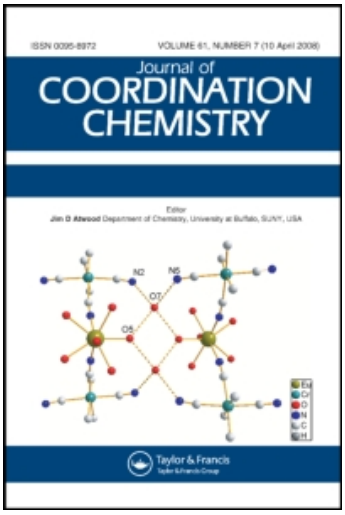

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Synthesis, characterization, antitumor, and cytotoxic activity of mononuclear Ru(II) complexes

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Synthesis, characterization, antitumor, and cytotoxic activity of mononuclear Ru(II) complexes

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In the search for antitumor active metal complexes several ruthenium complexes have been reported to be promising. A series of mononuclear Ru(II) complexes, $[Ru(T)_2(S)]^{2+}$, where T = 2,2′-bipyridine/1,10-phenanthroline and S = CH_3-bitsz, Cl-bitsz, Br-bitsz, tmtsz, dmtsz, have been prepared and characterized by UV-Vis, IR, ^{1}H-NMR, FAB-mass spectroscopy, and elemental analysis. The complexes were subjected to *in vivo* anticancer activity against a transplantable murine tumor cell line Ehrlich's ascitic carcinoma (EAC) and *in vitro* cytotoxic activity against human cancer cell line Molt 4/C_8, CEM, and murine tumor cell line L1210. Ruthenium complexes showed promising biological activity especially in decreasing tumor volume and viable ascitic cell counts. Treatment with these complexes prolonged the life span of EAC-tumor-bearing mice by 10–48%. *In vitro* evaluation of these ruthenium complexes revealed cytotoxic activity from 0.21 to 24 μmol L^{-1} against Molt 4/C_8, 0.16–19 μmol L^{-1} against CEM, and 0.75–32 μmol L^{-1} against L1210 cell proliferation, depending on the nature of the compound.

Keywords: Ru(II) complexes; Antitumor; EAC; Cytotoxicity

1. Introduction

Metal ions show good effect on cellular process, not only natural processes, such as cell division and gene expression, but also toxicity, carcinogenicity, and antitumor chemistry. In the search for antitumor metal complexes several ruthenium complexes have shown promise as anticancer drugs. A series of mononuclear Ru(II) complexes of the type $[Ru[M]_2[U]]^{2+}$, where M = 2,2′-bipyridine/1,10-phenanthroline and U = tpl, 4-Cl-tpl, 4-CH_3-tpl, 4-OCH_3-tpl, 4-NO_2-tpl, and pai [1].

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The objectives of the present investigations were to develop analogs of $[Ru[T]_2[S]]$ principally as candidate cytotoxins. The discovery of antitumor properties of cisplatin in 1965 marked the development of metallo-pharmaceuticals in cancer chemotherapy [2]. Platinum drugs induce cytotoxicity by cross-linking DNA, causing changes to the DNA structure resulting in the inhibition of replication and protein synthesis. However, the application of platinum drugs suffers from their high general toxicity leading to severe toxic side effects. In comparison, ruthenium complexes have attracted attention in the last 20 years as potential antitumor agents. Some exhibit very encouraging pharmacological profiles [3].

Ruthenium compounds are regarded as promising alternatives to platinum compounds and offer many approaches to metallo-pharmaceuticals; the compounds are known to be stable and to have predictable structures both in the solid state and in solution with the tuning of ligand affinities and steadily increasing knowledge of the biological effects of ruthenium compounds [4]. The first systematic investigation of ruthenium compounds and their antitumor properties was done in the beginning of the 1980s with *fac*-$[RuCl_3(NH_3)_3]$ and *cis*-$[RuCl_2(NH_3)_4]Cl$ [5] preceded by the discovery that ruthenium red possesses antitumor properties in the 1970s [6, 7]. Since then compounds, such as *trans*-$(IndH)[Ru(ind)_2Cl_4]$ (Ind = indazole), *mer*-$[Ru(terpy)Cl_3$ (terpy = 2,2′-terpyridine) [8–10], $[Ru(dmso)_4Cl_2]$ [11], $ImH[Ru(im)Cl_5]$ [12], $ImH[Ru(im)_2$-$Cl_4]$ [13], and $ImH[Ru(im)(dmso)Cl_4]$ [14] NAMI-A (im = imidazole) are also well-known antitumor agents.

Ruthenium(II) arene complexes show remarkable cytotoxic properties *in vitro* as well as *in vivo* [15, 16]. A series of complexes with general formula $[Ru(\eta^6$-arene$)Cl(en)][PF_6]$ (en = ethylene diamine, arene = benzene, *p*-cymene, tetrahydroanthracene, etc.) have been studied for their *in vitro* anticancer activity [17].

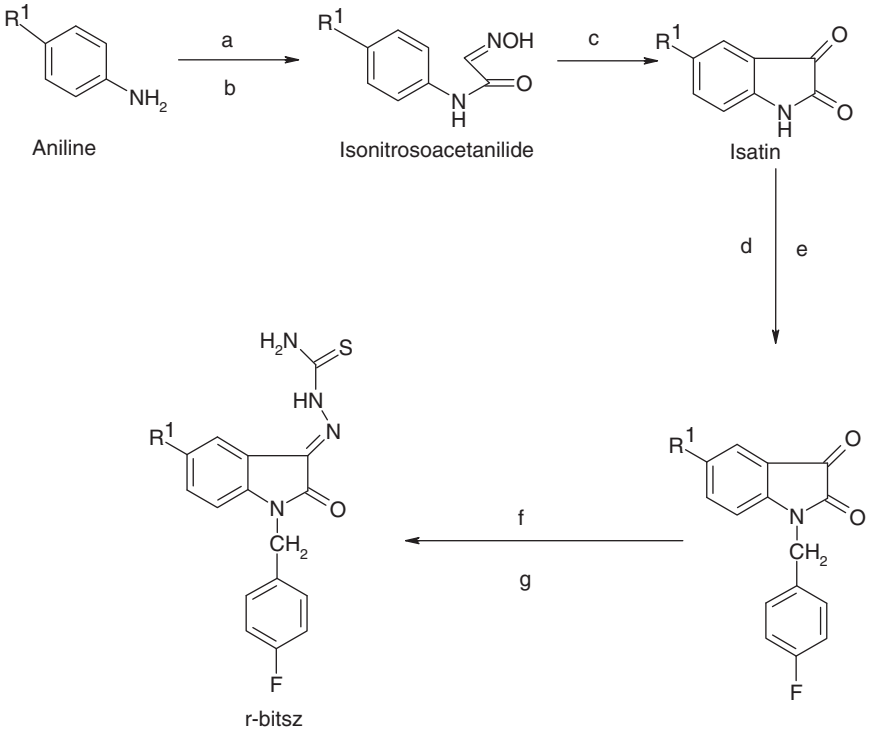
Ruthenium compounds with bidentate ligands show intercalation properties with DNA [18]. Ru(II) compounds are kinetically more reactive than Ru(III) [19]. So, we have reported that Ru(II) compounds bearing thiosemicarbazides (TSC), 8-hydroxy quinolines, have *in vivo* anticancer and *in vitro* antibacterial activity [20, 21]. Recently we reported Ru(II) compounds bearing isatin thiosemicarbazones, chloro-fluorophenyl imino methyl phenol have *in vivo* anticancer and *in vitro* cytotoxic activity [22]. In this work, we describe the synthesis and characterization of some ruthenium complexes, their *in vitro* cytotoxic activity against human cancer cell line Molt $4/C_8$, CEM, and murine tumor cell line L1210, and their *in vivo* anticancer activity against transplantable murine tumor cell line Ehrlich's ascitic carcinoma (EAC). Our research has focused on the complexes of the general formula $[Ru[T]_2[S]]Cl_2$, where T = 2,2′-bipyridine/1,10-phenanthroline and S = CH_3-bitsz, Cl-bitsz, Br-bitsz, tmtsz, and dmtsz.

