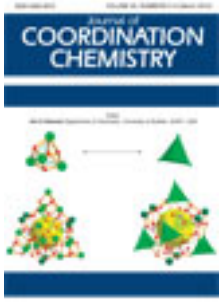

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Synthesis, spectroscopic characterization, antineoplastic, in vitro-cytotoxic, and antibacterial activities of mononuclear ruthenium(II) complexes

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Synthesis, spectroscopic characterization, antineoplastic, *in vitro*-cytotoxic, and antibacterial activities of mononuclear ruthenium(II) complexes

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The synthesis, antineoplastic, cytotoxic, and antibacterial activities of Ru(II) complexes derived from quinazoline and thiosemicarbazone ligands are reported. These complexes have been prepared and characterized by UV-Vis, IR, ^{1}H-NMR, FAB-mass spectroscopy, and elemental analysis. The ligands and resulting complexes were subjected to *in vivo* antineoplastic activity against a transplantable murine tumor cell line Ehrlich ascites carcinoma (EAC) and *in vitro* cytotoxic activity against human cancer cell line Molt 4/C_8, CEM, and murine tumor cell line L 1210. The ruthenium complexes show promising biological activity especially in decreasing tumor volume and viable ascitic cell counts. These complexes prolonged the life span of mice bearing EAC tumors by 10–52%. *In vitro* evaluation of these ruthenium complexes revealed cytotoxic activity from 0.29 to 2.9 μmol L^{-1} against Molt 4/C_8, 0.22 to 2.1 μmol L^{-1} against CEM and 0.42 to 4.7 μmol L^{-1} against L1210 cell proliferation, depending on the nature of the compound. The metal complexes are more active than the parent ligand and exhibit mild to moderate antibacterial activity.

Keywords: Antineoplastic; Quinazoline; Proliferation; Ru(II) complexes

1. Introduction

A large number of transition metal complexes with heterocyclic ligands containing nitrogen, oxygen, and sulfur have pharmacological importance. Antineoplastic is said of a drug that inhibits or prevents maturation and proliferation of neoplasms that may become malignant, by targeting the DNA; most chemotherapy drugs are antineoplastic. In searching for antineoplastic active metal complexes several ruthenium compounds have been reported to be promising as anticancer drugs, including series of

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mononuclear Ru(II) complexes $[Ru[M]_2[U]]^{2+}$, 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, pai [1].

The objectives of the present investigation were to develop analogs of $[Ru[T]_2[S]]^{2+}$, where T = 2,2′-bipyridine/1,10-phenanthroline and S = 4-NO_2-PQZ, 4-CH_3-PQZ, 3,4-di-OCH_3-PQZ, 3-OCH_3, 4-OH-PQZ, 4-OH-PQZ, 4-$N(CH_3)_2$-PQZ, 4-NO_2-PTSZ, 3,4,5-tri-OCH_3-PTSZ as candidate cytotoxins. The discovery of anticancer activity of cisplatin in 1965 marked the development of metallopharmaceuticals in cancer chemotherapy [2]. 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 as potential antineoplastic agents [3].

The first systematic investigation of ruthenium compounds and their antineoplastic properties was done in the early 1980s with *fac*-$[RuCl_3(NH_3)_3]$ and *cis*-$[RuCl_2(NH_3)_4]Cl$ [4]. Several ruthenium complexes have been investigated for potential antitumor activity such as *trans*-$[RuCl_4(Im)(DMSO)]$ ImH(NAMI-A) [5], (H_2ind) [*trans*-$RuCl_4(Hind)_2$] (Hind = 1 *H*-indazole) [6], imidazolium[*trans*-tetrachloro (1 *H*-imidazole) (S-dimethyl sulfoxide) ruthenate(III)] (NAMI-A) [7], [ImH] *trans*-$[RuCl_4(Im)(dmso\text{-}s)]$ (NAMI-A, Im = imidazole) [8], tethering Ru-arene drugs to macromolecules [9], [Ru(η^6-p-arene)Cl_2 (pta)] [10], [IndH] *trans*-$[RuCl_4(Ind)_2]$ (KP1019) [11].

Ruthenium(II) arene complexes show remarkable cytotoxic properties *in vitro* as well as *in vivo* [12, 13]. In comparison with Ru(III) complexes, Ru(II) complexes are kinetically more reactive [14]. We have reported that Ru(II) compounds bearing thiosemicarbazides and 8-hydroxy quinolines have *in vivo* anticancer and *in vitro* antibacterial activities [15, 16]. In this work, we describe the synthesis and characterization of some ruthenium complexes, their *in vitro* cytotoxic activity against human cancer cell line CEM, Molt 4/C_8, and murine tumor cell line L 1210, their *in vivo* anticancer activity, and *in vitro* antibacterial activity against transplantable murine tumor cell line Ehrlich ascites carcinoma (EAC). Our research has focused on complexes of general formula $[Ru[T]_2[S]]Cl_2$, where T = 2,2′-bipyridine/1,10-phenanthroline and S = 4-NO_2-PQZ, 4-CH_3-PQZ, 3,4-di-OCH_3-PQZ, 3-OCH_3, 4-OH-PQZ, 4-OH-PQZ, 4-$N(CH_3)_2$-PQZ [17], 4-NO_2-PTSZ, 3,4,5-tri-OCH_3-PTSZ [18, 19].

2. Experimental

2.1. *General methods*

AR grade solvents were obtained from E. Merck, Mumbai, and SD Fine Chem. All solvents were distilled prior to use. Fluka and E. Merck supplied the reagents (puriss grade). Anthranilic acid was purchased from Merck (Germany) and used as received. Hydrated ruthenium trichloride was purchased from Loba Chemie, Mumbai, and used as received. Silica gel plates were used for the TLC analysis by using $CHCl_3 : CH_3OH$ as mobile phase. UV-Vis spectra were recorded on a Jasco spectrophotometer. IR spectra (in KBr pellets) were recorded on the Vertex 70 Bruker apparatus. NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 on a Bruker Ultraspec 500 MHz/AMX 400 MHz/300 MHz spectrometer using TMS as internal standard. The content of C, H, and N were done with an ECS-40-10-Costech micro-dosimeter after drying the complexes.

FAB mass spectra were recorded on a JEOL JMS600 spectrometer with an mNBA matrix.

2.2. *General procedure for synthesis of 3-(aryldeneamino)-2-phenylquinazolin-4(3H)-ones [17]*

Step 1. Synthesis of 2-phenyl-3,1-benzoxazin-4-one: To a solution of anthranilic acid (0.1 mol) dissolved in pyridine (60 mL), benzoyl chloride (0.2 mol) was added. The mixture was stirred for 2 h followed by treatment with 5% $NaHCO_3$ (15 mL). The solid obtained was crystallized from ethanol.

Step 2. Synthesis of 3-amino-2-phenyl quinazolin-4(3H)-one: A mixture of 2-phenyl-3-benzoxazin-4-one (0.05 mol) and hydrazine hydrate (0.05 mol) in ethanol was refluxed for 3 h and cooled. The separated solid was recrystallized from ethanol.

Step 3. Synthesis of 3-(arylideneamino)-2-phenyl-quinazolin-4(3H)-one: A mixture of 3-amino-2-phenyl quinazolin-4(3 H)-one (0.01 mol), the appropriate aromatic aldehyde (0.01 mol), and ethanol (20 mL) was refluxed for 4–6 h. The resulting mixture was cooled and poured into ice water. The separated solid was filtered, washed with water, and recrystallized from ethanol.

2.2.1. 4-NO_2-PQZ. Yield 82%, m.p. 241–242°C. IR (KBr) cm^{-1}: 3144 (C–H), 2924 (C–H), 1679 (C=O). Calcd for $C_{21}H_{14}O_3N_4$ (%): C, 68.11; H, 3.78; N, 15.13. Found (%): C, 67.96; H, 3.69; N, 15.04. λ_{max} nm (MeOH): 232, 245, 355. ^{1}H-NMR (DMSO-d_6): $\delta = 8.88$ (1-H, s), 8.04 (2-H, d), 7.91 (2-H, d), 7.58 (5-H, m), 7.72 (2-H, d, $J = 8.6$ Hz), 7.31 (2-H, d).

2.2.2. 4-CH_3-PQZ. Yield 69%, m.p. 210–211°C. IR (KBr) cm^{-1}: 3157 (C–H), 2983 (C–H), 1669 (C=O). Calcd for $C_{22}H_{17}ON_3$ (%): C, 77.87; H, 5.01; N, 12.38. Found (%): C, 77.48; H, 4.99; N, 12.28. λ_{max} nm (MeOH): 236, 295, 374. ^{1}H-NMR (DMSO-d_6): $\delta = 9.02$ (1-H, s), 8.24 (2-H, d), 8.11 (1-H, s), 8.08 (2-H, d), 7.86 (4-H, m), 7.64 (2-H, d, $J = 8.6$ Hz), 7.59 (2-H, d), 1.58 (3-H, s).

