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ORIGINAL ARTICLE

Synthesis, antineoplastic and cytotoxic activities of some mononuclear Ru(II) complexes

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Abstract

A series of mononuclear Ru(II) complexes of the type $[Ru(S)_2(K)]^{2+}$, where S = 1,10-phenanthroline/2,2′-bipyridine and K = 4-OH-btsz, 4-CH_3-btsz, 3,4-di-OCH_3-btsz, 4-OH-binh, 4-CH_3-binh, 3,4-di-OCH_3-binh, were prepared and characterized by elemental analysis, FTIR, ^{1}H-NMR, and mass spectroscopy. The complexes displayed metal–ligand charge transfer (MLCT) transitions in the visible region. These ligands formed bidentate octahedral ruthenium complexes. The title complexes were evaluated for their *in vivo* anticancer activity against a transplantable murine tumor cell line, Ehrlisch's ascites carcinoma (EAC), and *in vitro* cytotoxic activity against human cancer cell lines Molt 4/C_8 and CEM and murine tumor cell line L1210. The ruthenium complexes showed promising biological activity especially in decreasing tumor volume and viable ascites cell counts. Treatment with 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 0.21 to 24 μM against Molt 4/C_8, 0.16 to 19 μM aginst CEM, and 0.75 to 32 μM against L1210.

Keywords: *Ruthenium complexes; anticancer; isonicotinyl hydrazones; thiosemicarbazones*

Introduction

The success of cisplatin and related platinum complexes as anticancer agents has stimulated a search for other active transition metal complexes, and ruthenium in particular has attracted research[1]. Metal complexes of ruthenium containing nitrogen and oxygen donor ligands are found to be effective catalysts for oxidation, reduction, hydrolysis, and other organic transformations[2]. The coordination environment around ruthenium plays a key role in stabilizing its different oxidation states and hence dictates the redox properties of the control atoms[3,4].

Ruthenium compounds are regarded as promising alternatives to platinum compounds, and offer many approaches to innovative metallopharmaceuticals. The compounds are known to be stable and have predictable structures both in the solid state and in solution. The tuning of ligand affinities is accompanied by a steadily increasing knowledge of the biological effects of ruthenium compounds[1,5]. The first systematic investigation of ruthenium compounds and their antitumor property was done at the beginning of the 1980s with the compounds fac-$[RuCl_3(NH_3)_3]$ and cis-$[RuCl_2(NH_3)_4]Cl$[6], preceded by the discovery in the 1970s that ruthenium red possesses antitumor properties[7,8]. Since then, compounds such as trans-(IndH)$[Ru(ind)_2Cl_4]$ (Ind = indazole), mer-$[Ru(terpy)Cl_3]$ (terpy = 2,2′-terpyridine)[9–11], $[Ru(dmso)_4Cl_2]$ (dmso = dimethyl sulfoxide)[12], ImH$[Ru(im)Cl_5]$[13], ImH$[Ru(im)_2$-$Cl_4]$[14], and ImH$[Ru(im)(dmso)Cl_4]$[15] (NAMI-A) (im = imidazole) have also become well-known antitumor agents.

Although the mechanism of action of ruthenium compounds is not fully understood, it is thought that, for certain species, it is similar to that of platinum drugs[16,17]. NAMI-A has high selectivity for solid tumor metastasis and low host toxicity at pharmacologically active doses[18], and it was the first ruthenium compound to enter clinical trials[19]. It has a remarkably low general toxicity[20,21] and shows marked efficacy against metastases[22,23]. It does not affect primary tumor growth[24,25] and does not exhibit cytotoxicity against tumor

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cells *in vitro*. A related ruthenium(III) compound, indazolium trans[tetrachlorobis (1H-indazole) ruthenate(III)], KP1019[26], has also entered clinical trials, since it was found to exhibit antiproliferative activity *in vitro* in human colon carcinoma cell lines[27].

In comparing the general toxicity of ruthenium compounds with platinum drugs, ruthenium has lower toxicity, which has been attributed to the ability of ruthenium compounds to specifically accumulate in cancer tissues. The higher specificity of these compounds for their targets may be linked to selective uptake by the tumor compared with healthy tissue[28,29] and selective activation by reduction to cytotoxic species within the tumor[30].

Ruthenium compounds with bidentate ligands show intercalation properties with DNA[31]. The Ru(II) compounds are kinetically more reactive than Ru(III)[32]. We have reported that Ru(II) compounds bearing thiosemicarbazides, 8-hydroxyquinolines, and 4-substituted thiopicolinanalides have *in vivo* anticancer and *in vitro* antibacterial activity[33–35]. Recently, we have reported that Ru(II) compounds bearing isatin thiosemicarbazones and chloro-fluoro-phenyl imino methyl phenol have *in vivo* anticancer and *in vitro* cytotoxic activity[36]. In this work, we describe the synthesis and characterization of some ruthenium complexes, their *in vitro* cytotoxic activity against human cancer cell lines Molt $4/C_8$ and CEM and murine tumor cell line L1210, and their *in vivo* anticancer activity against transplantable murine tumor cell line EAC (Ehrlisch's ascites carcinoma).

