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Synthesis, anticancer, and cytotoxic activities of some mononuclear Ru(II) compounds

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Abstract—The synthesis and characterization of ruthenium compounds (Ru1–Ru12) of the type $[Ru(S)_2(K)]$, (where S = 1,10-phenanthroline/2,2′-bipyridine and K = itsz, MeO-btsz, 4-Cl-btsz, 2-Cl-btsz, 2-F-btsz, hfc and itsz = isatin-3-thiosemicarbazone, MeO-btsz = 1-(4′-methoxy-benzyl)-thiosemicarbazone, hfc = 2-{[3-chloro-4-fluoro-phenylimino]methyl}phenol, 4-Cl-btsz = 1-(4′-chlorobenzyl)-thiosemicarbazone, 2-Cl-btsz = 1-(2′-chloro benzyl)-thiosemicarbazone, 2-F-btsz = 1-(2′-fluorobenzyl)-thiosemicarbazone) are described. These ligands form bidentate octahedral ruthenium compounds. The title compounds were subjected to in vivo anticancer activity against a transplantable murine tumor cell line *Ehrlich's Ascites Carcinoma* (EAC) and in vitro cytotoxic activity against human cancer cell line Molt 4/C8, CEM and murine tumor cell line L1210. Ruthenium compounds (Ru1–Ru12) showed promising biological activity especially in decreasing tumor volume and viable ascites cell counts. Treatment with these compounds prolonged the life span of mice bearing EAC tumor by 10–43%. In vitro evaluation of these ruthenium compounds revealed cytotoxic activity from 0.24 to 27 μM against Molt 4/C8, 0.27 to 48 μM against CEM, and 0.94 to 248 μM against L1210. Their ligands alone failed to show cytotoxic activity at the concentrations tested (68–405 μM).

1. Introduction

Compounds of transition elements of the platinum group hold promise in the design of new anticancer agents. Success of *cis*-diamine-dichloro-platinum(II) [cisplatin] and *cis*-diamine-(cyclobutane-1,1-dicarboxylato)platinum(II) [carboplatin] and [cyclohexane-1,2-diamine-*N*,*N*′-oxalate(2-)-*O*,*O*]platinum(II) [oxaliplatin] are the first antitumor drugs of this type. Nedapkatin, lobaplatin, and heptaplatin have been approved as anticancer agents only in Japan, China, and South Korea, respectively.[1–3] Cisplatin represents one of the most active and clinically useful agents used in the treatment of cancer, with high response rates in ovarian and small cell lung cancer. However in common with many other cytotoxic drugs, cisplatin induces normal tissue toxicity, particularly to the kidney, and the development of acquired drug resistance can occur in initially responsive disease types (ovarian and small cell lung) or be present as intrinsic drug resistance in less-responsive disease types (non-small cell lung and colon).

Therefore alternative metal compounds are presently being evaluated in clinical trials.[4–6] One of the most promising metals is ruthenium.[7] 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 to have predictable structures both in the solid state and in solution: tuning of ligand affinities and accompanied by a steadily increasing knowledge of the biological effects of ruthenium compounds.[4,5] The first systematic investigation of ruthenium compounds and their antitumor property was done in beginning of 1980s with the compounds *fac*-$[RuCl_3(NH_3)_3]$ and *cis*-$[RuCl_2(NH_3)_4]Cl$[8] preceded by the discovery that ruthenium red possesses antitumor properties made in the 1970s.[9,10] Since then compounds such as *trans*-(InH)$[Ru(ind)_2Cl_4]$ (Ind = indazole), *mer*-$[Ru(terpy)Cl_3$ (ter = 2,2′-terpyridine),[11–13] $[Ru(dmso)_4Cl_2]$[14] (dmso = dimethyl sulfoxide), $ImH[Ru(im)Cl_5]$,[15] $ImH[Ru(im)_2Cl_4]$[16], and $ImH[Ru(im)(dmso)Cl_4]$[17] NAMI-A (im = imidazole) are also well-known antitumor agents.