2.2.3. 3,4-di-OCH_3-PQZ. Yield 84%, m.p. 232–233°C. IR (KBr) cm^{-1}: 3132 (C–H), 2912 (C–H), 1680 (C=O). Calcd for $C_{23}H_{19}O_3N_3$ (%): C, 71.68; H, 4.94; N, 10.91. Found (%): C, 71.66; H, 4.89; N, 10.84. λ_{max} nm (MeOH): 217, 303, 371. ^{1}H-NMR (DMSO-d_6): $\delta = 8.94$ (1-H, s), 8.36 (2-H, d), 8.21 (2-H, d), 8.04 (5-H, m), 7.98 (1-H, s), 7.67 (2-H, d, $J = 8.6$ Hz), 3.71 (3-H, m), 3.65 (3-H, m).

2.2.4. 3-OCH_3,4-OH-PQZ. Yield 74%, m.p. 154–155°C. IR (KBr) cm^{-1}: 3531 (O–H), 3153 (C–H), 2924 (C–H), 1680 (C=O). Calcd for $C_{22}H_{17}O_3N_3$ (%): C, 71.16; H, 4.58; N, 11.32. Found (%): C, 71.06; H, 4.52; N, 11.24. λ_{max} nm (MeOH): 240, 292, 367. ^{1}H-NMR (DMSO-d_6): $\delta = 10.01$ (1-H, s), 9.12 (1-H, s), 8.44 (2-H, d), 8.28 (2-H, d), 8.01 (1-H, s), 7.98 (5-H, m), 7.76 (2-H, d, $J = 8.6$ Hz), 3.74 (3-H, m).

2.2.5. 4-OH-PQZ. Yield 76%, m.p. 166–167°C. IR (KBr) cm^{-1}: 3528 (O–H), 3042 (C–H), 2904 (C–H), 1682 (C=O). Calcd for $C_{21}H_{15}O_2N_3$ (%): C, 73.90; H, 4.39; N, 12.32. Found (%): C, 73.72; H, 4.32; N, 12.29. λ_{max} nm (MeOH): 263, 305, 356. ^{1}H-NMR (DMSO-d_6): $\delta = 10.28$ (1-H, s), 9.04 (1-H, s), 8.21 (2-H, d), 8.10 (5-H, m), 7.98 (2-H, d), 7.74 (2-H, d, $J = 8.6$ Hz), 7.53 (2-H, d).

2.2.6. 4-*N*-$(CH_3)_2$-PQZ. Yield 70%, m.p. 177–178°C. IR (KBr) cm^{-1}: 3148 (C–H), 2929 (C–H), 1680 (C=O). Calcd for $C_{23}H_{20}ON_4$ (%): C, 75.00; H, 5.43; N, 15.22. Found (%): C, 74.88; H, 5.39; N, 15.18. λ_{max} nm (MeOH): 252, 295, 369. ^{1}H-NMR (DMSO-d_6): $\delta = 8.89$ (1-H, s), 8.34 (2-H, d), 8.14 (5-H, m), 7.92 (2-H, d), 7.68 (2-H, d, $J = 8.6$ Hz), 7.41 (2-H, d), 2.84 (3-H, m), 2.78 (3-H, m).

2.3. *General procedure for synthesis of aryl substituted thiosemicarbazones*

Aryl substituted thiosemicarbazones [18, 19] were prepared according to the literature method.

2.3.1. 4-NO_2-PTSZ. Yield 84%, m.p. 242–243°C (lit., 242°C). IR (KBr) cm^{-1}: 3417–3380 (NH_2 and NH), 3136 (C–H), 2901 (C–H), 1370 (C=S). Calcd for $C_8H_8O_2N_4S$ (%): C, 42.85; H, 3.57; N, 25.00. Found (%): C, 42.69; H, 3.52; N, 24.94. λ_{max} nm (MeOH): 230, 307, 375. ^{1}H NMR (DMSO-d_6): $\delta = 10.36$ (1-H, s), 9.02 (1-H, s), 8.04 (2-H, d), 7.83 (2-H, d), 7.24 (2-H, d, $J = 8.6$ Hz).

2.3.2. 3,4,5-tri-OCH_3-PTSZ. Yield 85%, m.p. 187–188°C (lit., 187.5°C). IR (KBr) cm^{-1}: 3528 (O–H), 3042 (C–H), 2904 (C–H), 1682 (C=O). Calcd for $C_{11}H_{15}O_3N_3$ (%): C, 49.08; H, 5.57; N, 15.62. Found (%): C, 49.02; H, 5.48; N, 15.59. λ_{max} nm (MeOH): 240, 285, 372. ^{1}H NMR (DMSO-d_6): $\delta = 10.14$ (1-H, s), 9.16 (1-H, s), 8.43 (2-H, d), 7.44 (2-H, d, $J = 8.6$ Hz), 3.78 (3-H, m), 3.72 (3-H, m), 3.64 (3-H, m).

2.4. *Preparation of cis-[bis(T)dichlororuthenium(II)] cis-[$Ru(T)_2Cl_2$] [20] (where T = 2,2′-bipyridine/1,10-phenanthroline)*

A mixture of ligand T (5 mmol) and $RuCl_3 \cdot XH_2O$ 1.15 g (2.5 mmol), DMF (50 mL) was heated under reflux for 3 h under nitrogen atmosphere. After the reaction was completed the reddish-brown solution slowly turned purple and the product precipitated. The solution was kept overnight in the refrigerator at 0°C and then the crystalline mass was filtered off. The residue was repeatedly washed with 30% LiCl solution and finally recrystallized from ethanol. The product was dried 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) and S = 4-NO$_2$-PQZ, 4-CH$_3$-PQZ, 3,4-di-OCH$_3$-PQZ, 3-OCH$_3$,4-OH-PQZ, 4-OH-PQZ, 4-N(CH$_3$)$_2$-PQZ, 4-NO$_2$-PTSZ, 3,4,5-tri-OCH$_3$-PTSZ)*

A mixture of excess ligand (2.5 mmol), black microcrystalline *cis*-Ru(T)$_2$Cl$_2$ (2 mmol), and ethanol (100 mL) was heated under reflux for 5 h under nitrogen. The initial colored solution slowly changed to brownish orange at the end of the reaction and verified by TLC on silica plates. Then the excess ethanol was distilled off and silica gel (60–120 mesh) added to this solution. The residue was purified by column chromatography; orange red band was collected using silica gel as stationary phase and chloroform-methanol (8 : 2 ratio) as mobile phase.

2.5.1. [Ru[phen]$_2$[4-NO$_2$-PQZ]Cl$_2$. 45%, black crystals, IR (KBr) cm^{-1}: 3083 (C–H), 2931 (C–H), 1681 (C=O). Calcd for $C_{45}H_{30}Cl_2N_8O_3Ru_1$ (%): C, 59.80; H, 3.32; N, 12.41. Found (%): C, 59.74; H, 3.29; N, 12.38. ^{1}H NMR (DMSO-d$_6$): δ ppm: 8.91 (s, 1-H), 8.64 (s, 1-H), 8.58 (2-H, t), 8.44 (d, 2-H), 8.28 (t, 3-H), 8.12 (d, $J = 8.4$ Hz, 2-H), 7.93 (d, 2-H), 7.87 (m, 3-H),7.76 (m, 3-H), 7.68 (m, 5-H), 7.52 (d, 2-H), 7.24 (m, 4-H), FAB-MS (mNBA): 902 [Ru(phen)$_2$(4-NO$_2$-PQZ)]$^{2+}$(Cl$_2$)$^-$; 831 [Ru(phen)$_2$(4-NO$_2$-PQZ)]$^{2+}$; 651 [Ru(phen)(4-NO$_2$-PQZ)]$^{2+}$; 462 [Ru(phen)$_2$].