Materials and methods

Chemistry

AR grade solvents were obtained from S.D. Fine-Chem, Mumbai, and E. Merck, Mumbai. Puriss grade reagents were obtained from Fluka and E. Merck.

Hydrated ruthenium trichloride was purchased from Loba Chemie, Mumbai, and used as received. Ultraviolet (UV)-visible spectra were recorded on a Jasco spectrophotometer. Fourier transform infrared (FTIR) spectra were recorded in KBr powder on a Jasco V410 FTIR spectrometer by the diffuse reflectance technique. $^1H/^{13}C$-nuclear magnetic resonance (NMR) spectra were measured in $CDCl_3$ and dimethyl sulfoxide (DMSO)-d_6 on Bruker Ultraspec 500 MHz/AMX 400 MHz/300 MHz spectrometers. The reported chemical shifts were against that of tetramethylsilane (TMS). Fast atom bombardment (FAB) mass spectra were recorded on a Jeol JMS600 spectrometer with *meta*-nitrobenzylalcohol (mNBA) matrix. Substituted thiosemicarbazones were prepared according to the literature method.

General procedure for preparing substituted benzyl thiosemicarbazones (r-btsz)

A mixture of substituted benzaldehyde (1 mmol) and thiosemicarbazide (1 mmol) in 100 mL of ethanol was refluxed for 3 h and left overnight. The solid that separated was filtered and dried. The crude solid was purified by recrystallization from alcohol to give crystals.

4-OH-btsz Yield 56%, m.p. 224–225°C (lit., 226°C). IR (KBr) cm^{-1}: 3469–3320 (NH_2 and NH), 3200–2700 (O-H), 3133 (C-H), 1610 (N-H), 1328 (C=S). Calcd. for $C_8H_9N_3OS$: C, 49.21; H, 4.64; N, 21.52. Found C, 49.20; H, 4.62; N, 21.28%. λ_{max} nm (MeOH): 242, 321, 398. ^1H NMR (DMSO-d_6): δ = 12.6 (1H, s), 11.24 (1H, s), 8.07 (1H, s), 7.99 (1H, s), 7.89 (1H, s, -OH), 7.73 (2H, d, *J* = 8.6 Hz), 6.95 (2H, d, *J* = 8.6 Hz).

4-CH_3-btsz Yield 79%, m.p. 160–162°C (lit., 160–161°C). IR (KBr) cm^{-1}: 3416–3321 (NH_2 and NH), 3151 (C-H), 1615 (N-H), 1325 (C=S). Calcd. for $C_9H_{11}N_3S$: C, 55.93; H, 5.74; N,21.74. Found C, 55.87; H, 5.62; N, 21.53%. λ_{max} nm (MeOH): 234, 325, 389. ^1H NMR (DMSO-d_6): δ = 11.41 (1H, s), 8.10 (1H, s), 7.98 (1H, s), 7.78 (1H, s), 7.71 (2H, d, *J* = 8.9 Hz), 6.98 (2H, d, *J* = 8.9 Hz), 1.64 (3H, s, CH_3).

3,4-di-OCH_3-btsz Yield 56%, m.p. 194–195°C (lit., 195°C). IR (KBr) cm^{-1}: 3406–3320 (NH_2 and NH), 3133 (C-H), 1610 (N-H), 1332 (C=S). Calcd. for $C_{10}H_{13}N_3O_2S$: C, 50.19; H, 5.47; N,17.56. Found C, 50.21; H, 5.61; N, 17.43%. λ_{max} nm (MeOH): 239, 331, 395. ^1H NMR (DMSO-d_6): δ = 11.32 (1H, s), 8.16 (1H, s), 8.02 (1H, s) 7.97 (1H, s) 7.51 (1H, d), 7.13 (1H, dd, *J* = 8.6 Hz), 6.94 (1H, d, *J* = 8.3 Hz), 3.81 (3H, s, -OCH_3), 3.78 (3H, s, -OCH_3).

General procedure for preparing substituted benzyl isonicotinyl hydrazones (r-binh)

A mixture of substituted benzaldehyde (1 mmol) and isoniazid (1 mmol) in 100 mL of ethanol was refluxed for 3 h and left overnight. The solid that separated was filtered and dried. The crude solid was purified by recrystallization from alcohol to give crystals.

4-OH-binh Yield 65%, m.p. 287–288°C (lit., 287°C). IR (KBr) cm^{-1}: 3328(NH), 3180–2750 (O-H) 3148 (C-H),1683 (C=O), 1615 (N-H). Calcd. for $C_{13}H_{11}N_3O_2$: C, 60.22; H, 5.05; N,16.21. Found C, 60.17; H, 5.03; N, 16.07%. λ_{max} nm (MeOH): 233, 315, 391. ^1H NMR (DMSO-d_6): δ = 11.52 (1H, s), 11.27 (1H, s), 8.03 (1H, s, O-H), 7.78 (2H, d, *J* = 8.7 Hz), 6.95 (2H, d, *J* = 8.7 Hz), 7.76 (2H, d, *J* = 8.4 Hz), 6.87 (2H, d, *J* = 8.4 Hz).

