_7ORuS$: C, 54.62; H, 4.06; N, 16.7. Found C, 53.91; H,3.75; N,17.29%. FAB-MS (mNBA): 769 $[Ru(bpy)_2(BTSC)]^{2+}Cl_2^-$; 698 $[Ru(bpy)_2\ (BTSC)]^{2+}$; 542 $[Ru(bpy)\ (BTSC)]^{2+}$; 413 $[Ru(bpy)]$.

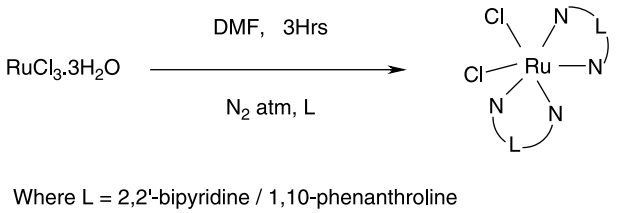
Anticancer activity [13]

Male Swiss albino mice were divided into nine groups (n = 8). EAC cells were collected from the donor mouse and suspended in sterile isotonic saline. The viable EAC cells were counted by Trypan blue exclusion assay and were adjusted to 2×10^6 cells/ml. The EAC cells were injected (i.p.) to all the groups except for Group I on day zero (day 0). A day of incubation was allowed for the multiplication of the cells. Control animals received only vehicle (Group I) and Group II was kept as an EAC control. Group III was treated with the standard drug cisplatin (2 mg/kg b.w.). All the ruthenium complexes were administered at a dose of 2 mg /kg bodyweight in groups IV-IX for 9 days. Food and water were withheld 18 h before the animals were sacrificed. On day 10, half of the animals (n = 4) in each group were sacrificed and the remaining animals were kept to observe the life span of the hosts.

The effect of ruthenium complexes on tumor growth was monitored by recording the daily mortality and % increase in life span (ILS) was calculated using the following formula: ILS (%) = [(Mean survival of treated group)/Mean survival of control group) – 1] × 100.

Tumor Volume and Viable count [14]. The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The tumor volume was measured by placing it in a graduated centrifuge tube and determining the packed cell volume by centrifuging at 1000 × g for 5 min. The viable and nonviable cells were counted by the Trypan blue exclusion method (0.4% in normal saline) using a Neubauer counting chamber.

Hematological parameters [14]. Blood was collected 24 h after the last dose from the tail vein and drawn into a RBC or WBC pipette and counted in a Neubauer counting chamber. The hemoglobin concentration was



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

determined by Sahli's hemoglobinometer method. A differential count of leukocytes was performed on a freshly drawn blood smear using Leishman's stain.

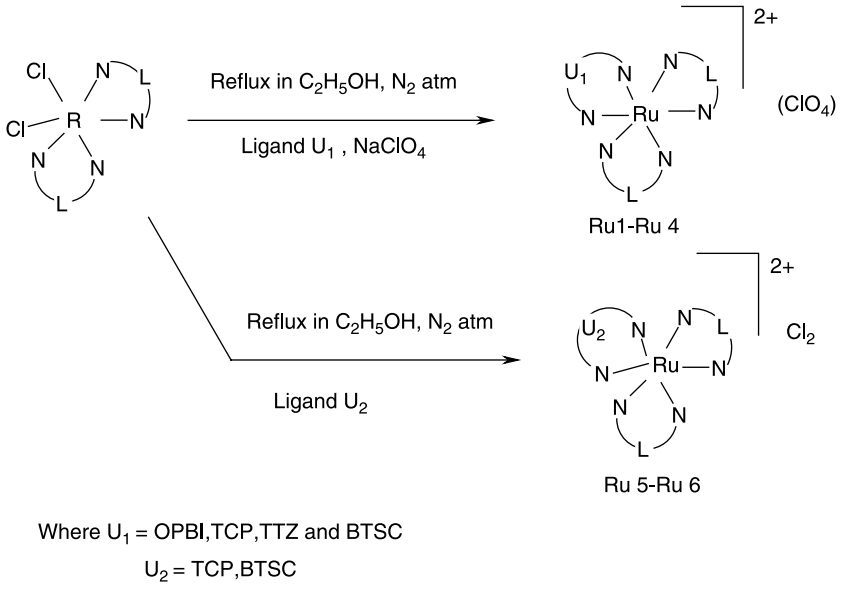
Assay for Anti-hemolytic activity [15]. Blood was obtained from male Swiss albino mice of (20–22 g). The erythrocytes were separated from the plasma and the buffy coat was washed three times with 10 ml of 10 mM phosphate buffer saline (PBS) at pH 7.4 and centrifuged at 1500 × g for 5 min. During the last washing, the erythrocytes were obtained by centrifugation at 1500 × g for 10 min. Then 0.1 ml of 20% suspension of erythrocytes in PBS was added to 0.2 ml of 200 mM 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH) solution (in PBS) and 0.1 ml of the ruthenium complex in 1% DMSO at different concentrations (20–120 μg/ml). The reaction mixture was shaken gently while being incubated at 37°C for 3 h and then diluted with 0.8 ml of PBS and centrifuged at 1000 × g for 10 min; the absorbance of the supernatant was then measured at 540 nm by spectrophotometer (Absorbance A). Similarly, the reaction mixture was treated with 0.8 ml of distilled water to achieve complete hemolysis and the absorbance of the supernatant obtained after centrifugation was measured at 540 nm (Absorbance B). The % inhibition of hemolysis was calculated from: % hemolysis inhibition = (1 − A/B) × 100.