Keywords: Ruthenium compounds; Anticancer; Cytotoxicity; Thiosemicarbazones.
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Although the mechanism of action of ruthenium compounds is not fully understood, it is thought that for certain species, similar to platinum drugs.[18,19] NAMI-A has high selectivity for solid tumor metastasis and low host toxicity at pharmacologically active doses[20] and it was the first ruthenium compound to enter clinical trials.[21] It has a remarkably low general toxicity[22,23] and shows marked efficacy against metastases.[24,25] It does not affect primary tumor growth[26,27] and does not exhibit cytotoxicity against tumor cells in vitro. A related ruthenium(III) compound, indazolium *trans*-[tetrachlorobis(1H-indazole)ruthenate(III)] KP1019,[28] has also entered clinical trials, since it was found to exhibit antiproliferative activity in vitro in human colon carcinoma cell lines.[29]

By comparing the general toxicity of ruthenium compounds with platinum drugs the ruthenium has lower toxicity, that is, has been attributed to the ability of ruthenium compounds to specifically accumulate in cancer tissues.[30] The higher specificity of these compounds for their targets may also be linked to the selective uptake by the tumor compared with healthy tissue[31,32] and because of a selective activation by reduction to cytotoxic species within the tumor.[33]

The ruthenium compounds with bidentate ligands show intercalation properties with DNA.[34] The Ru(II) compounds are kinetically more reactive than Ru(III).[35] So recently, we have reported that Ru(II) compounds bearing thiosemicarbazides, 8-hydroxy quinolines, 4-substituted thiopicolinanalides have in vivo anticancer and in vitro antibacterial activity.[36–38] In this work, we describe the synthesis and characterization of some ruthenium compounds, their in vitro cytotoxicity against human cancer cell line Molt 4/C8, CEM and murine cell line L1210, and their in vivo anticancer activity against transplantable murine tumor cell line EAC.

2. Results and discussion

2.1. Chemistry

The ligand itsz (isatin thiosemicarbazone) was prepared by reacting isatin with thiosemicarbazide in alcohol in the presence of acetic acid. Other ligands like r-btsz (r-btsz = substituted benzyl thiosemicarbazones) were prepared by reacting substituted benzaldehydes with thiosemicarbazide in alcohol at 1:1 molar ratio (Scheme 1). The hfc ligand was prepared by reacting salicylaldehyde with 3-chloro-4-fluoro-aniline in alcohol at 1:1 molar ratio. All ligands were confirmed for their purity by their melting point, elemental analysis, and other spectral studies.

The details of the synthetic strategy adopted for the synthesis of these ruthenium homoleptic compounds was as follows. The starting material for the synthesis of the compounds are *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).[44] (Scheme 2). The third ligand was introduced in alcohol in the presence of nitrogen atmosphere (Scheme 3).