Preparation of cis-[bis(S)dichlororuthenium(II)] cis-$[Ru(S)_2Cl_2]$[37] (where S = 2,2′-bipyridine/1, 10-phenanthroline)

$RuCl_3.H_2O$, 1g (2.5 mmol) and ligand S (5 mmol) were refluxed in 50 mL dimethylformamide (DMF) for 3 h under a nitrogen atmosphere. The reddish brown solution slowly turned purple and the product precipitated in the reaction mixture. The solution was cooled overnight at 0°C. A fine microcrystalline mass was filtered off. The residue was repeatedly washed with 30% LiCl solution and finally recrystallized from the same. The product was dried and stored in a vacuum desiccator over P_2O_5 for further use (yield 75%).

General procedure for preparing -$[Ru(S)_2(K)Cl_2]$ (where S = 1,10-phenanthroline (*Ru 1*)/2,2′-bipyridne (*Ru 2*); where K = 4-OH-btsz, 4-CH_3-btsz, 3,4-di-OCH_3-btsz, 4-OH-binh, 4-CH_3-binh, 3,4-di-OCH_3-binh)

To the black microcrystalline cis-bis(S)dichlororuthenium(II) {cis-$Ru(S)_2Cl_2$} (2 mmol), excess of ligand (r-btsz and r-binh)

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(2.5 mmol) was added and refluxed in ethanol under a nitrogen atmosphere. The initial colored solution slowly changed to brownish orange at the end of the reaction, which was verified by TLC on silica plates. Then the excess of ethanol was distilled off and to the remaining solution was added silica gel (60–120 mesh). The product was purified by column chromatography using silica gel as the stationary phase and chloroform–methanol as the mobile phase.

Ru 1 46%, black crystals, IR (KBr) cm^{-1}: 3402–3329 (NH_2 & N-H), 3210–2700 (O-H) 3036 (C-H), 1611 (N-H), 1328 (C=S). Calcd. for $C_{32}H_{25}Cl_2N_7ORuS$: C, 52.81; H, 3.43; N, 13.48. Found C, 52.26; H, 3.39; N, 13.32%. ^{1}H NMR (DMSO-d_6): δ ppm: 10.02 (d, *J* = 5.1 Hz, 1H), 9.03 (s, 1H), 8.91 (d, *J* = 4.9 Hz, 1H), 8.84 (t, *J* = 8.6 Hz, 2H), 8.63 (d, *J* = 8.4 Hz, 1H), 8.49 (d, *J* = 8.4 Hz, 1H), 8.34–8.20 (m, 6H), 8.15–8.08 (m, 2H), 7.91 (d, *J* = 5.0 Hz, 1H), 7.81–7.75 (m, 2H), 7.68–7.64 (s, 1H, O-H), 7.49–7.45 (m, 1H), 6.91 (s, 2H, br, NH_2), 6.73 (d, *J* = 14.6 Hz, 2H), 6.13 (s, 1H). FAB-MS (mNBA): 727 $[Ru(phen)_2(4\text{-}OH\text{-}btsz)]^{2+}(Cl_2)^-$; 656 $[Ru(phen)_2(4\text{-}OH\text{-}btsz)]^{2+}$; 475 $[Ru(phen)(4\text{-}OH\text{-}btsz)]^{2+}$; 462 $[Ru(phen)_2]$.

Ru 2 42%, black crystals, IR (KBr) cm^{-1}: 3401–3238 (NH_2 & N-H), 3200–2700 (O-H) 3041 (C-H), 1621 (N-H), 1344 (C=S). Calcd. for $C_{28}H_{25}Cl_2N_7ORuS$: C, 49.48; H, 3.68; N, 14.43. Found C, 49.24; H, 3.59; N, 14.32%. ^{1}H NMR (DMSO-d_6): δ ppm: 10. (d, *J* = 4.9 Hz, 1H), 9.15 (s, 1H), 8.90 (d, *J* = 5.0 Hz, 1H), 8.72–8.42 (m, 5H), 8.12–7.98 (m, 2H), 7.82–7.53 (m, 3H), 7.45–7.32 (m, 2H), 7.22–7.16 (m, 1H), 7.09–6.99 (m, 2H), 6.92–6.72 (m, 3H), 6.61 (s, 2H, br, NH_2), 6.34–6.13 (m, 2H). FAB-MS (mNBA): 679 $[Ru(bpy)_2(4\text{-}OH\text{-}btsz)]^{2+}(Cl_2)^-$; 608 $[Ru(bpy)_2(4\text{-}OH\text{-}btsz)]^{2+}$; 452 $[Ru(bpy)(4\text{-}OH\text{-}btsz)]^{2+}$; 413 $[Ru(bpy)_2]$.