Results and discussion

Chemistry

Synthesis of ruthenium complexes were carried out as shown in Schemes 1 and 2. Nitrogen, sulfur and oxygen centered ligands were used for the synthesis of the complexes and the yield of compounds **Ru1**-**Ru6** was 60–75%.

All the complexes were non-hygroscopic, insoluble in water but soluble in dimethylsulfoxide and acetonitrile and contained a 2:1 metal to ligand ratio. The melting points of all the complexes was above 300°C. Elemental analysis of the complexes was in accord with the theoretical values. The ligands used in this study are capable of forming bidentate complexes and their purity was confirmed by m.p., elemental analysis, FTIR spectroscopy. Ruthenium chloride undergoes reduction in a number of organic solvents. It was refluxed in dimethylformamide in the presence of 1,10-phenanthroline or 2,2′-bipyridine in excess of the stoichiometric amount, which afforded the final product of *cis*-(bis(1,10-phenanthroline) dichloroRu(II) or *cis*-bis(2,2′-bipyridine) dichloro-Ru(II) [16]. The introduction of the third ligand was carried out in the presence of anhydrous alcohol. The final complex formed had an ionic chloride in the molecule and hence a polar solvent was used to complete the aforesaid reaction. The IR spectra of the



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

complexes showed characteristic changes when compared to those of the free ligands. The ligand OPBI showed a broadband in the region 3100–2700 cm^{-1} due to the phenolic hydroxyl group. The band of medium intensity around 1612 cm^{-1} in the spectrum of the free ligands may be attributed to νC=N stretching vibrations. The νOH vibration disappeared in the IR spectra of the ruthenium complex which indicates the deprotonation of the νOH group upon coordination to the metal. The νC=N group band observed in the spectra of the ligand was shifted to lower frequency upon complexation. In the case of TCP, the appearance of a new band at 1591 cm^{-1}, assigned to the (νC=N) linkage, suggested condensation and formation of the proposed ligands. The shifting of this band to the lower frequency region (10–15 cm^{-1}) provided further evidence in support of the involvement of this nitrogen in coordination with the metal atom. The IR spectra showing a band at 1340 cm^{-1} which was shifted to lower frequency region in the metal complexes, confirming the coordination of the ligand to the metal ion through a νC=S moiety.

A comparison of the IR spectra of the TTZ ligand and its complex indicates that the ligand was coordinated to the metal ion by both imine nitrogens but not with a sulfur atom. The band around 728 cm^{-1} for νC–S was not changed in the spectra of ligand and complex of TTZ. In the IR spectra of the BTSC complex, a strong band near 1392 cm^{-1} which corresponded to a νC=S stretching vibration was changed in the spectra of the complex. The band around 1601 cm^{-1} which was shifted to higher frequency in the case of their complexes suggested coordination through the νC=N group. These observations clearly indicate the participation of a νC=N group and thiocarbamoyl sulfur in coordination to the metal ion. It is well known that the BTSC ligand in the free state exists in the *trans* configuration with respect to the thiocarbamoyl sulfur and the terminal nitrogen atom of the thiosemicarbazone moiety, but during complex formation they became *cis* to each other. The other bands in the spectrum of each complex were similar to those in the corresponding ligand spectrum, except for slight shifts in their positions and changes in their intensities due to coordination.