2. Experimental

2.1. *General methods*

AR grade solvents 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-Vis spectra were on a Jasco spectrophotometer. FTIR spectra were recorded in KBr powder on a Jasco V410 FTIR spectrometer by diffuse reflectance technique. $^{1}H/^{13}C$ NMR spectra were measured in $CDCl_3$ and DMSO-d_6 on a Bruker Ultraspec 500 MHz/AMX 400/300 MHz spectrometer. The reported chemical shifts were against that of TMS. FAB-mass spectra were recorded on a JEOL JMS600 spectrum with mNBA matrix. Substituted *N*-benzyl isatin thiosemicarbazones [23, 24] were prepared according to the literature method.



2.2. *General procedure for preparing thiophene-2-methylene thiosemicarbazone*

In a round bottom flask fitted with a reflux condenser thiophene-2-carboxyaldehyde (0.01 mol) and TSC (0.01 mol) were added. The mixture was refluxed in anhydrous alcohol for 4–5 h and left overnight. The solid that separated was filtered and dried. The crude solid was purified by recrystallization from alcohol to give white crystals.

2.2.1. CH_3-bitsz. Yield 74%, m.p. 259–260°C (lit., 260°C). IR (KBr) cm^{-1}: 3416–3262 (NH_2 and NH), 3031 (C–H), 2924 (C–H), 1693 (C=O), 1341 (C=S). Calcd for $C_{17}H_{15}O_1N_4S_1F_1$ (%): C, 59.64; H, 4.42; N, 16.36. Found (%): C, 59.20; H, 4.35; N, 16.28. λ_{max} nm (MeOH): 270, 366. ^{1}H NMR (DMSO-d_6): $\delta = 10.64$ (1H, s), 8.07 (1H, s), 7.99 (2H, d), 7.87 (2H, s), 7.73 (2H, d, $J = 8.6$ Hz), 7.62 (2H, d, NH_2), 6.45 (2H, d, $J = 8.6$ Hz), 1.56 (3H, s, CH_3).

2.2.2. Cl-bitsz. Yield 69%, m.p. 243–245°C (lit., 245°C). IR (KBr) cm^{-1}: 3427–3272 (NH_2 and NH), 3069 (C–H), 2922 (C–H), 1687 (C=O), 1335 (C=S). Calcd for $C_{16}H_{12}O_1N_4S_1F_1Cl_1$ (%): C, 52.97; H, 3.33; N, 15.44. Found (%): C, 52.76; H, 3.31; N, 15.29. λ_{max} nm (MeOH): 250, 287, 369. ^{1}H NMR (DMSO-d_6): $\delta = 10.29$ (1H, s), 8.04 (2H, d), 7.98 (2H, s), 7.87 (2H, d, $J = 8.6$ Hz), 7.80 (1H, s), 7.74 (2H, d, NH_2), 6.58 (2H, d, $J = 8.6$ Hz).

2.2.3. Br-bitsz. Yield 85%, m.p. 241–242°C (lit., 242°C). IR (KBr) cm^{-1}: 3422–3238 (NH_2 and NH), 3143 (C–H), 1682 (C=O), 1346 (C=S). Calcd for $C_{16}H_{12}O_1N_4S_1F_1Br_1$ (%): C, 47.19; H, 2.97; N, 13.76. Found (%): C, 47.16; H, 2.94; N, 13.69. λ_{max} nm (MeOH): 242, 277, 358. ^{1}H NMR (DMSO-d_6): $\delta = 10.01$ (1H, s), 8.12 (2H, d), 8.00 (2H, s), 7.89 (2H, d, $J = 8.6$ Hz), 7.72 (1H, s), 7.56 (2H, d, NH_2), 6.39 (2H, d, $J = 8.6$ Hz).

2.2.4. tmtsz. Yield 78%, m.p. 182–184°C. IR (KBr) cm^{-1}: 3447–3263 (NH_2 and NH), 3021 (C–H), 1326 (C=S). Calcd for $C_6H_7N_3S_2$ (%): C, 38.90; H, 3.81; N, 22.68. Found (%): C, 38.86; H, 3.76; N, 22.49. λ_{max} nm (MeOH): 267, 369. ^{1}H NMR (DMSO-d_6): $\delta = 10.45$ (1H, s), 8.89 (1H, s, CH=N), 7.89 (2H, d, NH_2, $J = 8.6$ Hz), 7.72–7.43 (3H, m).

2.3. *General procedure for preparing dichloro phenyl methylene thiosemicarbazone*

In a round bottom flask fitted with a reflux condenser 2,4-dichlorobenzaldehyde (0.01 mol) and TSC (0.01 mol) were added. The mixture was refluxed in anhydrous alcohol for 4–5 h and left overnight. The solid that separated was filtered and dried. The crude solid was purified by recrystallization from alcohol to give off white crystals.

2.3.1. dmtsz. Yield 80%, m.p. 234–235°C. IR (KBr) cm^{-1}: 3414–3238 (NH_2 and NH), 3016 (C–H), 2894 (C–H), 1339 (C=S). Calcd for $C_8H_7N_3Cl_2S_1$ (%): C, 38.72; H, 2.84; N, 16.93. Found (%): C, 38.66; H, 2.78; N, 16.89. λ_{max} nm (MeOH): 238, 366. ^{1}H NMR (DMSO-d_6): $\delta = 10.08$ (1H, s), 8.98 (1H, s, CH=N), 8.01 (2H, d), 7.62 (2H, d, NH_2, $J = 8.6$ Hz), 7.34 (1H, s).

2.4. *Preparation of* cis-*[*bis*(S)dichlororuthenium(II)]* cis-*[Ru(T)$_2$Cl$_2$] (where T = 2,2′-bipyridine/1,10-phenanthroline) [21]*

$RuCl_3 \cdot H_2O$, 1 g (2.5 mmol) and ligand T (5 mmol) was refluxed in 50 mL DMF for 3 h under nitrogen. 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, repeatedly washed with 30% LiCl solution, and finally recrystallized from the same. The product was dried and stored in a vacuum desiccator over P_2O_5 for further use (yield 75%).