Ru 3 44%, black crystals, IR (KBr) cm^{-1}: 3318 (N-H), 3200–2700 (O-H), 3041 (C-H), 1601 (N-H), 1681 (C=O). Calcd. for $C_{37}H_{27}Cl_2N_7O_2Ru$: C, 57.43; H, 3.49; N, 12.67. Found C, 57.26; H, 3.34; N, 12.32%. ^{1}H NMR (DMSO-d_6): δ ppm: 10.01 (d, *J* = 5.1 Hz, 1H), 9.02 (s, 1H), 8.87 (d, *J* = 5.6 Hz, 1H),8.64 (d, *J* = 8.3 Hz, 1H), 8.46 (d, *J* = 8.6 Hz, 1H), 8.37–8.19 (m, 6H), 8.13–8.07 (m, 2H), 7.93 (d, *J* = 5.1 Hz, 2H), 7.84–7.78 (m, 2H), 7.64–7.60 (s, 1H, O-H), 7.46–7.43 (m, 2H), 7.38–7.32 (m, 2H), 6.93 (s, 2H, br, NH_2), 6.77 (d, *J* = 15.2 Hz, 2H), 6.11 (s, 1H). FAB-MS (mNBA): 773 $[Ru(phen)_2(4\text{-}OH\text{-}binh)]^{2+}(Cl_2)^-$; 702 $[Ru(phen)_2(4\text{-}OH\text{-}binh)]^{2+}$; 521 $[Ru(phen)(4\text{-}OH\text{-}binh)]^{2+}$; 462 $[Ru(phen)_2]$.

Ru 4 44%, black crystals, IR (KBr) cm^{-1}: 3312 (N-H), 3200–2700 (O-H), 3041 (C-H), 1615 (N-H), 1675 (C=O). Calcd. for $C_{33}H_{27}Cl_2N_7O_2Ru$: C, 54.62; H, 3.72; N, 13.52. Found C, 53.89; H, 3.55; N, 13.28%. ^{1}H NMR (DMSO-d_6): δ ppm: 9.98. (d, *J* = 4.9 Hz, 1H), 9.18 (s, 1H), 8.91 (d, *J* = 5.3 Hz, 1H), 8.74–8.44 (m, 5H), 8.11–7.97 (m, 2H), 7.93–7.89 (m, 2H), 7.80–7.51 (m, 3H), 7.46–7.22 (m, 2H), 7.21–7.15 (s,1H, O-H), 7.10–7.01 (m, 2H), 6.94–6.72 (m, 3H), 6.63 (s, 2H, br, NH_2), 6.36–6.15 (m, 2H). FAB-MS (mNBA): 725 $[Ru(bpy)_2(4\text{-}OH\text{-}binh)]^{2+}(Cl_2)^-$; 654 $[Ru(bpy)_2(4\text{-}OH\text{-}binh)]^{2+}$; 498 $[Ru(bpy)(4\text{-}OH\text{-}binh)]^{2+}$; 413 $[Ru(bpy)_2]$.

Ru 5 44%, black crystals, IR (KBr) cm^{-1}: 3414–3224 (NH_2 & N-H), 3032 (C-H), 1632 (N-H), 1331 (C=S). Calcd. for $C_{33}H_{27}Cl_2N_7RuS$: C, 54.62; H, 3.72; N, 13.52. Found C, 53.26; H, 3.72, N, 14.47%. ^{1}H NMR (DMSO-d_6): δ ppm: 10.15–10.04 (m, 2H), 9.41 (s, 1H), 8.87–8.83 (m, 2H), 8.71 (s, 1H, br), 8.53–8.51 (m, 1H), 8.46–8.34 (d, *J* = 5.7 Hz, 3H), 8.31–8.24 (m, 4H), 8.01 (s, 2H, br, NH_2), 7.91–7.85 (m, 4H), 7.59–7.45 (dd, 1H, *J* = 8.2, 8.1 Hz), 7.51–7.42 (m, 2H), 7.23 (d, *J* = 8.3 Hz, 2H, br), 6.95 (d, 1H, *J* = 8.5 Hz), 6.13 (s, 1H). FAB-MS (mNBA): 725 $[Ru(phen)_2(4\text{-}CH_3\text{-}btsz)]^{2+}(Cl_2)^-$; 654 $[Ru(phen)_2(4\text{-}CH_3\text{-}btsz)]^{2+}$; 474 $[Ru(phen)(4\text{-}CH_3\text{-}btsz)]^{2+}$; 462 $[Ru(phen)_2]$.

Ru 6 44%, black crystals, IR (KBr) cm^{-1}: 3409–3219 (NH_2 & N-H), 3035 (C-H), 1615 (N-H), 1327 (C=S). Calcd. for $C_{29}H_{27}Cl_2N_7RuS$: C, 51.41; H, 3.98; N, 14.47. Found C, 50.98; H, 3.79; N, 14.35%. ^{1}H NMR (DMSO-d_6): δ ppm: 10.01 (m, 1H), 8.82–8.76 (m, 2H), 8.70 (d, 1H, *J* = 5.6 Hz), 8.61 (d, 1H, *J* = 8.0 Hz), 8.43 (d, 1H, *J* = 8.0 Hz), 8.06–8.00 (m, 3H, 7.79–7.73 (m, 2H), 7.65–7.59 (m, 2H), 7.46 (d, 1H, *J* = 5.6 Hz), 7.31–7.22 (m, 3H), 7.19–7.16 (mt, 3H, *J* = 12.0 Hz), 6.97 (d, 2H, *J* = 12.0 Hz), 6.22 (s, 2H, br, NH_2), 1.61(s, 3H, -CH_3) FAB-MS (mNBA): 677 $[Ru(bpy)_2(4\text{-}CH_3\text{-}btsz)]^{2+}(Cl_2)^-$; 606 $[Ru(bpy)_2(4\text{-}CH_3\text{-}btsz)]^{2+}$; 452 $[Ru(bpy)(4\text{-}CH_3\text{-}btsz)]^{2+}$; 413 $[Ru(bpy)_2]$.