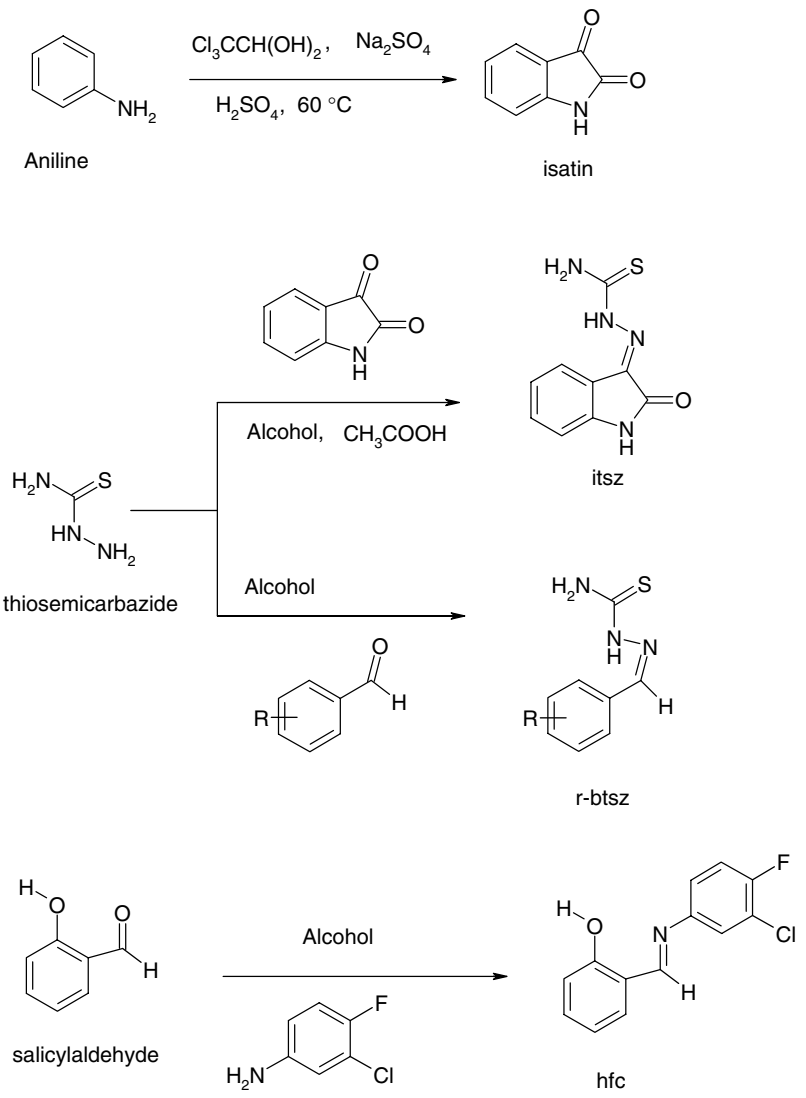
The structures of the ligands especially itsz, r-btsz, and hfc were capable of exhibiting bidentate behavior (Fig. 1). There were very few cases in which the thiosemicarbazide acts as monodentate ligand binding to the metal center through the sulfur atom.[45,46]

In case of itsz, the chelating mode was via sulfur atom and imine nitrogen atom but not with amide carbonyl oxygen. In other ligands (r-btsz), the chelating mode was via sulfur atom and imine nitrogen by coordination covalent bond. In hfc ligand the covalent bond formed between metal ion and oxygen atom of phenyl group and coordinate covalent bond with imine nitrogen.

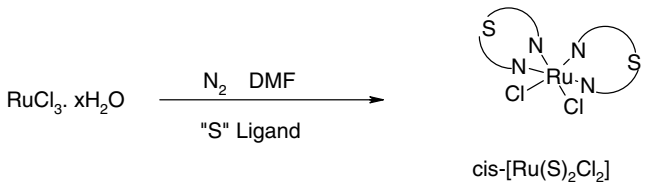
The infrared spectra of all the ligands and their ruthenium (II) compounds were recorded in KBr powder by diffuse reflectance technique and are reported in their respective titles by tentative assignments. itsz ligand showed vibrational frequency from 3422 to 3200 cm^{-1} which was assigned for NH_2 and N–H stretching and at 1682 cm^{-1} for amide carbonyl group and at 1342 cm^{-1} for C=S stretching. The r-btsz ligands showed vibrational frequency from 3400 to 3200 cm^{-1} for NH_2 and N–H stretching and from 1325 to 1320 cm^{-1} for C=S stretching. In hfc ligand the vibrational frequency for O–H stretching was observed at 3200–2520 cm^{-1} (bonded), and other minor peaks were observed at 3062 cm^{-1} for C–H stretching and 752 cm^{-1} for C–Cl.

A comparison of IR spectra of ligand itsz with ruthenium compound indicates this was coordinated to the metal center by sulfur and imine nitrogen but not with amide carbonyl oxygen, which was confirmed by the IR spectra, which indicates no change in vibrational frequency of amide carbonyl groups at 1677 cm^{-1}. In the compounds such as Ru3–Ru10, the coordination had occurred via sulfur and imine nitrogen but not with terminal amine group, which was confirmed by the spectra, which indicates no change in vibrational frequency of NH_2 group between 3400 and 3300 cm^{-1}. In the IR spectra of hfc and its compounds Ru11 and Ru12, the bond formation took place between oxygen of hydroxy group and imine nitrogen, which was confirmed by the absence of O–H vibration peak.