2.5.2. [Ru[bpy]$_2$[4-NO$_2$-PQZ]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3102 (C–H), 2987 (C–H), 1679 (C=O). Calcd for $C_{41}H_{30}Cl_2N_8O_3Ru_1$ (%): C, 57.61; H, 3.51; N, 13.11. Found (%): C, 57.54; H, 3.49; N, 13.09. ^{1}H NMR (DMSO-d$_6$): δ ppm: 9.18 (s, 8.66 (2-H, d), 8.46 (d, $J = 4.9$ Hz, 2-H), 8.38 (m, $J = 8.4$-Hz, 4-H), 8.26 (d, 2-H), 8.14 (d, 2-H), 8.04 (d, $J = 5.0$ Hz, 2-H), 7.92 (m, 4-H), 7.84 (m, 5-H), 7.54 (d, 2-H), 7.62 (m, 4-H). FAB-MS (mNBA): 854 [Ru(bpy)$_2$(4-NO$_2$-PQZ)]$^{2+}$(Cl$_2$)$^-$; 783 [Ru(bpy)$_2$ (4-NO$_2$-PQZ)]$^{2+}$; 627 [Ru(bpy)(4-NO$_2$-PQZ)]$^{2+}$; 413 [Ru(bpy)$_2$].

2.5.3. [Ru[phen]$_2$[4-CH$_3$-PQZ]Cl$_2$. 45%, black crystals, IR (KBr) cm^{-1}: 3134 (C–H), 2986 (C–H), 1681 (C=O). Calcd for $C_{46}H_{33}Cl_2N_7O_1Ru_1$ (%): C, 63.37; H, 3.78; N, 11.25. Found (%): C, 63.28; H, 3.69; N, 11.22. ^{1}H NMR (DMSO-d$_6$): δ ppm: 10.01 (s, 1-H), 8.86 (s, 1-H), 8.52 (d, 2-H), 8.46 (d, 2-H), 8.34 (3-H, m), 8.22 (d, 2-H), 8.01 (d, $J = 8.4$ Hz, 2-H), 7.96 (t, 3-H), 7.82 (s, 1-H), 7.64 (m, 5-H), 7.54 (m, 4-H), 6.49 (m, 4-H), 1.56 (s, 3-H). FAB-MS (mNBA): 871 [Ru(phen)$_2$(4-CH$_3$-PQZ)]$^{2+}$(Cl$_2$)$^-$; 800 [Ru(phen)$_2$(4-CH$_3$-PQZ)]$^{2+}$; 620 [Ru(phen)(4-CH$_3$-PQZ)]$^{2+}$; 462 [Ru(phen)$_2$].

2.5.4. [Ru[bpy]$_2$[4-CH$_3$-PQZ]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3139 (C–H), 2928 (C–H), 1683 (C=O). Calcd for $C_{42}H_{33}Cl_2N_7O_1Ru_1$ (%): C, 61.23; H, 4.01; N, 11.91. Found (%): C, 61.14; H, 3.99; N, 11.87. ^{1}H NMR (DMSO-d$_6$): δ ppm: 10.45 (s, 1-H), 8.72 (1-H, s), 8.44 (m, $J = 4.9$ Hz, 4-H), 8.26 (d, $J = 8.4$ Hz, 2-H), 8.18 (d, 2-H), 8.02 (d, 2-H), 7.96 (d, $J = 5.0$ Hz, 2-H), 7.78 (m, 4-H), 7.68 (m, 4-H), 7.54 (m, 4-H), 6.37 (m, 4-H), 1.58 (m, 3-H). FAB-MS (mNBA): 823 [Ru(bpy)$_2$(4-CH$_3$-PQZ)]$^{2+}$(Cl$_2$)$^-$; 752 [Ru(bpy)$_2$(4-CH$_3$-PQZ)]$^{2+}$; 596 [Ru(bpy)(4-CH$_3$-PQZ)]$^{2+}$; 413 [Ru(bpy)$_2$].

2.5.5. [Ru[phen]$_2$[3,4-di-OCH$_3$-PQZ]Cl$_2$. 45%, black crystals, IR (KBr) cm^{-1}: 3121 (C–H), 2990 (C–H), 1668 (C=O). Calcd for $C_{47}H_{35}Cl_2N_7O_3Ru_1$ (%): C, 61.51; H, 3.82; N, 10.68. Found (%): C, 61.46; H, 3.79; N, 10.64. ^{1}H NMR (DMSO-d$_6$): δ ppm: 10.12 (s, 1-H), 8.58 (s, 1-H), 8.52 (1-H, s), 8.38 (d, 2-H), 8.34 (d, 2-H), 8.24 (d, 2-H), 8.16 (t, 3-H), 8.01 (m, 5-H), 7.96 (m, 5-H), 7.78 (d,2-H), 7.67 (m, 3-H), 6.46 (d, 2-H), 3.81 (m, 3-H), 3.76 (m, 3-H). FAB-MS (mNBA): 917 [Ru(phen)$_2$(3,4-di-OCH$_3$-PQZ)]$^{2+}$(Cl$_2$)$^-$; 846 [Ru(phen)$_2$(3,4-di-OCH$_3$-PQZ)]$^{2+}$; 666 [Ru(phen)(3,4-di-OCH$_3$-PQZ)]$^{2+}$; 462 [Ru(phen)$_2$].

2.5.6. [Ru[bpy]$_2$[3,4-di-OCH$_3$-PQZ]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3109 (C–H), 2916 (C–H), 1678 (C=O). Calcd for $C_{43}H_{35}Cl_2N_7O_3Ru_1$ (%): C, 59.37; H, 4.03; N, 11.27. Found (%): C, 59.34; H, 3.99; N, 11.21. ^{1}H NMR (DMSO-d$_6$): δ ppm: 9.92 (s, 1-H), 8.62 (4-H, m), 8.54 (s, 1-H), 8.44 (d, $J = 4.9$ Hz, 2-H), 8.36 (d, $J = 8.4$ Hz, 2-H), 8.28 (m, 4-H), 8.14 (m, 5-H), 8.01 (d, $J = 5.0$ Hz, 2-H), 7.86 (m, 4-H), 7.64 (d, 2-H), 6.23 (d, 2-H), 3.78 (m, 3-H), 3.65 (m, 3-H). FAB-MS (mNBA): 869 [Ru(bpy)$_2$(3,4-di-OCH$_3$-PQZ)]$^{2+}$(Cl$_2$)$^-$; 798 [Ru(bpy)$_2$(3,4-di-OCH$_3$-PQZ)]$^{2+}$; 642 [Ru(bpy)(3,4-di-OCH$_3$-PQZ)]$^{2+}$; 413 [Ru(bpy)$_2$].

2.5.7. [Ru[phen]$_2$[3-OCH$_3$,4-OH-PQZ]Cl$_2$. 45%, black crystals, IR (KBr) cm^{-1}: 3490 (O–H), 3086 (C–H), 2913 (C–H), 1681 (C=O). Calcd for $C_{46}H_{33}Cl_2N_7O_3Ru_1$ (%): C, 61.13; H, 3.65; N, 10.85. Found (%): C, 61.06; H, 3.61; N, 10.74. ^{1}H NMR (DMSO-d$_6$): δ ppm: 10.84 (s, 1-H), 10.16 (s, 1-H), 8.66 (d, 2-H), 8.52 (s, 1-H), 8.41 (d, 2-H), 8.34 (d, 2-H), 8.22 (t, 3-H), 8.01 (d, 2-H), 7.94 (m, 3-H), 7.82 (m, 5-H), 7.74 (m, 3-H), 7.58 (t, 3-H), 6.93 (d, 2-H), 3.65 (m, 3-H). FAB-MS (mNBA): 903 [Ru(phen)$_2$(3-OCH$_3$, 4-OH-PQZ)]$^{2+}$(Cl$_2$)$^-$; 832 [Ru(phen)$_2$(3-OCH$_3$,4-OH-PQZ)]$^{2+}$; 652 [Ru(phen)(3-OCH$_3$, 4-OH-PQZ)]$^{2+}$; 462 [Ru(phen)$_2$].

2.5.8. [Ru[bpy]$_2$[3-OCH$_3$,4-OH-PQZ]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3511 (O–H), 3099 (C–H), 2936 (C–H), 1666 (C=O). Calcd for $C_{42}H_{33}Cl_2N_7O_3Ru_1$ (%): C, 58.95; H, 3.86; N, 11.46. Found (%): C, 58.89; H, 3.85; N, 11.41. ^{1}H NMR (DMSO-d$_6$): δ ppm: 10.68 (s, 1-H), 9.87 (s, 1-H), 8.62 (d, 1-H), 8.42 (d, 2-H), 8.38 (m, 4-H), 8.24 (d, $J = 8.4$ Hz, 2-H), 8.06 (m, 4-H), 7.92 (d, $J = 5.0$ Hz, 2-H), 7.88 (d, 2-H), 7.74 (m, 5-H), 7.54 (m, 4-H), 6.55 (d, 2-H), 3.62 (m, 3-H). FAB-MS (mNBA): 855 [Ru(bpy)$_2$(3-OCH$_3$,4-OH-PQZ)]$^{2+}$(Cl$_2$)$^-$; 784 [Ru(bpy)$_2$(3-OCH$_3$,4-OH-PQZ)]$^{2+}$; 628 [Ru(bpy)(3-OCH$_3$,4-OH-PQZ)]$^{2+}$; 413 [Ru(bpy)$_2$].