2.5. *General procedure for preparing [Ru(T)$_2$(S)Cl$_2$] (where T = 1,10-phenanthroline (Ru^1)/2,2′-bipyridine (Ru^2); S = CH$_3$-bitsz, Cl-bitsz, Br-bitsz, tmtsz, dmtsz)*

To the black microcrystalline *cis*-bis(T)dichloro ruthenium(II){*cis*-Ru(T)$_2$Cl$_2$} (2 mmol) excess of ligand (2.5 mmol) was added and refluxed in ethanol under nitrogen. The initial colored solution slowly changed to brownish orange at the end of the reaction, which was verified by TLC on silica plates. Then excess ethanol distilled off and silica gel (60–120 mesh) added to this solution. The product was purified by column chromatography by using silica gel as stationary phase and chloroform–methanol as mobile phase.

2.5.1. [Ru[phen]$_2$[CH$_3$-bitsz]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3438–3254 (NH_2 and N–H), 3098 (C–H), 2946 (C–H), 1682 (C=O), 1334 (C=S). Calcd for $C_{41}H_{31}Cl_2N_8O_1Ru_1S_1F_1$ (%): C, 56.29; H, 3.54; N, 12.82. Found (%): C, 56.24; H, 3.49; N, 12.79. ^{1}H NMR (DMSO-d$_6$): δ ppm: 10.42 (s, 1H), 8.86 (2H, t), 8.65 (d, $J = 4.9$ Hz, 1H), 8.51 (d, $J = 8.4$ Hz, 1H), 8.31–8.19 (m, 6H), 8.09 (s, 1H), 8.00 (d, $J = 5.0$ Hz, 2H), 7.82 (d, 2H), 7.76 (d, 2H), 7.22 (d, 2H, NH_2), 7.10 (d, 2H), 7.01 (d, $J = 14.6$ Hz, 2H), 6.73 (d, 2H), 6.47 (d, 2H), 1.58 (3H, s, CH_3). FAB-MS (mNBA): 874 [Ru(phen)$_2$(CH$_3$-bitsz)]$^{2+}$(Cl$_2$)$^-$; 803 [Ru(phen)$_2$(CH$_3$-bitsz)]$^{2+}$; 623 [Ru(phen)(CH$_3$-bitsz)]$^{2+}$; 461 [Ru(phen)$_2$].

2.5.2. [Ru[bpy]$_2$[CH$_3$-bitsz]Cl$_2$. 42%, black crystals, IR (KBr) cm^{-1}: 3426–3267 (NH_2 and N–H), 3124 (C–H), 2958 (C–H), 1686 (C=O), 1342 (C=S). Calcd for $C_{37}H_{31}Cl_2N_8O_1Ru_1S_1F_1$ (%): C, 53.75; H, 3.75; N, 13.56. Found (%): C, 53.64; H, 3.69; N, 13.42. ^{1}H NMR (DMSO-d$_6$): δ ppm: 10.29 (s, 1H), 8.71 (2H, d), 8.59 (d, $J = 4.9$ Hz, 2H), 8.47 (d, $J = 8.4$ Hz, 2H), 8.36–8.22 (m, 4H), 8.10 (s, 1H), 8.02 (d, $J = 5.0$ Hz, 2H), 7.87 (d, 2H), 7.64 (d, 2H), 7.12 (d, 2H, NH_2), 7.09 (d, 2H), 7.00 (d, $J = 14.6$ Hz, 2H), 6.68 (d, 2H), 6.39 (d, 2H), 1.62 (3H, s, CH_3). FAB-MS (mNBA): 826 [Ru(bpy)$_2$(CH$_3$-bitsz)]$^{2+}$(Cl$_2$)$^-$; 755 [Ru(bpy)$_2$(CH$_3$-bitsz)]$^{2+}$; 599 [Ru(bpy)(CH$_3$-bitsz)]$^{2+}$; 413 [Ru(bpy)$_2$].

2.5.3. [Ru[phen]$_2$[Cl-bitsz]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3417–3238 (NH_2 and N–H), 3079 (C–H), 2958 (C–H), 1680 (C=O), 1328 (C=S). Calcd for $C_{40}H_{28}Cl_3N_8O_1Ru_1S_1F_1$ (%): C, 53.69; H, 3.13; N, 12.52. Found (%): C, 53.56; H, 3.10; N, 12.44. ^{1}H NMR (DMSO-d$_6$): δ ppm: 10.18 (s, 1H), 8.73 (2H, t), 8.59 (d, $J = 4.9$ Hz, 1H), 8.47 (d, $J = 8.4$ Hz, 1H), 8.26–8.18 (m, 6H), 8.14 (s, 1H), 8.06 (d, $J = 5.0$ Hz, 2H), 7.96 (d, 2H), 7.79 (d, 2H), 7.02 (d, 2H, NH_2), 7.00 (d, 2H), 6.89 (d, $J = 14.6$ Hz, 2H), 6.72 (d, 2H), 6.38 (d, 2H). FAB-MS (mNBA): 894 [Ru(phen)$_2$(Cl-bitsz)]$^{2+}$(Cl$_2$)$^-$; 823 [Ru(phen)$_2$(Cl-bitsz)]$^{2+}$; 643 [Ru(phen)(Cl-bitsz)]$^{2+}$; 461 [Ru(phen)$_2$].

2.5.4. [Ru[bpy]$_2$[Cl-bitsz]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3451–3242 (NH_2 and N–H), 3082 (C–H), 2983 (C–H), 1688 (C=O), 1344 (C=S). Calcd for $C_{36}H_{28}Cl_3N_8O_1Ru_1S_1F_1$ (%): C, 51.06; H, 3.31; N, 13.24. Found (%): C, 51.01; H, 3.28; N, 13.19. ^{1}H NMR (DMSO-d$_6$): δ ppm: 10.27 (s, 1H), 8.87 (2H, d), 8.69 (d, $J = 4.9$ Hz, 2H), 8.58 (d, $J = 8.4$ Hz, 2H), 8.42–8.17 (m, 4H), 8.06 (s, 1H), 8.01

(d, $J = 5.0$ Hz, 2H), 7.91 (d, 2H), 7.68 (d, 2H), 7.15 (d, 2H, NH_2), 7.09 (d, 2H), 7.00 (d, $J = 14.6$ Hz, 2H), 6.61 (d, 2H), 6.37 (d, 2H). FAB-MS (mNBA): 846 $[Ru(bpy)_2(Cl\text{-}bitsz)]^{2+}(Cl_2)^-$; 775 $[Ru(bpy)_2(Cl\text{-}bitsz)]^{2+}$; 619 $[Ru(bpy)(Cl\text{-}bitsz)]^{2+}$; 413 $[Ru(bpy)_2]$.