In the ^{1}H-NMR spectra of the complexes, there were well resolved resonance peaks at low field at δ 13.17 (s,br, NH), 8.70 (d, Ar-H). Thus in the case of

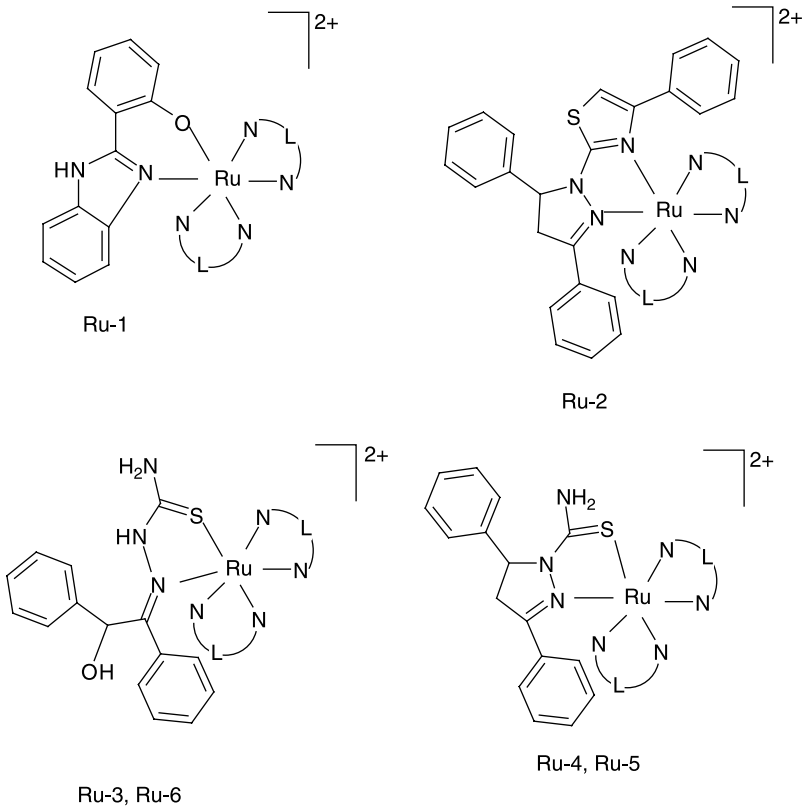


Figure 1. Structures of the ruthenium(II) complexes.

Ru1 there were 16 well resolved resonance peaks (δ7.01–9.20, aromatic) and 18 well resolved peaks (δ 6.55–9.75, aromatic) for **Ru4**.

The mass spectra of the complexes confirmed the suggested formula by their molecular ion peaks. The spectrum showed numerous peaks representing successive degradation of the molecule. FAB mass spectroscopic data clearly suggested that mononuclear complexes had been formed in each case, the first fragment being due to $[Ru(R)_2(L)]^{2+}$ Cl^-/ClO_4^- ion pair. The complex also showed a peak due to the complex cation $[Ru(R)_2(L)]^{2+}$ and others due to $[Ru(R)(L)]^{2+}$, $[Ru(R)_2]^{2+}$ respectively [where R = 2,2′-bipyridine /1,10-phenanthroline and L = OPBI, TCP, TTZ and BTSC]. This type of fragmentation was reported for $[Ru(phen)_2(nmit)]Cl_2$ and $[Ru(bpy)_2(ihqs)]Cl_2$, where phen = 1,10-phenanthroline, bpy = 2,2′-bipyridine, nmit = N-methyl isatin thiosemicarbazone, ihqs = 7-iodo-8-hydroxy quinoline-5-sulfonic acid [17]. Thus, based on the above observations, the proposed structures of the complexes are as shown in Figure 1.

Biological activity and Discussion

The pharmacological data was analyzed by ANOVA followed by Dunnett's test of significance and the results are summarized in Tables I–III. Statistical significance was considered only when $p < 0.05$ and $F > F_{critical}$. Antitumor activity of ruthenium complexes against EAC-tumor bearing mice was assessed by parameters such as tumor volume, cell count (viable and nonviable), mean survival time and % increase of life span. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirements of tumor cells. The tumor volume and viable cell counts were found to be increased significantly and the non-viable cell count was significantly low in EAC control animals. Administration of the ruthenium complexes at a dose of 2 mg/kg significantly decreased the tumor volume, packed cell volume and viable cell count in ruthenium-treated animals in comparison with the EAC control animals. A reliable criteria for judging the value of any anticancer drugs are the prolongation in the life span of the animals. Treatment with the Ru complexes increased the life span of the tumor-bearing mice.