2.5.9. [Ru[phen]$_2$[4-OH-PQZ]Cl$_2$. 45%, black crystals, IR (KBr) cm^{-1}: 3520 (O–H), 3128 (C–H), 2901 (C–H), 1678 (C=O). Calcd for $C_{45}H_{31}Cl_2N_7O_2Ru_1$ (%): C, 61.85; H, 3.55; N, 11.23. Found (%): C, 61.78; H, 3.49; N, 11.20. ^{1}H NMR (DMSO-d$_6$): δ ppm: 11.03 (s, 1-H), 9.45 (s, 1-H), 8.59 (d, 2-H), 8.36 (3-H, m), 8.21 (t, 3-H), 8.09 (d, $J = 8.4$ Hz, 2-H), 8.01 (d, 2-H), 7.92 (m, 5-H), 7.86 (t, 3-H), 7.78 (d, 2-H), 7.64 (m, 3-H), 7.54 (d, 2-H), 6.87 (d, 2-H). FAB-MS (mNBA): 873 [Ru(phen)$_2$(4-OH-PQZ)]$^{2+}$(Cl$_2$)$^-$; 802 [Ru(phen)$_2$(4-OH-PQZ)]$^{2+}$; 622 [Ru(phen)(4-OH-PQZ)]$^{2+}$; 462 [Ru(phen)$_2$].

2.5.10. [Ru[bpy]$_2$[4-OH-PQZ]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3513 (O–H), 3094 (C–H), 2915 (C–H), 1680 (C=O). Calcd for $C_{41}H_{31}Cl_2N_7O_2Ru_1$ (%): C, 59.88; H, 3.76; N, 11.88. Found (%): C, 59.84; H, 3.70; N, 11.79. ^{1}H NMR (DMSO-d$_6$): δ ppm: 10.91 (s, 1-H), 9.24 (s, 1-H), 8.76 (4-H, m), 8.12 (d, $J = 4.9$ Hz, 2-H), 8.04 (d, $J = 8.4$ Hz, 2-H), 7.98 (m, 5-H), 7.88 (m, 4-H), 7.82 (d, 2-H), 7.64 (m, 2-H), 7.54 (m, 4-H), 7.52 (d, 2-H), 6.37 (d, 2-H). FAB-MS (mNBA): 825 [Ru(bpy)$_2$(4-OH-PQZ)]$^{2+}$(Cl$_2$)$^-$; 754 [Ru(bpy)$_2$(4-OH-PQZ)]$^{2+}$; 598 [Ru(bpy)(4-OH-PQZ)]$^{2+}$; 413 [Ru(bpy)$_2$].

2.5.11. [Ru[phen]$_2$[4-*N*-(CH$_3$)$_2$-PQZ]Cl$_2$. 45%, black crystals, IR (KBr) cm^{-1}: 3136 (C–H), 2943 (C–H), 1679 (C=O). Calcd for $C_{47}H_{36}Cl_2N_8O_1Ru_1$ (%): C, 62.66; H, 4.00; N, 12.45. Found (%): C, 62.58; H, 3.91; N, 12.39. ^{1}H NMR (DMSO-d$_6$): δ ppm: 10.04 (s, 1-H), 8.56 (d, 2-H), 8.48 (d, 2-H), 8.28 (5-H, m), 8.16 (m, 3-H), 8.10 (d, $J = 8.4$ Hz, 2-H), 8.04 (d, 2-H), 7.98 (m, 3-H), 7.84 (t, 3-H), 7.56 (m, 3-H), 6.86 (d, 2-H), 6.45 (d, 2-H), 2.89 (s, 3-H), 2.82 (s, 3-H). FAB-MS (mNBA): 900 [Ru(phen)$_2$(4-N-(CH$_3$)$_2$-PQZ)]$^{2+}$(Cl$_2$)$^-$; 829 [Ru(phen)$_2$(4-N-(CH$_3$)$_2$-PQZ)]$^{2+}$; 649 [Ru(phen)(4-N-(CH$_3$)$_2$-PQZ)]$^{2+}$; 462 [Ru(phen)$_2$].

2.5.12. [Ru[bpy]$_2$[4-*N*-(CH$_3$)$_2$-PQZ]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3133 (C–H), 2908 (C–H), 1661 (C=O). Calcd for $C_{43}H_{36}Cl_2N_8O_1Ru_1$ (%): C, 60.56; H, 4.23; N, 13.15. Found (%): C, 60.45; H, 4.19; N, 13.09. ^{1}H NMR (DMSO-d$_6$): δ ppm: 10.11 (s, 1-H), 8.58 (2-H, d), 8.52 (d, $J = 4.9$ Hz, 2-H), 8.32 (m, $J = 8.4$ Hz, 4-H), 8.22 (m, 5-H), 8.04 (d, 2-H), 7.94 (m, 4-H), 7.62 (d, 2-H), 7.50 (m, 4-H), 6.96 (d, 2-H), 6.46 (d, 2-H), 2.81 (s, 3-H), 2.78 (s, 3-H). FAB-MS (mNBA): 852 [Ru(bpy)$_2$(4-N-(CH$_3$)$_2$-PQZ]$^{2+}$(Cl$_2$)$^-$; 781 [Ru(bpy)$_2$(4-N-(CH$_3$)$_2$-PQZ)]$^{2+}$; 625 [Ru(bpy)(4-N-(CH$_3$)$_2$-PQZ)]$^{2+}$; 413 [Ru(bpy)$_2$].

2.5.13. [Ru[phen]$_2$[4-NO$_2$-PTSZ]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3417–3380 (NH$_2$&N–H), 3136 (C–H), 2958 (C–H), 1370 (C=S). Calcd for $C_{32}H_{24}Cl_2N_8O_2Ru_1S_1$ (%): C, 50.79; H, 3.17; N, 14.81. Found (%): C, 50.65; H, 3.15; N, 14.73. ^{1}H NMR (DMSO-d$_6$): δ ppm: 12.49 (s, 1-H), 9.04 (s, 1-H), 8.58 (3-H, t), 8.36 (d, $J = 4.9$ Hz, 2-H), 8.26 (d, $J = 8.4$ Hz, 2-H), 8.06 (t, 3-H), 8.01 (d, $J = 5.0$ Hz, 2-H), 7.96 (m, 3-H), 7.74 (t, 3-H), 7.62 (d, 2-H, NH$_2$), 6.23 (d, 2-H). FAB-MS (mNBA): 756 [Ru(phen)$_2$(4-NO$_2$-PTSZ)]$^{2+}$(Cl$_2$)$^-$; 685 [Ru(phen)$_2$(4-NO$_2$-PTSZ)]$^{2+}$; 505 [Ru(phen)(4-NO$_2$-PTSZ)]$^{2+}$; 462 [Ru(phen)$_2$].

2.5.14. [Ru[bpy]$_2$[4-NO$_2$-PTSZ]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3424–3347 (NH$_2$&N–H), 3175 (C–H), 2983 (C–H), 1328 (C=S). Calcd for $C_{28}H_{24}Cl_2N_8O_2Ru_1S_1$ (%): C, 47.46; H, 3.39; N, 15.82. Found (%): C, 47.38; H, 3.34; N, 15.76. ^{1}H NMR (DMSO-d$_6$): δ ppm: 11.24 (s, 1-H), 9.26 (1-H, s), 8.46 (m, 4-H), 8.34 (d, $J = 4.9$ Hz, 2-H), 8.14 (m, 4-H), 8.01 (d, 2-H), 7.87 (m, 4-H), 7.78 (s, NH$_2$, 2-H), 7.69 (d, 2-H), 6.43 (d, 2-H). FAB-MS (mNBA): 708 [Ru(bpy)$_2$(4-NO$_2$-PTSZ)]$^{2+}$(Cl$_2$)$^-$; 637 [Ru(bpy)$_2$ (4-NO$_2$-PTSZ)]$^{2+}$; 481 [Ru(bpy)(4-NO$_2$-PTSZ)]$^{2+}$; 413 [Ru(bpy)$_2$].