2.5.5. [Ru[phen]$_2$[Br-bitsz]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3411–3303 (NH_2 and N–H), 3125 (C–H), 2995 (C–H), 1681 (C=O), 1324 (C=S). Calcd for $C_{40}H_{28}Cl_2N_8O_1Ru_1S_1F_1Br_1$ (%): C, 51.17; H, 2.98; N, 11.94. Found (%): C, 51.12; H, 2.92; N, 11.90%. ^{1}H NMR (DMSO-d_6): δ ppm: 10.07 (s, 1H), 8.79 (2H, t), 8.68 (d, $J = 4.9$ Hz, 1H), 8.54 (d, $J = 8.4$ Hz, 1H), 8.39–8.21 (m, 6H), 8.12 (s, 1H), 8.04 (d, $J = 5.0$ Hz, 2H), 7.96 (d, 2H), 7.73 (d, 2H), 7.31 (d, 2H, NH_2), 7.24 (d, 2H), 7.11 (d, $J = 14.6$ Hz, 2H), 6.81 (d, 2H), 6.35 (d, 2H). FAB-MS (mNBA): 938 $[Ru(phen)_2(Br\text{-}btsz)]^{2+}(Cl_2)^-$; 867 $[Ru(phen)_2(Br\text{-}btsz)]^{2+}$; 687 $[Ru(phen)(Br\text{-}btsz)]^{2+}$; 461 $[Ru(phen)_2]$.

2.5.6. [Ru[bpy]$_2$[Br-bitsz]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3467–3232 (NH_2 and N–H), 3058 (C–H), 2927 (C–H), 1682 (C=O), 1341 (C=S). Calcd for $C_{36}H_{28}Cl_2N_8O_1Ru_1S_1F_1Br_1$ (%): C, 48.53; H, 3.14; N, 12.58. Found (%): C, 48.46; H, 3.11; N, 12.52%. ^{1}H NMR (DMSO-d_6): δ ppm: 10.00 (s, 1H), 8.62 (2H, d), 8.59 (d, $J = 4.9$ Hz, 2H), 8.48 (d, $J = 8.4$ Hz, 2H), 8.39–8.26 (m, 4H), 8.18 (s, 1H), 8.07 (d, $J = 5.0$ Hz, 2H), 7.99 (d, 2H), 7.56 (d, 2H), 7.19 (d, 2H, NH_2), 7.08 (d, 2H), 6.93 (d, $J = 14.6$ Hz, 2H), 6.67 (d, 2H), 6.37 (d, 2H). FAB-MS (mNBA): 890 $[Ru(bpy)_2(Br\text{-}bitsz)]^{2+}(Cl_2)^-$; 819 $[Ru(bpy)_2(Br\text{-}bitsz)]^{2+}$; 663 $[Ru(bpy)(Br\text{-}bitsz)]^{2+}$; 413 $[Ru(bpy)_2]$.

2.5.7. [Ru[phen]$_2$[tmtsz]Cl$_2$. 46%, black crystals, IR (KBr) cm^{-1}: 3418–3226 (NH_2 N–H), 3035 (C–H), 1339 (C=S). Calcd for $C_{30}H_{23}Cl_2N_7Ru_1S_2$ (%): C, 50.21; H, 3.21; N, 13.67. Found (%): C, 50.11; H, 3.18; N, 13.62. ^{1}H NMR (DMSO-d_6): δ ppm: 10.43 (d, $J = 5.2$ Hz, 1H), 8.98 (s, 1H, CH=N), 8.80 (d, $J = 8.8$ Hz, 2H), 8.64 (d, $J = 8.6$ Hz, 1H), 8.51 (d, $J = 8.6$ Hz, 1H), 8.40–8.20 (m, 6H), 8.11–8.03 (m, 2H), 7.89 (d, $J = 5.0$ Hz, 2H), 7.67–7.48 (m, 3H), 7.42 (d, 2H), 6.98 (s, 2H, br, NH_2). FAB-MS (mNBA): 717 $[Ru(phen)_2(tmtsz)]^{2+}(Cl_2)^-$; 646 $[Ru(phen)_2(tmtsz)]^{2+}$; 466 $[Ru(phen)(tmtsz)]^{2+}$; 461 $[Ru(phen)_2]$.

2.5.8. [Ru[bpy]$_2$[tmtsz]Cl$_2$. 43%, black crystals, IR (KBr) cm^{-1}: 3472–3284 (NH_2 N–H), 3050 (C–H), 1342 (C=S). Calcd for $C_{26}H_{23}Cl_2N_7Ru_1S_2$ (%): C, 46.64; H, 3.43; N, 14.65. Found (%): C, 46.57; H, 3.41; N, 14.42. ^{1}H NMR (DMSO-d_6): δ ppm: 10.52 (d, $J = 5.2$ Hz, 1H), 9.03 (s, 1H, CH=N), 8.83 (d, $J = 8.8$ Hz, 2H), 8.59 (d, $J = 8.6$ Hz, 1H), 8.52 (d, $J = 8.6$ Hz, 1H), 8.39–8.26 (m, 6H), 8.14–8.01 (m, 2H), 7.73 (d, $J = 5.0$ Hz, 2H), 7.54–7.43 (m, 3H), 7.32 (d, 2H), 6.87 (s, 2H, br, NH_2). FAB-MS (mNBA): 669 $[Ru(bpy)_2(tmtsz)]^{2+}(Cl_2)^-$; 598 $[Ru(bpy)_2(tmtsz)]^{2+}$; 442 $[Ru(bpy)(tmtsz)]^{2+}$; 413 $[Ru(bpy)_2]$.

2.5.9. [Ru[phen]$_2$[dmtsz]Cl$_2$. 46%, black crystals, IR (KBr) cm^{-1}: 3482–3220 (NH_2 and N–H), 3072 (C–H), 2957 (C–H), 1329 (C=S). Calcd for $C_{32}H_{23}Cl_4N_7Ru_1S_1$ (%): C, 49.29; H, 2.95; N, 12.58. Found (%): C, 49.23; H, 2.88; N, 12.52. ^{1}H NMR (DMSO-d_6):

δ ppm: 10.13 (d, $J = 5.2$ Hz, 1H), 9.11 (s, 1H, CH=N), 8.84 (d, $J = 8.8$ Hz, 2H), 8.66 (d, $J = 8.6$ Hz, 1H), 8.54 (d, $J = 8.6$ Hz, 1H), 8.47–8.21 (m, 6H), 8.10–8.01 (m, 2H), 7.81 (d, $J = 5.0$ Hz, 2H), 7.62–7.44 (m, 4H), 7.39 (d, 1H), 6.84 (s, 2H, br, NH_2). FAB-MS (mNBA): 779 $[Ru(phen)_2(dmtsz)]^{2+}(Cl_2)^-$; 708 $[Ru(phen)_2(dmtsz)]^{2+}$; 528 $[Ru(phen)(dmtsz)]^{2+}$; 461 $[Ru(phen)_2]$.