Ru 9 46%, black crystals, IR (KBr) cm^{-1}: 3418–3226 (NH_2 & N-H), 3042 (C-H), 1608 (N-H), 1339 (C=S). Calcd. for $C_{34}H_{29}Cl_2N_7O_2RuS$: C, 52.91; H, 3.76; N, 12.71. Found C, 52.87; H, 3.68; N, 12.42%. ^{1}H NMR (DMSO-d_6): δ ppm: 10.09 (d, *J* = 5.2 Hz, 1H), 8.98 (d, *J* = 5.6 Hz, 1H), 8.80 (t, *J* = 8.8 Hz, 2H), 8.68 (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), 7.88 (d, *J* = 5.0 Hz, 1H), 7.83–7.77 (m, 2H), 7.67–7.63 (m, 1H), 7.46–7.42 (m, 1H), 6.98 (s, 2H, br, NH_2), 6.75 (d, *J* = 14.9 Hz, 2H), 3.68 (s, 3H, -OCH_3), 3.62 (s, 3H, -OCH_3), FAB-MS (mNBA): 771 $[Ru(phen)_2(3,4\text{-}di\text{-}OCH_3\text{-}btsz)]^{2+}(Cl_2)^-$; 700 $[Ru(phen)_2(3,4\text{-}di\text{-}OCH_3\text{-}btsz)]^{2+}$; 521 $[Ru(phen)(3,4\text{-}di\text{-}OCH_3\text{-}btsz)]^{2+}$; 461 $[Ru(phen)_2]$.

Ru 10 43%, black crystals, IR (KBr) cm^{-1}: 3406–3217 (NH_2 & N-H), 3025 (C-H), 1612 (N-H), 1322 (C=S). Calcd. for $C_{30}H_{29}Cl_2N_7O_2RuS$: C, 49.79; H, 4.01; N, 13.55. Found C, 49.56; H, 3.95; N, 13.42%. ^{1}H NMR (DMSO-d_6): δ ppm: 10.02 (d, *J* = 5.0 Hz, 1H), 8.73–8.72 (d, *J* = 5.4 Hz, 1H,), 8.63–8.41 (m, 5H), 8.10–8.03 (m, 3H), 7.88–7.70 (m, 6H), 7.46 (d, *J* = 4.9 Hz, 2H), 7.39–7.12 (m, 3H), 6.94 (s, 2H, br, NH_2), 3.76 (s, 3H, -OCH_3), 3.69 (s, 3H, -OCH_3), FAB-MS (mNBA): 723 $[Ru(bpy)_2(3,4\text{-}di\text{-}OCH_3\text{-}btsz)]^{2+}(Cl_2)^-$; 652 $[Ru(bpy)_2(3,4\text{-}di\text{-}OCH_3\text{-}btsz)]^{2+}$; 496 $[Ru(bpy)(3,4\text{-}di\text{-}OCH_3\text{-}btsz)]^{2+}$; 413$[Ru(bpy)_2]$.

Antineoplastic activity

Albino swiss mice (18–20 g body weight) were maintained in identical laboratory conditions and given standard food pellets (Hindustan Lever Ltd, Bombay, India) and water *ad libitum*. LD_{50} values of the synthesized compounds were determined according to the literature[38]. All compounds were dissolved in 10% DMSO solution. The animals were divided into 15 groups each containing 12 mice. Group I was the vehicle control group (5mL/kg body weight, i.p.) and group II was the EAC control group (2×10^6 EAC cells/mouse, i.p.). Group III were treated with the standard drug cisplatin (2 mg/kg body weight). All the compounds were administered (i.p.) at a dose of 2 mg/kg body weight in groups IV–XV, respectively. Mice were treated with the compounds and cisplatin daily for 9 days starting 24 h after tumor transplantation. Six animals from each group were sacrificed 18 h after the last

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dose. Ascitic fluid volume and Ascitic cell count parameters were noted. Mean survival time (MST) for the remaining six mice of each group was noted.

Tumor volume and viable cell count

Ascites volume was noted by taking it in a graduated centrifuge tube, and packed cell volume determined by centrifuging at 1000*g* for 5 min. The viability of ascitic cells was checked by Trypan blue (0.4% in normal saline) dye exclusion test and the count was taken in a Neubauer counting chamber. The effect of the ruthenium complexes on tumor growth was monitored by recording the mortality daily, and percentage increase in life span (ILS%) was calculated by the following formula:

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

Cytotoxic evaluation

The compounds prepared in the laboratory were evaluated against Molt $4/C_8$, CEM, and L1210 cells by a literature procedure[39].