2.5.15. [Ru[phen]$_2$[3,4,5-tri-OCH$_3$-PTSZ]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3426–3247 (NH$_2$&N–H), 3155 (C–H), 2987 (C–H), 1332 (C=S). Calcd for

$C_{35}H_{31}Cl_2N_7O_3Ru_1S_1$ (%): C, 52.43; H, 3.87; N, 12.24. Found (%): C, 52.38; H, 3.84; N, 12.21. ^{1}H NMR (DMSO-d_6): δ ppm: 12.24 (s, 1-H), 8.79 (2-H, d), 8.54 (d, 4-H), 8.12 (d, 2-H), 8.04 (d, $J = 5.0$ Hz, 2-H), 7.96 (d, 2-H), 7.73 (d, 2-H), 7.31 (d, 2-H, NH_2), 7.24 (m, 3-H), 6.81 (d, 2-H), 3.78 (s, 3-H), 3.66 (s, 3-H), 3.63 (s, 3-H). FAB-MS (mNBA): 801 $[Ru(phen)_2(3,4,5\text{-tri-}OCH_3\text{-PTSZ})]^{2+}(Cl_2)^-$; 730 $[Ru(phen)_2(3,4,5\text{-tri-}OCH_3\text{-PTSZ})]^{2+}$; 550 $[Ru(phen)(3,4,5\text{-tri-}OCH_3\text{-PTSZ})]^{2+}$; 462 $[Ru(phen)_2]$.

2.5.16. [Ru[bpy]$_2$[3,4,5-tri-OCH$_3$-PTSZ]Cl$_2$. 44%, black crystals, IR (KBr) cm^{-1}: 3444–3245 (NH_2&N–H), 3056 (C–H), 2931 (C–H), 1384 (C=S). Calcd for $C_{31}H_{31}Cl_2N_7O_3Ru_1S_1$ (%): C, 49.41; H, 4.12; N, 13.02. Found (%): C, 49.38; H, 4.08; N, 12.98. ^{1}H NMR (DMSO-d_6): δ ppm: 12.32 (s, 1-H), 8.62 (2-H, d), 8.48 (d, $J = 8.4$ Hz, 2-H), 8.39–8.26 (m, 4-H), 8.18 (s, 1-H), 8.07 (d, $J = 5.0$ Hz, 2-H), 7.99 (d, 2-H), 7.56 (d, 2-H), 7.19 (d, 2-H, NH_2), 6.93 (d, $J = 14.6$ Hz, 2-H), 6.13 (d, 2-H) 3.78 (s, 3-H), 3.66 (s, 3-H), 3.63 (s, 3-H). FAB-MS (mNBA): 753 $[Ru(bpy)_2(3,4,5\text{-tri-}OCH_3\text{-PTSZ})]^{2+}(Cl_2)^-$; 682 $[Ru(bpy)_2(3,4,5\text{-tri-}OCH_3\text{-PTSZ})]^{2+}$; 526 $[Ru(bpy)(3,4,5\text{-tri-}OCH_3\text{-PTSZ})]^{2+}$; 413 $[Ru(bpy)_2]$.

2.6. *Antineoplastic activity*

All the mice (Albino Swiss mice) body weight of 18–20 g were maintained in identical laboratory conditions and given standard food pellets (Hindustan Lever Ltd, Mumbai, India) and water *ad libitum*. LD_{50} value of the synthesized complexes was determined according to the literature [21]. The animals were divided into 19 groups each containing 10 mice group I was vehicle controls (5 mL kg^{-1} body weight ip) and group II was EAC control (2×10^6 EAC cells/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–XIX, respectively. All the ruthenium complexes and cisplatin were treated daily for 9 days starting 24 h after tumor transplantation. Six animals from each group were sacrificed 18 h after the last dose. The ascetic cell count parameters and ascetic fluid volume were noted. Mean survival time (MST) for the remaining six mice of each group was noted. *Tumor volume and viable count* ascites volume was noted according to the literature method.

2.7. *Biological assays*

The cytotoxic assays on inhibition of tumor cell proliferation in exponentially growing tumor cell cultures.

2.8. *Cytotoxic evaluation [22]*

Murine leukemia L1210 and human lymphocyte Molt4/C8 and CEM cells were seeded in 96-well microtiter plates at 50,000 (L1210) or 75,000 (CEM, Molt4/C8) cells per 200 µL-well in the presence of different concentrations of the test compounds. After 2 (L1210) or 3 (CEM, Molt4/C8) days, the viable cell number was counted using a Coulter counter apparatus. The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration required to inhibit tumor cell proliferation by 50%.

2.9. *Antibacterial activity [20]*

A stock solution of ruthenium complexes of 200 μg mL^{-1} was made in sterile containing 5% DMF under aseptic conditions and further dilutions were made with the same solvent in a similar manner. All the dilutions and stock 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 in mm zones of inhibition. Inoculum was prepared by washing a fresh 5 mL medium slant of test organism with 5 mL sterile water and further diluting the 1 mL washing to 10 mL. The suspension was added to 15 mL melted medium at a temperature 45–50°C and plates were prepared. Holes were dug into the agar plates with a sterile borer and filled with the drug. The plates were incubated at 35°C for 24 h. The results were compared with that of standard chloramphenicol.

3. Results and discussion

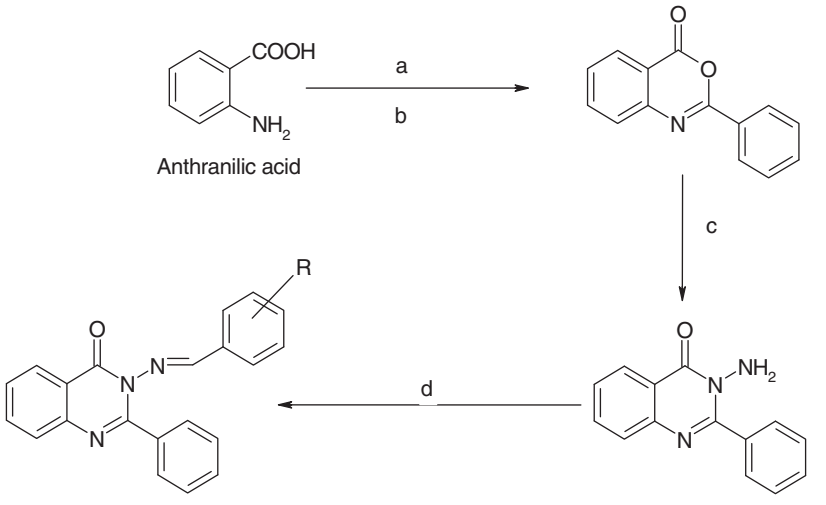
3.1. *Chemistry*

The crude ruthenium complexes were purified by column chromatography with the orange-red band collected by using silica gel as stationary phase and $CHCl_3 : CH_3OH$ (8 : 2) ratio as mobile phase.

The arylidene amino-2-phenyl quinazoline-4(3 H)-ones were prepared by reacting 3-amino-2-phenyl quinazolin-4(3 H)-one with appropriate substituted aromatic aldehyde in alcohol at 1 : 1 molar ratio and substituted aromatic thiosemicarbazones were prepared by reacting substituted benzaldehyde with thiosemicarbazide in alcohol at 1 : 1 molar ratio (schemes 1 and 2). Structures of the ligands and complexes (schemes 3 and 4) were established on the basis of UV-Vis, FT-IR, ^{1}H–NMR, ^{13}C-NMR, and mass spectral analysis (Supplementary figures S1–S9). In IR spectra, phenyl quinazoline ligands showed absorptions at 3150–3000 cm^{-1} for C–H aromatic stretching, 2980–2850 cm^{-1} for C–H aliphatic stretching, and 1685–1680 cm^{-1} for C=O stretching. The thiosemicarbazones showed absorptions at 3410–3200 cm^{-1} for NH_2 and NH stretch, from 3150 to 3000 cm^{-1} for C–H aromatic stretch, and from 1370 to 1320 cm^{-1} for C=S stretch. R_f values of all ligands were determined.

A comparison of IR spectra of ruthenium complexes with PQZ ligands confirms coordination to ruthenium by oxygen and nitrogen (Supplementary figures S1–S9). Thiosemicarbazones coordinate to ruthenium by sulfur and imino nitrogen, confirmed by the spectra. These compounds do not possess any C2-axis of symmetry. Such loss of C2-axis of symmetry was seen for $[Ru(L)_2(R)]$ (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.