2.5.10. [Ru[bpy]$_2$[dmtsz]Cl$_2$. 43%, black crystals, IR (KBr) cm^{-1}: 3450–3263 (NH_2 and N–H), 3061 (C–H), 2980 (C–H), 1338 (C=S). Calcd for $C_{28}H_{23}Cl_4N_7Ru_1S_1$ (%): C, 45.96; H, 3.14; N, 13.41. Found (%): C, 45.88; H, 3.09; N, 13.39. ^{1}H NMR (DMSO-d_6): δ ppm: 10.05 (d, $J = 5.2$ Hz, 1H), 9.18 (s, 1H, CH=N), 8.76 (d, $J = 8.8$ Hz, 2H), 8.62 (d, $J = 8.6$ Hz, 1H), 8.51 (d, $J = 8.6$ Hz, 1H), 8.39–8.22 (m, 6H), 8.20–8.03 (m, 2H), 7.86 (d, $J = 5.0$ Hz, 2H), 7.64–7.45 (m, 4H), 7.28 (d, 1H), 6.54 (s, 2H, br, NH_2). FAB-MS (mNBA): 731 $[Ru(bpy)_2(dmtsz)]^{2+}(Cl_2)^-$; 660 $[Ru(bpy)_2(dmtsz)]^{2+}$; 494 $[Ru(bpy)(dmtsz)]^{2+}$; 413 $[Ru(bpy)_2]$.

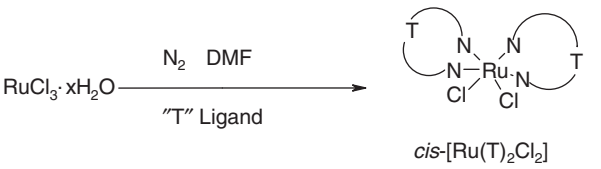
2.6. *Antineoplastic activity*

Albino swiss mice (18–20 g body weight) were maintained in identical laboratory conditions, given standard food pellets (Hindustan Lever Ltd, Bombay, India) and water *ad libitum*. LD_{50} value of the synthesized compound was determined according to the literature [25]. All compounds were dissolved in 10% DMSO solution. The animals were divided into 15 groups each containing 12 mice. Group I was vehicle controls (5 mL kg^{-1} body weight ip) and group II as EAC control (2×10^6 EAC cells per mouse ip). Group III was treated with standard drug cisplatin (2 mg kg^{-1} body weight). All the compounds were administered (ip) at a dose of 2 mg kg^{-1} body weight in groups IV–XIII, respectively. All the compounds and cisplatin treatments were provided daily for 9 days, starting at 24 h after tumor transplantation. Six animals from each group were sacrificed 18 h after the last dose. The ascitic fluid volume and ascitic cell count parameters were noted. Mean survival time (MST) for remaining six mice of each group was noted.

2.6.1. Tumor volume and viable count. Ascitic volume was noted by taking it in a graduated centrifuge tube and packed cell volume determined by centrifuging at $1000 \times g$ for 5 min. The viability of ascitic cells was checked by Trypan blue (0.4% in normal saline) dye exclusion test and the count was taken in 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:

$$\text{ILS}(\%) = [(\text{mean survival of treated group})/(\text{mean survival of control group}) - 1] \times 100.$$

2.6.2. Biological assays. The antiviral assays were based on the inhibition of virus-induced cytopathicity in confluent cell cultures and the cytostatic assays on the inhibition of tumor cell proliferation in exponentially growing tumor cell cultures.



where T = 2,2′-bipyridine/1,10-phenanthroline

Scheme 1. Preparation of *cis*-$[Ru(T)_2Cl_2]$.

2.6.3. Cytotoxic evaluation. The compounds prepared in laboratory were evaluated against Molt $4/C_8$, CEM, and L1210 cells by a literature procedure [26].

3. Results and discussion

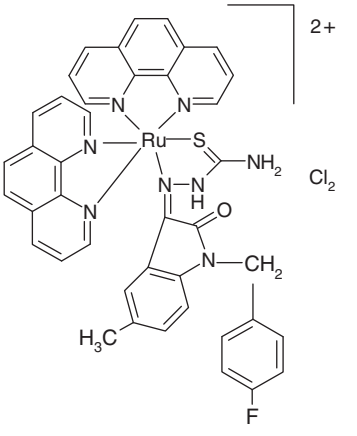
3.1. *Chemistry*

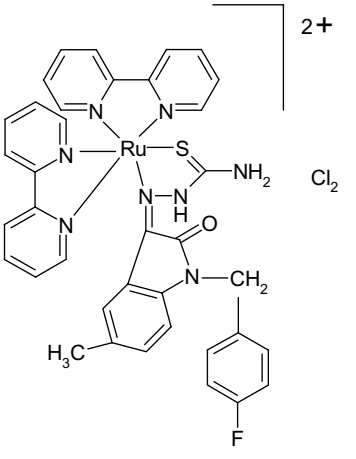
Ruthenium trichloride undergoes reduction when refluxed in DMF; when refluxed along with two-fold molar ratios of the bidentate ligand, namely (1,10-phenanthroline/2,2′-bipyridine), a homoleptic complex is formed. The product *cis*-bis(1,10-phenanthroline/2,2′-bipyridine)dichlororuthenate(II) is the starting material for the synthesis of complexes. The product *cis*-bis(1,10-phenanthroline/2,2′-bipyridine)dichlororuthenate(II) was then refluxed in ethanol in the presence of nitrogen with various ligands to yield the final octahedral ruthenium complexes.

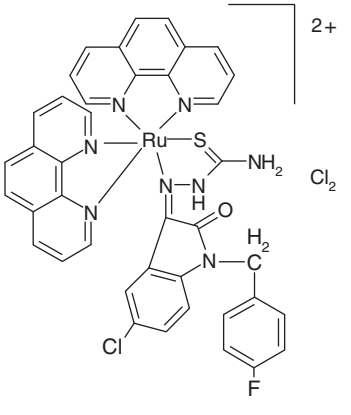
In this homoleptic chelate the first two ligands to enter the complex in a stepwise assembly were 1,10-phenanthroline/2,2′-bipyridine, respectively. Since both ligands are the same, a single step method was adopted for its synthesis. Ruthenium trichloride was refluxed in DMF in the presence of 1,10-phenanthroline/2,2′-bipyridine, in excess of the stoichiometric amount, which afforded the final product *cis*-bis(1,10-phenanthroline)dichlororuthenium(II)/*cis*-bis(2,2′-bipyridine)dichlororuthenium(II) (scheme 1). The introduction of the third ligand was carried out in the presence of alcohol. The final chelate formed had ionic chloride in the molecule.

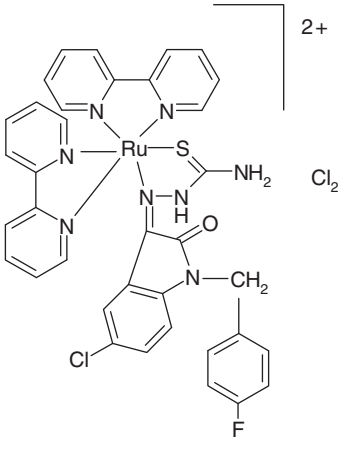
Ligands like *r*-bitsz (*r*-bitsz = *N*-benzyl isatin thiosemicarbazones) were prepared by reacting *N*-benzyl isatin with TSC in alcohol at 1 : 1 molar ratio (scheme 2). Tmtsz (tmtsz = thiophene methylene thiosemicarbazones) and dmtsz (dichloro methylene thiosemicarbazones) were prepared by reacting thiophene carboxyaldehyde, dichlorobenzaldehyde with TSC in alcohol at 1 : 1 molar ratio (scheme 3). All ligands were confirmed for their purity by their melting points, elemental analyses, and other spectral studies.