Results and discussion

Chemistry

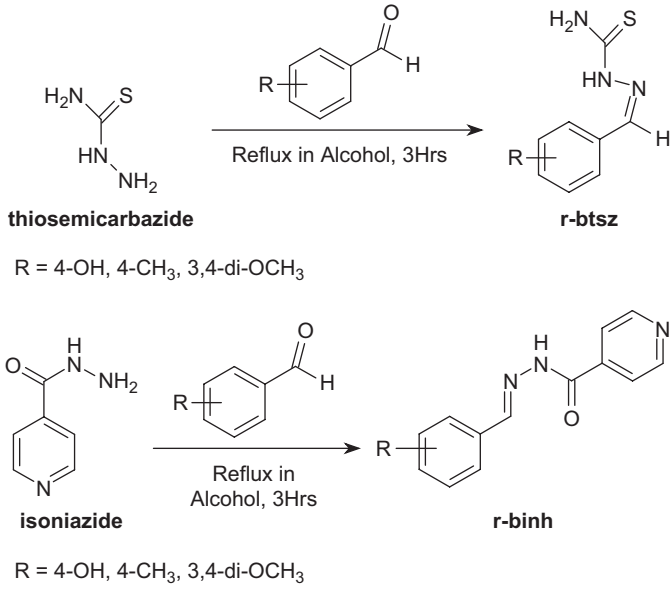
Ligands type r-binh (r-binh = substituted benzyl isonicotinyl hydrazones) were prepared by reacting substituted benzaldehydes with isoniazid in alcohol at 1:1 molar ratio (Scheme 1), and r-btsz (r-btsz = substituted benzyl thiosemicarbazones) were prepared by reacting substituted benzaldehydes with thiosemicarbazide in alcohol at 1:1 molar ratio (Scheme 1). All ligands were confirmed for their purity by their melting point, elemental analysis, and other spectral studies. Details of the strategy adopted for the synthesis of these ruthenium homoleptic compounds are as follows. The starting material for synthesis of the compounds was cis-bis(1,10-phenanthroline) dichlororuthenium(II)/cis-bis(2,2′-bipyridine) dichlororuthenium(II). Ruthenium trichloride was refluxed in DMF in the presence of 1,10-phenanthroline/2,2′-bipyridine and 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)[37] (Scheme 2). The third ligand was introduced in alcohol in the presence of a nitrogen atmosphere (Scheme 3).

The structures of the ligands, especially r-inh and r-btsz, were capable of exhibiting bidentate behavior. There are very few cases in which the thiosemicarbazide acts as a monodentate ligand, binding to the metal center through the sulfur atom[40,41]. In the case of r-btsz ligands the chelating mode was via the sulfur atom and imine nitrogen by a coordination covalent bond. In the case of r-binh ligands a covalent bond was formed between the metal ion and oxygen atom and a coordinate covalent bond with the imine nitrogen.

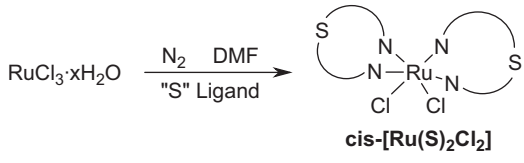
The infrared spectra of all ligands and their ruthenium(II) compounds were recorded in KBr powder by the diffuse reflectance technique, and are reported in their respective titles by tentative assignments. The r-btsz ligands showed vibrational frequency from 3400 to 3200 cm^{-1} for NH_2 and N-H stretching, and from 1335 to 1325 $cm^{-1}$for C=S stretching. The r-binh ligands showed vibrational frequency from 3320 to 3200 cm^{-1} for N-H stretching and from 1690 to 1670 cm^{-1} for C=O stretching.

A comparison of IR spectra of r-btsz ligands and ruthenium complexes confirmed coordination to the metal center by the sulfur atom and imine nitrogen. Comparing the IR spectra of r-binh ligands and ruthenium compounds confirmed coordination to the metal center by an oxygen atom and imine nitrogen. In complexes such as **Ru 1–Ru 2, Ru 5–Ru 6, Ru 9–Ru 10**, coordination occurred via the sulfur and imine nitrogen but not with the terminal amine group; this was confirmed by the spectra, which indicated no change in vibrational frequency of the NH_2 group between 3400 and 3300 cm^{-1}.

Coordination of ligands (K = r-binh, r-btsz) to ruthenium resulted in compounds such as $[Ru(S)_2(K)]^{2+}Cl_2$(**Ru 1–Ru 12**), respectively. These compounds did not possess any C2 axes of symmetry. Such a loss of C2 axis of symmetry was seen for $[Ru(L)_2(R)]$[33–35] (where L = 2,2′-bipyridine/1,10-phenanthroline and R = acetazolamide, 7-iodo-8-hydroxy-quinoline, 4-substituted thiopicolinanalide, etc.). All compounds had well-resolved resonance peaks, which corresponded to four



Scheme 1. Preparation of ligands (r-btsz and r-binh).



Where S = 2,2'-bipyridine/ 1,10-phenanthroline

Scheme 2. Preparation of cis-$[Ru(S)_2Cl_2]$.