In UV spectra the ruthenium complexes showed broad and intense visible bands between 320 and 530 nm due to metal-to-ligand charge transfer (MLCT) transition. In the UV region, bands at 270 and 300 nm were assigned to 2,2′-bipyridine/1,10-phenanthroline π–π^* charge transfer transitions. The same transition was found in



R = 4-NO_2, 4-CH_3, 3,4-di-OCH_3, 3-$OCH_3$4-OH, 4-OH, 4-N-$(CH_3)_2$

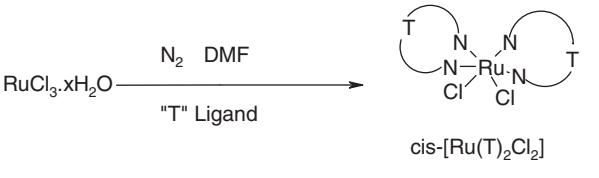
Reagents: a = C_6H_5COCl; b = C_5H_5N; c = $NH_2NH_2H_2O$; d = R-CHO

Scheme 1. Preparation of ligands (4-NO_2-PQZ, 4-CH_3-PQZ, 3,4-di-OCH_3-PQZ, 3-OCH_3, 4-OH-PQZ, 4-OH-PQZ, 4-*N*-$(CH_3)_2$-PQZ), (PQZ = phenyl quinazolines).

R = 4-NO_2, 3,4,5-tri-OCH_3

Reagents: a = $NH_2NHCSNH_2$; b = C_2H_5OH, CH_3COOH

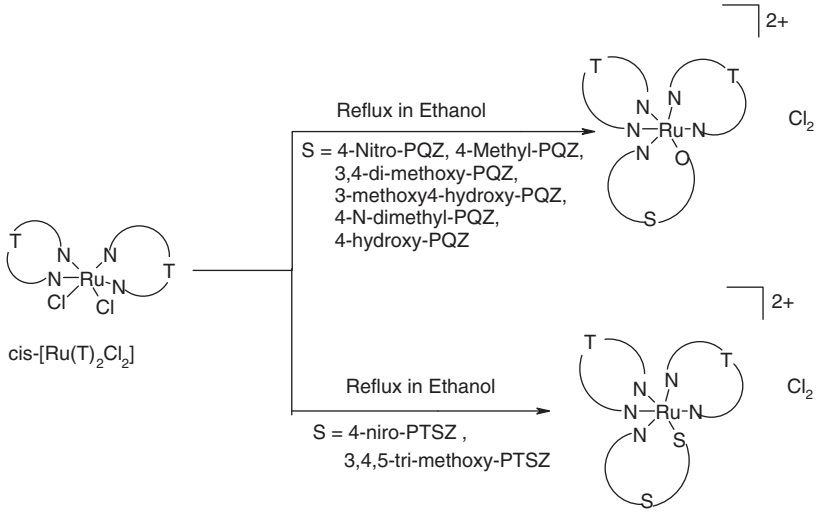
Scheme 2. Preparation of ligands (4-NO_2-PTSZ, 3,4,5-tri-OCH_3-PTSZ), (PTSZ = phenyl thiosemicarbazones).



where T = 2,2'-bipyridine/1,10-phenanthroline

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

free 2,2′-bipyridine/1,10-phenanthroline at 270 nm, so that coordination of the ligand resulted in a red shift. There were also two shoulders at 380 and 500 nm, which were tentatively attributed to MLCT transitions involving 2,2′-bipyridine, 1,10-phenanthroline, and the third ligand.



Scheme 4. Preparation of tris chelates from *cis*-$[Ru(T)_2Cl_2]$.

In ^{1}H-NMR spectra of the complexes, there were resonances at δ 12.49 (s, br, NH). For [Ru(phen)(4-NO2[Ru[phen]$_2$[4-NO_2-PTSZ]Cl_2 there were 24 resonances (δ 12.49–6.23) and 24 well-resolved peaks (δ 11.24–6.43) for [Ru[bpy]$_2$[4-NO_2-PTSZ]]Cl_2. There were also resonances at δ 8.91. Thus for [Ru[phen]$_2$[4-NO_2-PQZ]Cl_2 there were 30 resonances (δ 8.1–7.24) and 30 peaks (δ 9.18–7.54) also for [Ru[bpy]$_2$[4-NO_2-PQZ]]Cl_2.

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 molecules. FAB mass spectroscopic data in figure 1 clearly suggest that mononuclear complexes had 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 $[Ru(T)_2(S)]^{2+}$, $[Ru(T)_2]^{2+}$, respectively (where T = 1,10-phenanthroline/2,2′-bipyridine and S = 4-NO_2-PQZ, 4-CH_3-PQZ, 3,4-di-OCH_3-PQZ, 3-OCH_3,4-OH-PQZ, 4-OH-PQZ, 4-N$(CH_3)_2$-PQZ, 4-NO_2-PTSZ, 3,4,5-tri-OCH_3-PTSZ). This type of fragmentation was reported for $[Ru(phen)_2(tpl)]$, where tpl = thiopicolinanilide. In all the cases, loss of chloride was detected [1]. Thus, based on the above UV, IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectral data, the Ru(II) complexes show an octahedral geometry.

3.2. *Biological activity and discussion*

All the ruthenium complexes were tested for their antineoplastic activity in EAC bearing mice. Results are shown in table 1 and the pharmacological data were analyzed statistically by analysis of variance (ANOVA). The statistical significance was considered only when $p < 0.05$ and $F > F_{\text{critical}}$. This study clearly demonstrates tumor inhibitory activity of the ruthenium complexes against the transplantable murine

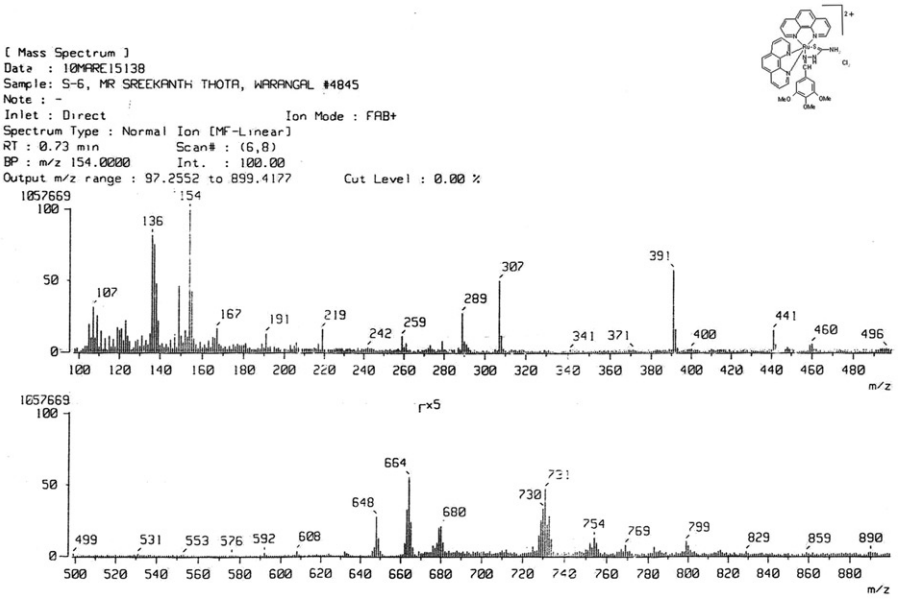


Figure 1. Mass spectrum of $[Ru(phen)_2(3,4,5\text{-tri-}OCH_3\text{-ptsz})]^{2+}Cl_2^-$ shows intense peak at 799, $[Ru(phen)_2(3,4,5\text{-tri-}OCH_3\text{-ptsz})]^{2+}$ shows intense peak at 731, $[Ru(phen)(Cl\text{-bitsz})]^{2+}$ shows intense peak at 550, $[Ru(phen)_2]$ shows intense peak at 461 by the loss of Cl_2.