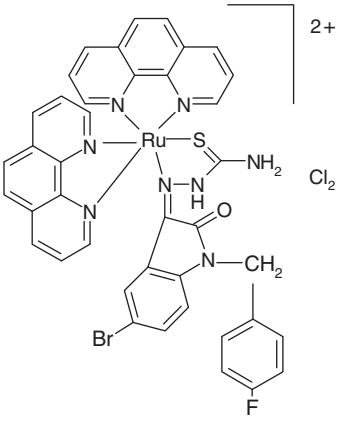
The structures of the ligands especially *r*-bitsz, tmtsz, and dmtsz are capable of exhibiting bidentate behavior. There are very few cases in which the TSC is monodentate binding to the metal through sulfur [27, 28]. In the case of *r*-bitsz, the chelating mode was *via* sulfur and imine nitrogen, not with amide carbonyl oxygen. For tmtsz and dmtsz, the chelating mode was *via* sulfur and imine nitrogen by coordination covalent bonds.

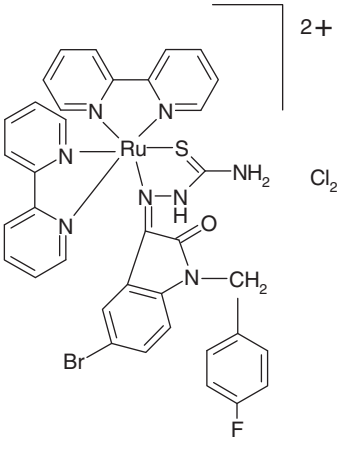


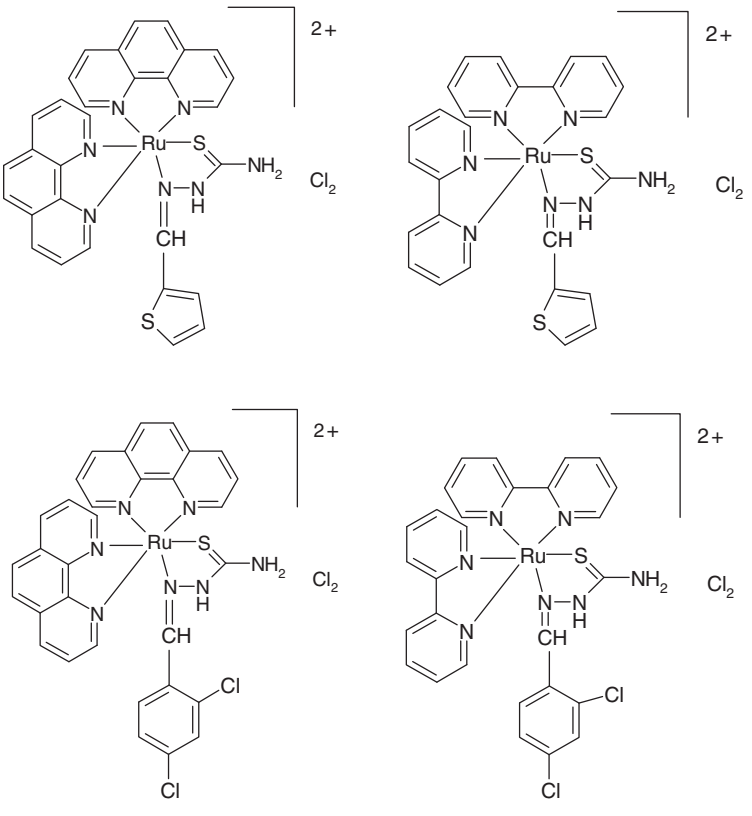








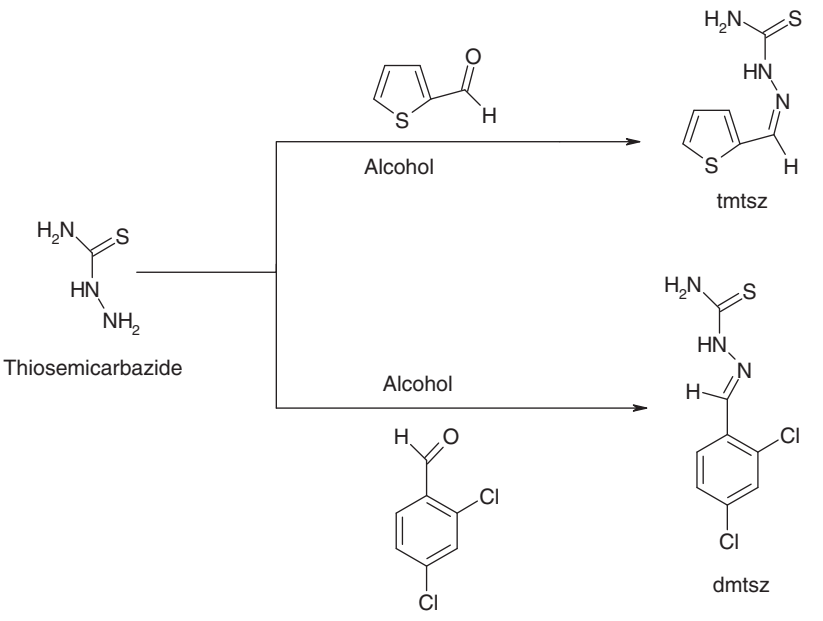




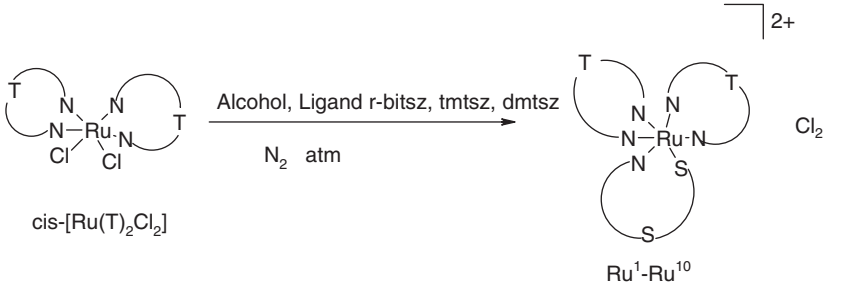
Infrared spectra of all the ligands and their ruthenium(II) compounds were recorded in KBr powder by diffuse reflectance technique and are reported with tentative assignments. The *r*-bitsz, tmtsz and dmtsz showed vibrational frequency from 3400 to 3200 cm^{-1} for NH_2 and N–H stretching, from 1690 to 1670 cm^{-1} for C=O stretching, and from 1345 to 1320 cm^{-1} for C=S stretching.