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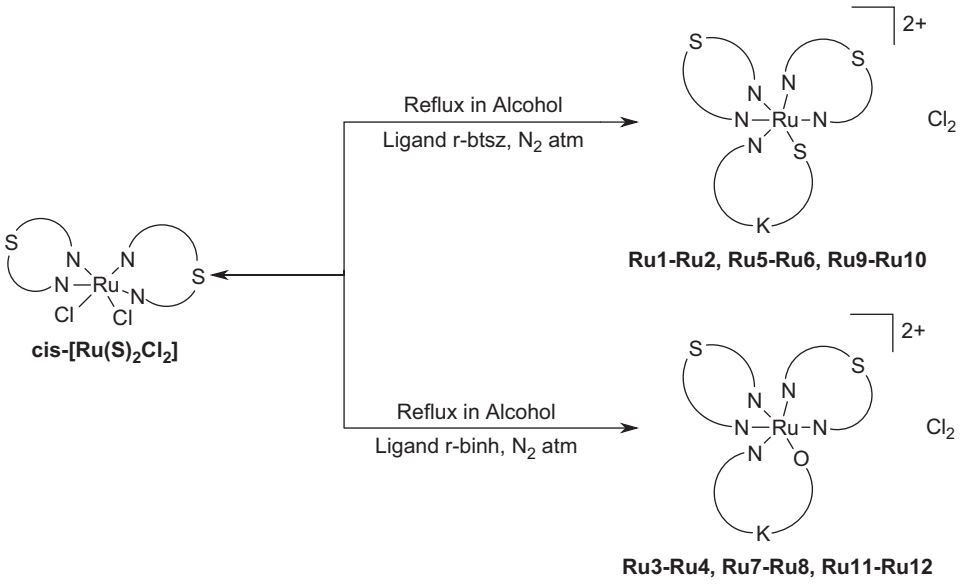

Scheme 3. Preparation of tris chelates from cis-$[Ru(S)_2Cl_2]$.

different aromatic ring protons of the two 2,2′-bipyridine/1,10-phenanthroline ligands and the third ligand.

These compounds showed broad and intense visible bands between 340 and 510 nm due to a metal–ligand charge transfer transition (MLCT). In the UV region the bands at 280 and 310 nm were assigned to 2,2′-bipyridine/1,10-phenanthroline ligand p–p* charge transfer transitions. The same transition was found in free 2,2′-bipyridine/1,10-phenanthroline at 270 nm, so that coordination of the ligand resulted in a red shift in the transition energy. There were also two shoulders at 380 and 500 nm, which were, tentatively, attributed to metal–ligand charge transfer transitions involving 2,2′-bipyridine, 1,10-phenanthroline, and the third ligand.

In the ^{1}H-NMR spectra of the complexes, there were resolved resonance peaks at low field at δ 10.02 (s, br, NH), 7.68 (s, 1H, O-H). Thus, in the case of **Ru 1**, there were 25 resonance peaks (δ 10.03–6.13), and 25 well-resolved peaks (δ 10.00–6.34) for **Ru 2**.

The mass spectra of the complexes confirmed the formulae suggested 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 the $[Ru(S)_2(K)]^{2+}$–Cl_2^- ion pair. The complex also showed a peak due to the complex cation $[Ru(S)_2(K)]^{2+}$ and others due to $[Ru(S)(K)]^{2+}$ and $[Ru(S)_2]^{2+}$ respectively (where S = 1,10-phenanthroline/2,2′-bipyridine and K = r-binh, r-btsz). This type of fragmentation has been 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-hydroxyquinoline-5-sulfonicacid)[33]. In all cases, the loss of chlorine ions was detected where S = 2,2′-bipyridine/1,10-phenanthroline and K = r-binh, r-btsz. Thus, based on the above observations, it is tentatively suggested that Ru(II) complexes show an octahedral geometry (Figure 1).

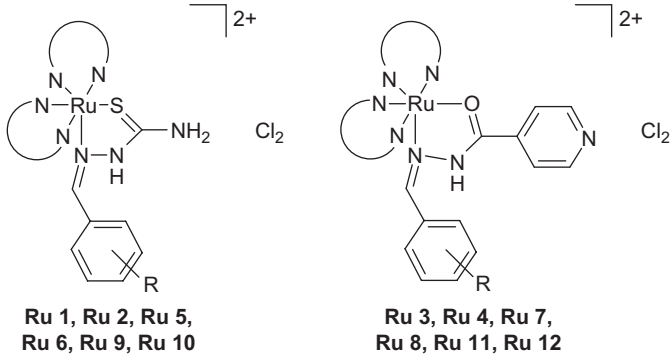

Figure 1. Structures of the ruthenium(II) complexes, where N = 1,10-phenanthroline/2,2′-bipyridine, R= 4-OH, 4-CH_3, 3,4-di-OCH_3.

Biological activity and discussion

Results are summarized in Tables 1 and 2 and the pharmacological data were analyzed statistically by ANOVA (analysis of variance). Statistical significance was considered only when $p < 0.05$ and $F > F_{critical}$. All the complexes were tested for their anticancer activity in mice bearing EAC tumors. **Ru 6** was found to increase the life span of the tumor hosts by 52%, while the remaining ruthenium complexes were able to increase the life span in the tumor hosts by 10–38% only. The results of the present study clearly demonstrated the tumor inhibitory activity of the ruthenium complexes against the transplantable murine tumor cell line (Table 1).

The *in vitro* cytotoxic activity was evaluated for all the synthesized ligands and the ruthenium complexes against human Molt $4/C_8$ and CEM T-lymphocytes as well as murine L1210 cells, and the results are summarized in Table 2. The relative potencies between ligands and their ruthenium complexes revealed the importance of ruthenium metal using the $4/C_8$ and CEM assays and murine L1210 assay. These determinations showed that in comparison to the ligands, the ruthenium complexes were more potent.