Table 1. Antineoplastic 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	22.4 ± 0.4	–	–	–	–
Group II	26.8 ± 0.7	21	–	3.8 ± 0.2	95.4 ± 4.1
Group III	20.2 ± 0.4	22	5	–	–
Group IV	23.4 ± 0.4	25	19	1.6 ± 0.06	46.4 ± 1.2
Group V	21.8 ± 0.8	24	14	1.4 ± 0.02	47.8 ± 1.1
Group VI	24.6 ± 0.6	29	38	0.8 ± 0.08	41.2 ± 1.6
Group VII	22.6 ± 0.5	23	10	1.8 ± 0.04	48.0 ± 1.2
Group VIII	23.2 ± 0.8	24	14	1.3 ± 0.03	47.2 ± 1.4
Group IX	22.8 ± 1.2	29	38	0.9 ± 0.06	40.8 ± 1.8
Group X	28.3 ± 0.3	31	48	0.6 ± 0.05	34.6 ± 1.2
Group XI	20.8 ± 0.4	24	14	1.4 ± 0.04	46.6 ± 1.2
Group XII	22.1 ± 0.5	25	19	1.7 ± 0.08	47.1 ± 1.4
Group XIII	25.2 ± 0.9	32	52	0.5 ± 0.03	31.4 ± 1.2
Group XIV	23.6 ± 0.6	23	10	1.8 ± 0.06	48.6 ± 1.5
Group XV	22.2 ± 0.6	31	48	0.7 ± 0.04	33.2 ± 1.4
Group XVI	24.2 ± 0.8	30	43	0.8 ± 0.02	36.8 ± 1.2
Group XVII	25.6 ± 0.6	24	14	1.3 ± 0.06	47.6 ± 1.2
Group XVIII	21.4 ± 0.8	32	52	0.6 ± 0.02	30.8 ± 1.6
Group XIX	20.8 ± 0.4	30	43	0.8 ± 0.06	36.4 ± 1.4

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 XIX, ruthenium complexes ($2\,mg\,kg^{-1}$) + EAC.

Table 2. Cytotoxic studies of ligands.

Compound	IC_{50}[a] ($\mu mol\,L^{-1}$)		
	L1210	Molt 4/C8	CEM
4-NO_2-PQZ	164 ± 28	116 ± 14	122 ± 16
4-CH_3-PQZ	78 ± 13	98 ± 16	86 ± 12
3,4-di-OCH_3-PQZ	86 ± 16	74 ± 14	104 ± 18
3-OCH_3,4-OH-PQZ	102 ± 18	116 ± 16	128 ± 12
4-OH-PQZ	188 ± 16	94 ± 21	98 ± 18
4-*N*-$(CH_3)_2$-PQZ	63 ± 25	76 ± 14	84 ± 16
4-NO_2-PTSZ	58 ± 14	84 ± 12	78 ± 12
3,4,5-tri-OCH_3-PTSZ	48 ± 12	66 ± 14	48 ± 14

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

Table 3. Cytotoxic studies of ruthenium compounds.

Compound	IC_{50}[a] ($\mu mol\,L^{-1}$)		
	L1210	Molt 4/C8	CEM
Ru[phen]$_2$[4-NO_2-PQZ]Cl_2	1.1 ± 0.4	0.98 ± 0.03	0.67 ± 0.2
Ru[bpy]$_2$[4-NO_2-PQZ]Cl_2	0.92 ± 0.06	1.6 ± 0.5	0.82 ± 0.04
Ru[phen]$_2$[4-CH_3-PQZ]Cl_2	1.9 ± 0.8	1.2 ± 0.05	1.2 ± 0.06
Ru[bpy]$_2$[4-CH_3-PQZ]Cl_2	0.88 ± 0.02	0.84 ± 0.4	0.49 ± 0.02
Ru[phen]$_2$[3,4-di-OCH_3-PQZ]Cl_2	2.3 ± 0.07	1.4 ± 0.2	1.1 ± 0.08
Ru[bpy]$_2$[3,4-di-OCH_3-PQZ]Cl_2	1.5 ± 0.8	0.95 ± 0.04	0.84 ± 0.06
Ru[phen]$_2$[3-OCH_3,4-OH-PQZ]Cl_2	0.42 ± 0.04	0.29 ± 0.05	0.22 ± 0.02
Ru[bpy]$_2$[3-OCH_3,4-OH-PQZ]Cl_2	0.89 ± 0.06	0.38 ± 0.04	0.28 ± 0.03
Ru[phen]$_2$[4-OH-PQZ]Cl_2	2.5 ± 0.6	1.3 ± 0.09	1.1 ± 0.07
Ru[bpy]$_2$[4-OH-PQZ]Cl_2	0.54 ± 0.05	0.42 ± 0.06	0.33 ± 0.04
Ru[phen]$_2$[4-*N*-$(CH_3)_2$-PQZ]Cl_2	3.4 ± 0.5	2.2 ± 0.3	2.1 ± 0.5
Ru[bpy]$_2$[4-*N*-$(CH_3)_2$-PQZ]Cl_2	1.8 ± 0.7	1.4 ± 0.4	1.0 ± 0.08
Ru[phen]$_2$[4-NO_2-PTSZ]Cl_2	0.58 ± 0.2	0.63 ± 0.05	0.46 ± 0.04
Ru[bpy]$_2$[4-NO_2-PTSZ]Cl_2	0.82 ± 0.05	0.94 ± 0.04	0.78 ± 0.5
Ru[phen]$_2$[3,4,5-tri-OCH_3-PTSZ]Cl_2	0.47 ± 0.03	0.52 ± 0.6	0.36 ± 0.04
Ru[bpy]$_2$[3,4,5-tri-OCH_3-PTSZ]Cl_2	0.79 ± 0.2	0.82 ± 0.04	0.48 ± 0.04
Cisplatin	0.28 ± 0.2	0.34 ± 0.04	0.26 ± 0.02

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

tumor cell line. Ru(bpy)$_2$(4-OH-pqz)Cl_2 and Ru(phen)$_2$(3,4,5-tri-OCH_3-ptsz)Cl_2 increase life span of the tumor hosts by 52%; the remaining ruthenium complexes increase life span in the tumor hosts by 14–48%.

The *in vitro* cytotoxic activity was evaluated for the ligands and ruthenium complexes against human 4/C8, CEM, T-lymphocytes as well as murine L1210 cells. The relative potencies between ligands and their ruthenium complexes revealed the importance of ruthenium metal using the $4/C_8$, CEM, and murine L1210 assays. These determinations showed that in comparison to ligands, the ruthenium complexes were more potent.

Table 4. Antibacterial activity of ruthenium compounds.

Compound	S.A 6571	S.A 8530	V.C 865	S.F
$Ru[phen]_2[4\text{-}NO_2\text{-}PQZ]Cl_2$	16	14	20	12
$Ru[bpy]_2[4\text{-}NO_2\text{-}PQZ]Cl_2$	18	12	14	14
$Ru[phen]_2[4\text{-}CH_3\text{-}PQZ]Cl_2$	09	08	10	09
$Ru[bpy]_2[4\text{-}CH_3\text{-}PQZ]Cl_2$	12	10	15	12
$Ru[phen]_2[3,4\text{-di-}OCH_3\text{-}PQZ]Cl_2$	16	14	18	10
$Ru[bpy]_2[3,4\text{-di-}OCH_3\text{-}PQZ]Cl_2$	10	09	09	10
$Ru[phen]_2[3\text{-}OCH_3,4\text{-OH-}PQZ]Cl_2$	14	15	16	15
$Ru[bpy]_2[3\text{-}OCH_3,4\text{-OH-}PQZ]Cl_2$	12	12	14	14
$Ru[phen]_2[4\text{-OH-}PQZ]Cl_2$	08	10	09	10
$Ru[bpy]_2[4\text{-OH-}PQZ]Cl_2$	11	10	12	11
$Ru[phen]_2[4\text{-}N\text{-}(CH_3)_2\text{-}PQZ]Cl_2$	12	14	12	12
$Ru[bpy]_2[4\text{-}N\text{-}(CH_3)_2\text{-}PQZ]Cl_2$	09	08	10	08
$Ru[phen]_2[4\text{-}NO_2\text{-}PTSZ]Cl_2$	14	12	14	12
$Ru[bpy]_2[4\text{-}NO_2\text{-}PTSZ]Cl_2$	12	08	10	09
$Ru[phen]_2[3,4,5\text{-tri-}OCH_3\text{-}PTSZ]Cl_2$	16	15	18	15
$Ru[bpy]_2[3,4,5\text{-tri-}OCH_3\text{-}PTSZ]Cl_2$	09	08	10	08
Chloramphenicol	26	26	30	20

S.A = *Staphylococcus. aureus*, V.C = *Vibrio cholera*, S.F = *Shigella flexneri*.
Zone of inhibition in mm (including bore size of 6 mm).

The cytotoxicity data in tables 2 and 3 reveal that ruthenium complexes have significant cytotoxic potencies (IC_{50} figures at 0.29–1.6 μmol L^{-1} for Molt 4/C8, 0.42–3.4 μmol L^{-1} for L1210, and 0.22–2.1 μmol L^{-1} for CEM), while for ligands the IC_{50} values were larger (66–116 μmol L^{-1} against Molt 4/C8, 48–188 μmol L^{-1} for L1210, and 48–128 μmol L^{-1} for CEM). $Ru(phen)_2(3\text{-}OCH_3,4\text{-OH-pqz})Cl_2$ showed cytotoxicity against all three cell lines in the range of 0.29, 0.42, and 0.22 μmol L^{-1} for Molt 4/C8, CEM, and L1210, respectively. $Ru(bpy)_2(4\text{-OH-pqz})Cl_2$ showed cytotoxicity against cell lines tested 0.42 μmol L^{-1} for Molt 4/C8, 0.33 for CEM, and 0.54 for L1210; $Ru(phen)_2(3,4,5\text{-tri-}OCH_3\text{-ptsz})Cl_2$ 0.52 μmol L^{-1} for Molt 4/C8, 0.47 for CEM, and 0.36 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 concentration.