A comparison of IR spectra of *r*-bitsz with ruthenium complexes indicates coordination to the metal center by sulfur and imine nitrogen. In $[Ru[phen]_2[CH_3\text{-bitsz}]Cl_2$, $[Ru[bpy]_2[CH_3\text{-bitsz}]Cl_2$, $[Ru[phen]_2[Cl\text{-bitsz}]Cl_2$, $[Ru[bpy]_2[Cl\text{-bitsz}]Cl_2$, $[Ru[phen]_2[Br\text{-bitsz}]Cl_2$, $[Ru[bpy]_2[Br\text{-bitsz}]Cl_2$, $[Ru[phen]_2[tmtsz]Cl_2$, $[Ru[bpy]_2[tmtsz]Cl_2$, $[Ru[phen]_2[dmtsz]Cl_2$, and $[Ru[bpy]_2[dmtsz]Cl_2$ the coordination occurred *via* sulfur and imine nitrogen but not with terminal amine group, which was confirmed by no change in vibrational frequency of NH_2 between 3400 and 3200 cm^{-1}.

The coordination of ligands (S = *r*-bitsz, tmtsz, dmtsz) to ruthenium results in $[Ru(T)_2(S)]^{2+}Cl_2$ (Ru^1–Ru^{10}), respectively. These compounds do not possess any C2 axes of symmetry. Such loss of C2 axis of symmetry was seen for $[Ru(L)_2(R)]$ [22, 23] (where L = 2,2′-bipyridine/1,10-phenanthroline and R = acetazolamide, 7-iodo-8-hydroxy-quinoline, etc.). All compounds had well-resolved resonances, which correspond to four different aromatic ring protons of the two 2,2′-bipyridine/1,10-phenanthroline ligands and the third ligand.



Scheme 2. Preparation of ligands (*r*-bitsz, tmtsz, dmtsz).



Scheme 3. Preparation of tris chelates from *cis*-$[Ru(T)_2Cl_2]$.

These compounds showed broad and intense visible bands between 330 and 520 nm due to metal-to-ligand charge-transfer (MLCT) transition. In the UV region the bands at 270 and 300 nm were assigned to 2,2′-bipyridine/1,10-phenanthroline p–p* charge-transfer transitions. The same transition was found in free 2,2′-bipyridine/1,10-phenanthroline at 270 nm, so that the coordination of the ligand resulted in a red shift in the transition energy. There were also shoulders at 380 and 500 nm, which were, tentatively, attributed to MLCT transitions involving 2,2′-bipyridine, 1,10-phenanthroline, and the third ligand.

In ^{1}H-NMR spectra of the complexes, there were resolved resonances at low field at δ 10.42 (s, br, NH), 1.58 (s, 3H, CH_3). Thus in the case of $[Ru[phen]_2[CH_3\text{-bitsz}]Cl_2$ there were 31 resonances (δ 10.42–1.58) and 31 well-resolved peaks (δ 10.29–1.62) for $[Ru[bpy]_2[CH_3\text{-bitsz}]]Cl_2$.

The mass spectra of the complexes confirmed the suggested formula by their molecular ion peaks. The spectrum showed numerous peaks representing the successive

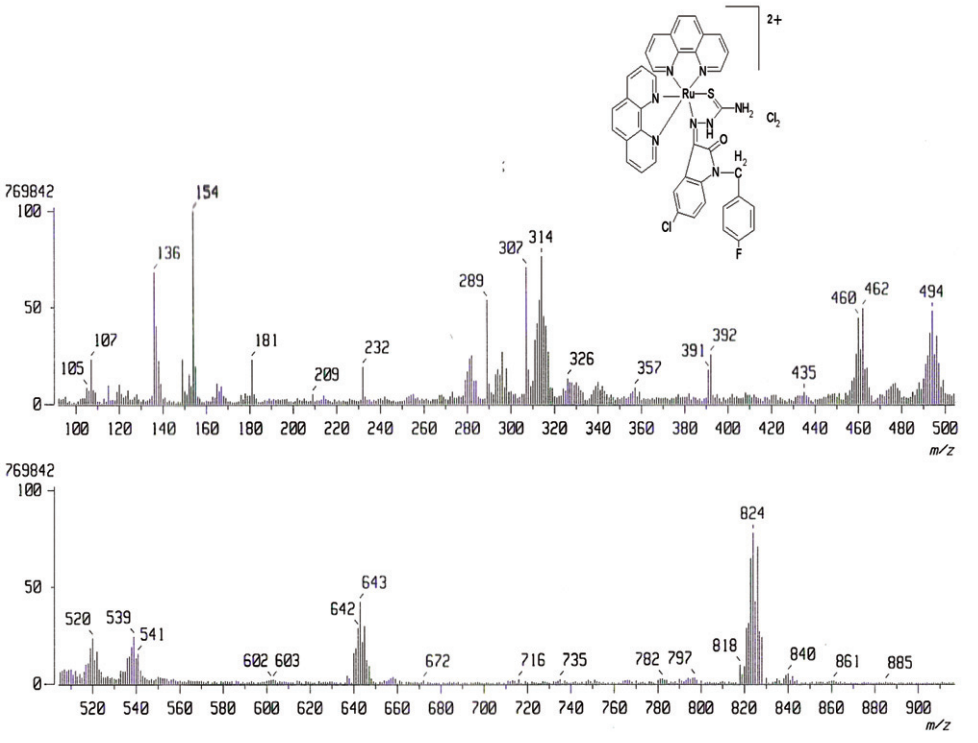


Figure 1. Mass spectrum of $[Ru(phen)_2(Cl\text{-}bitz)]^{2+}$ shows intense peak at 824, $[Ru(phen)(Cl\text{-}bitsz)]^{2+}$ shows intense peak at 643, and $[Ru(phen)_2]$ shows intense peak at 460 by loss of Cl_2.

degradation of the molecule. FAB mass spectroscopic data in figure 1 clearly suggested that mononuclear complexes had been formed in each case, the first fragment being due to $[Ru(T)_2(S)]^{2+}$ Cl_2^- ion pair. The complex also showed a peak due to the complex cation $[Ru(T)_2(S)]^{2+}$ and others due to $[Ru(T)(S)]^{2+}$, $[Ru(T)_2]^{2+}$, respectively [where T = 1,10-phenanthroline/2,2′-bipyridine and S = *r*-bitsz, tmtsz, dmtsz]. This type of fragmentation 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 thio semicarbazone, ihqs = 7-iodo-8-hydroxy quinoline-5-sulfonic acid. In all cases, the loss of chlorine ions was detected, where T = 2,2′-bipyridine/1,10-phenanthroline and S = *r*-bitsz, tmtsz, and dmtsz. Thus, based on the above observations, it is suggested that Ru(II) complexes have octahedral geometry.