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Table 1. Antineoplastic activity of ruthenium complexes against EAC bearing mice.

Parameter	Total body weight (g)	Mean survival time (days)	ILS%	Tumor volume (mL)	Viable cells in ascitic fluid (%)
Group I	24.2 ± 0.5	—	—	—	—
Group II	27.8 ± 0.6	21	—	3.4 ± 0.3	94.8 ± 3.8
Group III	19.6 ± 0.5	22	5	—	—
Group IV	22.4 ± 0.4	29	38	0.9 ± 0.07	36.2 ± 1.1
Group V	23.2 ± 0.7	26	24	1.1 ± 0.03	43.5 ± 1.4
Group VI	23.7 ± 0.8	25	19	1.4 ± 0.04	45.6 ± 1.2
Group VII	28.4 ± 0.6	28	33	1.0 ± 0.04	38.8 ± 1.7
Group VIII	25.3 ± 0.3	24	14	1.2 ± 0.03	46.9 ± 1.4
Group IX	26.8 ± 0.2	32	52	0.7 ± 0.03	28.4 ± 1.6
Group X	26.4 ± 0.5	26	24	1.1 ± 0.02	43.4 ± 1.3
Group XI	24.2 ± 0.5	25	19	1.4 ± 0.06	45.2 ± 1.4
Group XII	22.9 ± 0.4	28	33	1.0 ± 0.02	38.6 ± 1.8
Group XIII	24.8 ± 0.6	26	24	1.1 ± 0.04	43.8 ± 1.2
Group XIV	22.6 ± 0.8	23	10	1.3 ± 0.06	47.9 ± 1.5
GroupXV	23.8 ± 0.2	25	19	1.9 ± 0.04	45.1 ± 1.3

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

Table 2. Cytotoxic studies of ligands and ruthenium compounds.

	IC_{50}[a] (μM)		
Compound	L1210	Molt 4/C_8	CEM
4-OH-btsz	244 ± 8	328 ± 12	223 ± 4
4-CH_3-btsz	186 ± 21	126 ± 34	136 ± 22
3,4-di-OCH_3-btsz	72 ± 4	88 ± 12	84 ± 33
4-OH-binh	232 ± 12	180 ± 24	163 ± 26
4-CH_3-binh	94 ± 22	227 ± 13	128 ± 42
3,4-di-OCH_3-binh	64 ± 32	96 ± 28	202 ± 64
Ru 1	18 ± 4	3.1 ± 1.8	2.9 ± 0.8
Ru 2	32 ± 12	24 ± 0.6	19 ± 5
Ru 3	0.78 ± 0.6	0.21 ± 0.02	0.24 ± 0.21
Ru 4	8.7 ± 0.3	0.65 ± 0.11	0.96 ± 0.53
Ru 5	0.82 ± 0.04	0.39 ± 0.03	0.48 ± 0.16
Ru 6	1.8 ± 0.2	1.2 ± 0.4	0.19 ± 0.14
Ru 7	0.75 ± 0.06	0.29 ± 0.07	0.16 ± 0.09
Ru 8	5.9 ± 1.3	1.4 ± 0.1	2.1 ± 0.2
Ru 9	0.91 ± 0.08	0.26 ± 0.03	0.22 ± 0.02
Ru 10	3.9 ± 1.5	0.92 ± 0.24	2.3 ± 0.5
Ru 11	1.5 ± 0.3	0.36 ± 0.04	1.6 ± 0.4
Ru 12	12 ± 1.4	18 ± 12	10 ± 06

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

The cytotoxicity data in Table 2 revealed that most ruthenium complexes had significant cytotoxic potencies (IC_{50} values in the range 0.21–3.1 for Molt 4/C_8, and 0.75–5.9 μM for L1210). On the other hand, for the ligands, the IC_{50} values were in excess (84–223 μM against CEM, 96–328 μM for Molt 4/C_8, and 64–244 μM for L1210). Of the tested ligands and ruthenium complexes, **Ru 3** showed cytotoxicity against all three cell lines tested in the region of 0.21, 0.24, and 0.78 μM for Molt 4/C_8, CEM, and L1210, respectively. Another complex, **Ru 5**, showed cytotoxicity against the cell lines tested at 0.39 μM for Molt 4/C_8, 0.48 for CEM, and 0.82 for L1210. Yet another complex, **Ru 7**, showed cytotoxicity against the cell lines tested at 0.29 μM for Molt 4/C_8, 0.16 for CEM, and 0.75 for L1210. The remaining ruthenium complexes showed low-μM values for Molt 4/C_8 and CEM and higher-μM values for L1210. In comparison with the ruthenium complexes, the ligands displayed cytotoxicty at higher-μM concentration.

From the results presented in Table 2, it is clear that several ruthenium complexes exhibited a marked inhibitory effect on the proliferation of tumor cells, with IC_{50} values from as low as 0.21 μM for Molt 4/C_8, 0.16 μM for CEM, and 0.75 μM for L1210. Thus, the ruthenium complexes proved inhibitory to tumor growth at submicromolar concentration. Their ligands, however, were not antitumorally active.

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Declaration of interest

The authors declare no conflict of interest. The authors alone are responsible for the writing and content of this paper.

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