The mean survival time was increased to 38 and 35 days on administration of **Ru1** and **Ru2** at a dose of 2 mg/kg, respectively, but for cisplatin the survival time was only 23 days. A novel anticancer agent, NAMI-A (Imidazolium-*trans*-DMSO-imidazole-tet-

Table I. Anticancer activity 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	21.4 ± 0.9	–	–	–	–
Group II	28.0 ± 1.5	22	–	3.4 ± 0.3	96.5 ± 1.4
Group III	18.5 ± 0.6	23	5	–	–
Group IV	23.1 ± 0.9	38	73	0.8 ± 0.2	47.0 ± 1.7
Group V	22.1 ± 1.1	35	59	0.8 ± 0.3	45.6 ± 3.0
Group VI	24.4 ± 0.7	27	23	1.8 ± 0.3	67.5 ± 1.9
Group VII	25.1 ± 0.9	30	36	1.8 ± 0.3	75.4 ± 2.6
Group VIII	24.8 ± 0.3	31	41	1.5 ± 0.2	64.6 ± 1.5
Group IX	23.6 ± 0.8	25	14	1.4 ± 0.3	64.9 ± 2.2

Values are mean ± SEM.Group I: Vehicle (5 ml/kg); Group II: EAC (2×10^6 cells/mouse); Group III: Cisplatin (2 mg/kg) + EAC; Group IV-IX: Ruthenium complexes (2 mg/kg) + EAC.

Table II. Effect of ruthenium complexes on the hematological profile of EAC-bearing mice.

Treatment	Hemoglobin (g/dl)	RBC (cell count × 10^8)	WBC (cell count × 10^6)	Lymphocyte (%)	Monocyte (%)	Granulocyte (%)
Group I	12.2 ± 0.4	12.6 ± 0.5	6.6 ± 0.3	71.8 ± 0.5	2.6 ± 0.4	26.1 ± 0.6
Group II	10.0 ± 0.6	6.6 ± 0.6	17.7 ± 0.6	62.7 ± 0.7	33.4 ± 0.4	4.6 ± 0.5
Group III	11.0 ± 0.7	4.9 ± 0.7	10.8 ± 0.3	66.0 ± 0.78	29.4 ± 0.3	5.2 ± 0.2
Group IV	11.7 ± 0.6	6.7 ± 0.4	9.2 ± 0.8	77.8 ± 0.5	5.3 ± 0.2	18.0 ± 0.7
Group V	12.2 ± 0.6	6.0 ± 0.4	9.7 ± 0.7	77.0 ± 0.7	4.7 ± 0.1	17.5 ± 0.6
Group VI	10.2 ± 0.8	5.9 ± 0.8	11.9 ± 0.4	80.2 ± 0.7	8.7 ± 0.2	11.7 ± 0.4
Group VII	9.4 ± 0.9	5.8 ± 0.8	10.8 ± 0.9	81.0 ± 0.4	8.2 ± 0.2	11.8 ± 0.4
GroupVIII	9.8 ± 0.6	6.1 ± 0.6	10.7 ± 0.6	80.2 ± 1.0	7.3 ± 0.2	13.3 ± 0.6
Group IX	8.9 ± 0.5	5.6 ± 0.5	10.9 ± 0.9	79.0 ± 0.5	7.3 ± 0.2	14.6 ± 0.8

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

Table III. IC_{50} value of synthesized ruthenium complexes in the antihemolytic activity assay.

Synthesized complexes	IC_{50} Values (μg/ml)
Ru Complex – 1	76.15
Ru Complex – 2	73.28
Ru Complex – 3	89.62
Ru Complex – 4	94.37
Ru Complex – 5	85.51
Ru Complex – 6	84.24

rachlororuthenate), also shows remarkable antitumor activity and lower systemic toxicity than cisplatin [18,19]. Finally the changed body weights of the animals confirmed the tumor growth inhibiting property of the ruthenium complexes.

Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia [20,21]. Hematological parameters (Table II) for tumor-bearing mice on day 10 were found to be significantly altered when compared to the normal group. The total WBC count was found to be increased with a reduction in the hemoglobin content of RBC in EAC control mice. At the same time interval, the ruthenium complex treatment restored all the altered hematological parameters to almost near to normal. It may be concluded that the Ru complexes by decreasing the nutritional fluid volume and arresting the tumor growth, increased the life span of the EAC-bearing mice.

The biomembrane may be most susceptible to metal-induced toxicity due to its content of polyunsaturated fatty acids. The present study revealed that the ruthenium complexes stabilized the RBC membrane incubated with AAPH. Table III shows the anti-hemolytic properties of ruthenium complexes. The inhibitory effect was dose-dependent at a concentration of 20–120 μg/ml.

All these results clearly indicate that the ruthenium complexes had a remarkable capacity to inhibit the tumor growth of the EAC cell line in a dose–dependent manner in experimental animals.

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