3.2. *Biological activity and discussion*

Results are summarized in tables 1 and 2 and the pharmacological data were analyzed statistically by ANOVA. The statistical significance was considered only when $p < 0.05$ and $F > F_{critical}$. All the complexes were tested for their anticancer activity in EAC-bearing mice. $Ru(phen)_2(tmtsz)Cl_2$ was found to increase life span of the tumor hosts by 48%; remaining ruthenium complexes increased life span in the tumor hosts by 38% only. The results of this study clearly demonstrated the tumor-inhibitory activity

Table 1. Antitumor activity of ruthenium complexes against EAC-bearing mice.

Parameters	Total body weight (g)	MST (days)	ILS (%)	Tumor volume (mL)	Viable cells in ascitic fluid (%)
Group I	21.6 ± 0.3	–	–	–	–
Group II	27.2 ± 0.6	21	–	3.3 ± 0.3	94.8 ± 3.8
Group III	19.8 ± 0.5	22	5	–	–
Group IV	22.2 ± 0.9	24	14	1.5 ± 0.01	46.3 ± 1.5
Group V	23.1 ± 0.5	25	19	1.7 ± 0.05	44.9 ± 1.6
Group VI	23.8 ± 0.5	23	10	1.8 ± 0.03	47.9 ± 1.4
Group VII	24.3 ± 0.6	29	38	0.9 ± 0.05	40.1 ± 1.8
Group VIII	24.8 ± 0.5	26	24	1.2 ± 0.05	43.2 ± 1.2
Group IX	24.1 ± 0.5	25	19	1.3 ± 0.05	44.8 ± 1.5
Group X	23.6 ± 0.4	31	48	0.7 ± 0.03	33.8 ± 1.7
Group XI	22.9 ± 0.8	25	19	1.4 ± 0.04	45.1 ± 1.6
Group XII	24.5 ± 0.8	24	14	1.3 ± 0.06	46.5 ± 1.5
Group XIII	23.1 ± 0.6	29	38	0.9 ± 0.04	40.7 ± 1.8

Values are means ± SEM; ILS (%) = [(mean survival of treated group)/(mean survival of control group) − 1] × 100. Group I, vehicle (5 mL kg^{-1}); group II, EAC (2×10^6 cells/mouse); group III, cisplatin (2 mg kg^{-1}) + EAC; group IV, (Ru^1); group IV–group XIII, ruthenium complexes (2 mg kg^{-1}) + EAC.

Table 2. Cytotoxic studies of ligands and ruthenium compounds.

	IC_{50}[a] (μmol L^{-1})		
Compound	L1210	Molt 4/C8	CEM
CH_3-bitsz	241 ± 45	178 ± 6	144 ± 21
Cl-bitsz	98 ± 29	63 ± 31	49 ± 5
Br-bitsz	74 ± 8	84 ± 7	58 ± 13
tmtsz	102 ± 9	93 ± 12	76 ± 05
dmtsz	204 ± 19	186 ± 39	111 ± 16
$Ru[phen]_2[CH_3\text{-bitsz}]Cl_2$	15 ± 1	17 ± 8	9.1 ± 0.5
$Ru[bpy]_2[CH_3\text{-bitsz}]Cl_2$	1.4 ± 0.3	9.6 ± 0.9	2.8 ± 0.4
$Ru[phen]_2[Cl\text{-bitsz}]Cl_2$	0.98 ± 0.06	0.71 ± 0.05	0.89 ± 0.05
$Ru[bpy]_2[Cl\text{-bitsz}]Cl_2$	2.2 ± 0.3	1.9 ± 0.6	1.4 ± 0.3
$Ru[phen]_2[Br\text{-bitsz}]Cl_2$	0.86 ± 0.08	0.45 ± 0.03	0.34 ± 0.04
$Ru[bpy]_2[Br\text{-bitsz}]Cl_2$	2.5 ± 0.4	1.1 ± 0.4	1.8 ± 0.6
$Ru[phen]_2[tmtsz]Cl_2$	1.6 ± 0.4	0.98 ± 0.05	12 ± 0.9
$Ru[bpy]_2[tmtsz]Cl_2$	0.89 ± 0.06	0.38 ± 0.04	0.28 ± 0.02
$Ru[phen]_2[dmtsz]Cl_2$	8.7 ± 0.9	2.4 ± 0.4	1.6 ± 0.2
$Ru[bpy]_2[dmtsz]Cl_2$	1.2 ± 0.3	1.8 ± 0.5	0.94 ± 0.06

[a]50% Inhibitory concentration, required to inhibit tumor cell proliferation by 50%.

of the ruthenium complexes against transplantable murine tumor cell line. The *in vitro* cytotoxic activity was evaluated for all the synthesized ligands and ruthenium complexes against human Molt 4/C8, CEM, T-lymphocytes as well as murine L1210 cells, and its results are summarized in table 2. The relative potencies between ligands and their ruthenium complexes revealed the importance of ruthenium using the Molt 4/C8, CEM assays, and murine L1210 assays. These determinations showed that in comparison to ligands, the ruthenium complexes were more potent.

The cytotoxicity data in table 2 reveal that most ruthenium complexes have significant cytotoxic potencies (IC_{50} figures in the 0.38-17 for Molt 4/C8,

0.86–15 μmol L^{-1} for L1210 and 0.34–9.1 μmol L^{-1} for CEM). For ligands, the IC_{50} values were in excess (49–144 μmol L^{-1} against CEM, 63–186 μmol L^{-1} for Molt 4/C8, and 74–241 μmol L^{-1} for L1210). Of the tested ligands and ruthenium complexes, $Ru(phen)_2(Br\text{-}bitsz)Cl_2$ showed cytotoxicity against all three cell lines tested in range of 0.45, 0.34, and 0.86 μmol L^{-1} for Molt 4/C8, CEM, and L1210, respectively. $Ru(bpy)_2(tmtsz)Cl_2$ shows cytotoxicity against cell lines tested, 0.38 μmol L^{-1} for Molt 4/C8, 0.28 μmol L^{-1} for CEM, and 0.89 μmol L^{-1} for L1210; $Ru(phen)_2(Cl\text{-}bitsz)Cl_2$ shows cytotoxicity against cell lines tested, 0.71 μmol L^{-1} for Molt 4/C8, 0.89 μmol L^{-1} for CEM, and 0.98 μmol L^{-1} for L1210; remaining ruthenium complexes for Molt 4/C8 and CEM (low) μmol L^{-1} and L1210 (higher) μmol L^{-1}. On comparison to ruthenium complexes the ligands displayed the cytotoxicity at higher μmol L^{-1} concentration.

From the results presented in table 2, it is clear that several ruthenium complexes exhibit a marked inhibitory effect on the proliferation of tumor cells: IC_{50} from as low as 0.38 μmol L^{-1} for Molt 4/C8, 0.28 μmol L^{-1} CEM, and 0.86 μmol L^{-1} for L1210. Thus, the ruthenium complexes proved inhibitory to tumor growth at submicromolar concentrations. Their ligands however were not antitumorally active.

The mechanism of action of the ruthenium complexes is not known but organoruthenium antitumor agents contain an electron-deficient metal atom that acts as a magnet for electron-rich DNA nucleophiles. Organoruthenium complexes are bifunctional and can accept electrons from two DNA nucleophiles.

Acknowledgments

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