Several ruthenium complexes exhibit marked inhibitory effect on the proliferation of tumor cells with IC_{50} as low as 0.29 μmol L^{-1} for Molt 4/C8, 0.22 μmol L^{-1} for CEM, and 0.42 μmol L^{-1} for L1210, inhibiting tumor growth at submicromolar concentration. The ligands were not antitumorally active. The mechanism of action of the ruthenium complexes is not known but ruthenium antitumor agents contain an electron deficient metal that acts as a magnet for electron-rich DNA nucleophiles.

The synthesized complexes were also evaluated for their antibacterial activity (table 4) by the cup–plate method. Moderate antibacterial activity was observed for $Ru(phen)_2(4\text{-}NO_2\text{-PQZ})$, $Ru(phen)_2(3,4,5\text{-tri-}OCH_3\text{-PTSZ})$ against microorganisms such as *Staphylococcus aureus*, *Vibrio cholera*, and *Shigella flexneri*. However some of the complexes show mild antibacterial activity against tested organism. All the results of the complexes were compared with that of the standard chloramphenicol.

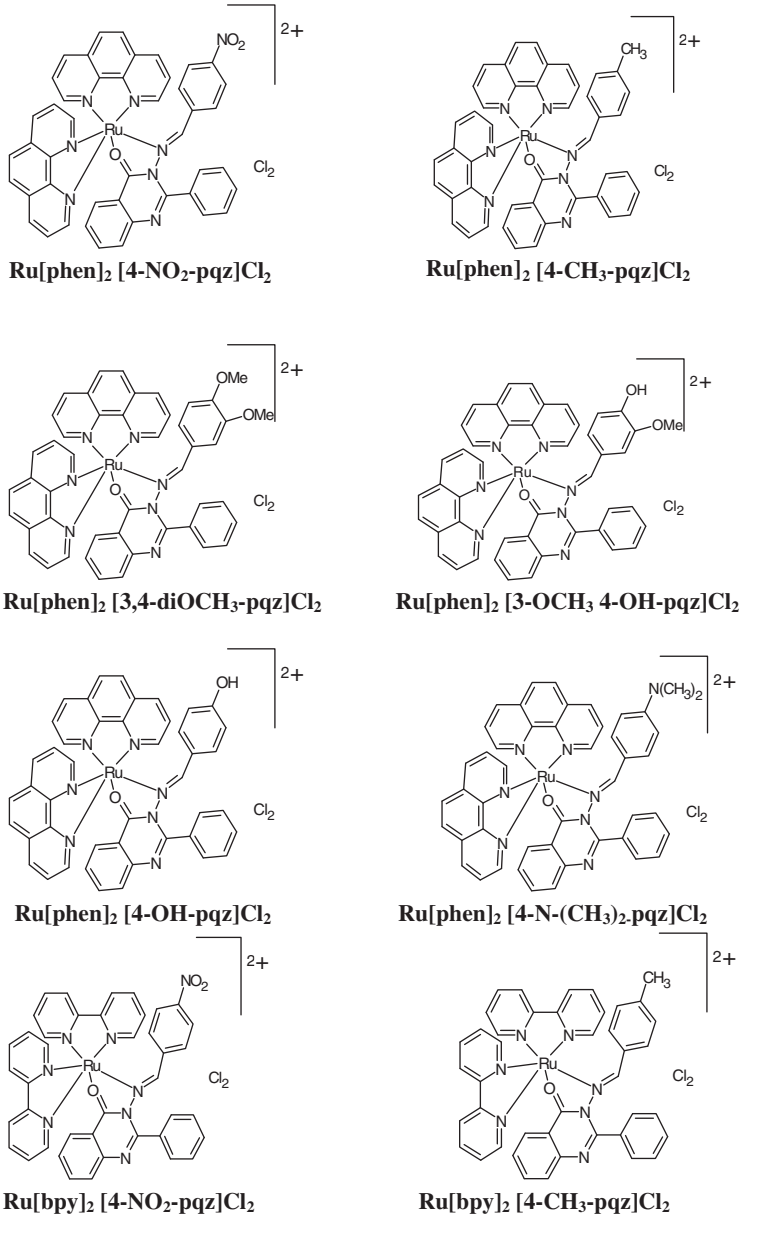
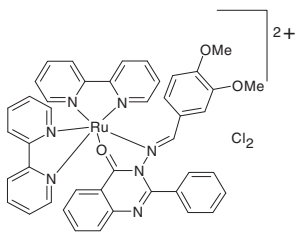


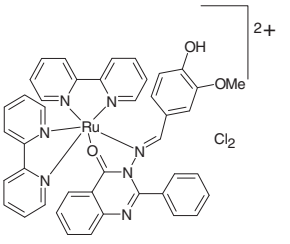
Figure 2. Schematic presentation of the 16 complexes.

4. Conclusion

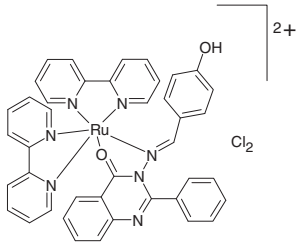
Sixteen ruthenium complexes (figure 2) bearing 2,2′-bipyridine and 1,10-phenanthroline with r-pqz or r-ptsz were synthesized in ethanol in the presence of nitrogen atmosphere. The coordination involved for Ru is bidentate. Several ruthenium complexes exhibited marked inhibitory effect on the proliferation of tumor cells with IC_{50} as low as



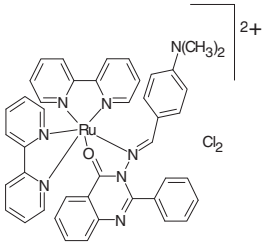
Ru[bpy]$_2$ [3,4-diOCH$_3$-pqz]Cl$_2$



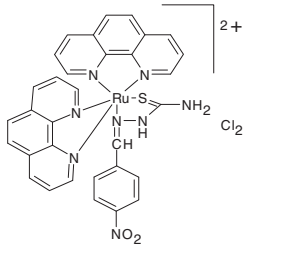
Ru[bpy]$_2$ [3-OCH$_3$ 4-OH-pqz]Cl$_2$



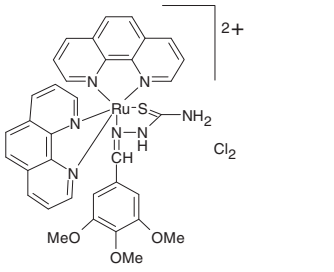
Ru[bpy]$_2$ [4-OH-pqz]Cl$_2$



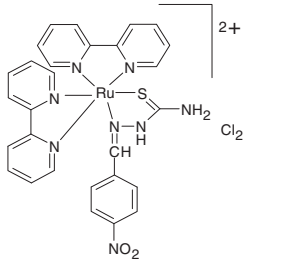
Ru[bpy]$_2$ [4-N-(CH$_3$)$_2$-pqz]Cl$_2$



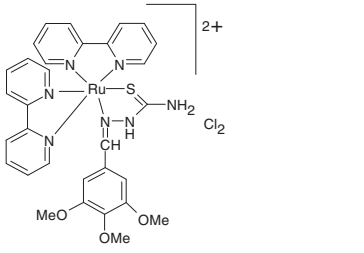
Ru[phen]$_2$ [4-NO$_2$-ptsz]Cl$_2$



Ru[phen]$_2$ [3,4,5-tri-OCH$_3$-ptsz]Cl$_2$



Ru[bpy]$_2$ [4-NO$_2$-ptsz]Cl$_2$



Ru[bpy]$_2$ [3,4,5-tri-OCH$_3$-ptsz]Cl$_2$

Figure 2. Continued.

0.29 μmol L^{-1} for Molt 4/C8, 0.42 μmol L^{-1} for L1210, and 0.22 μmol L^{-1} for CEM, inhibiting tumor growth at submicromolar concentration. The ligands were not antitumorally active. Ruthenium complexes have significant *in vitro* antibacterial activity. In comparison to previously reported ruthenium complexes most of the newly synthesized Ru(II)complexes show significant antineoplastic activity and antibacterial activity [23–27].

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