Coordination of ligands (K = itsz, r-btsz, and hfc) to ruthenium results in compounds such as $[Ru(S)_2(K)]^{2+}Cl_2$ (Ru1–Ru10) and $[Ru(S)_2(K)]^{+}Cl$ (Ru11–Ru12), respectively. All these compounds do not possess any C2 axes of symmetry. Such a loss of C2 axis of symmetry was seen for $[Ru(L)_2(R)]$[36–38] (where L = 2,2′-bipyridine/1,10-phenanthroline and R = acetazolamide, 7-iodo-8-hydroxy-quinoline, 4-substituted thiopicolinanalide, etc.). All compounds



Scheme 1. Preparation of ligands (itsz, r-btsz, and hfc).



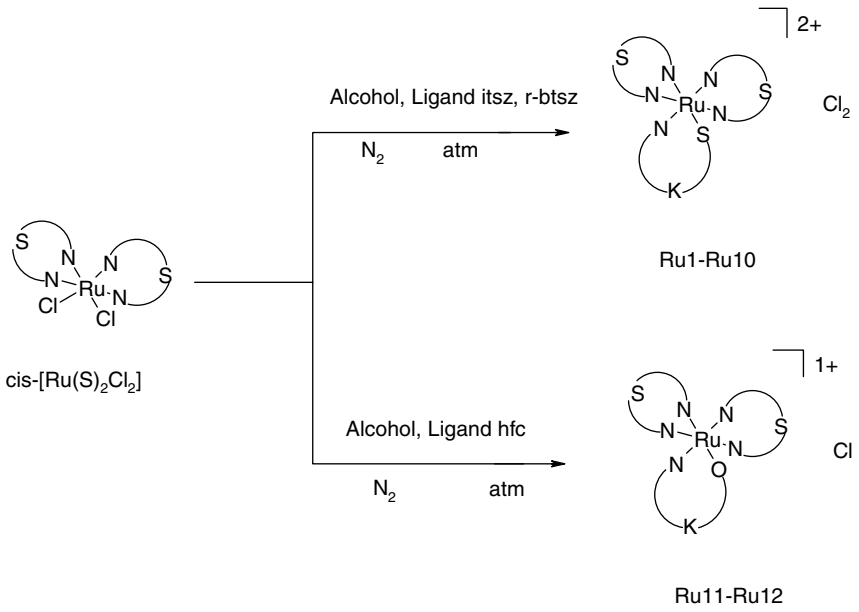
Scheme 2. Preparation of *cis*-$[Ru(S)_2Cl_2]$.

had well-resolved resonance peaks, which correspond to four different aromatic ring protons of the two 2,2′-bipyridine/1,10-phenanthroline ligands and third ligand.

These compounds showed broad and intense visible bands between 350 and 500 nm due to metal to ligand charge transfer transition (MLCT). In the UV region the bands at 290 and 310 nm were assigned to 2,2-bipyridine/1,10-phenanthroline ligand π–π^* charge transfer transitions. The same transition was found in free 2,2′-bipyridine/1,10-phenanthroline at 280 nm, so that coordination of the ligand resulted in a red shift in the transition energy. There were also two shoulders at 390 and 500 nm, which were, tentatively, attributed to metal to ligand charge transfer transitions involving 2,2′-bipyridine, 1,10-phenanthroline, and the third ligand.

The most significant *m*/*z* peaks in TOF mass spectra of the free ligands and their respective metal compounds were recorded on a Qtof Micro YA263 high resolution mass spectrometer. The MS analyses of the free ligands show the molecular peak of each compound. The ruthenium compounds' (Ru1–Ru12) mass spectra show intense peaks assigned to $[Ru(S)_2(K)]$. In all the cases, the loss of chlorine ions was detected (Fig. 2) where S = 2,2-bipyridine/1,10-phenanthroline and K = itsz, r-btsz, and hfc.

Thus, based on the above observations, it is tentatively suggested that Ru(II) compounds showed an octahedral geometry (Fig. 3).



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

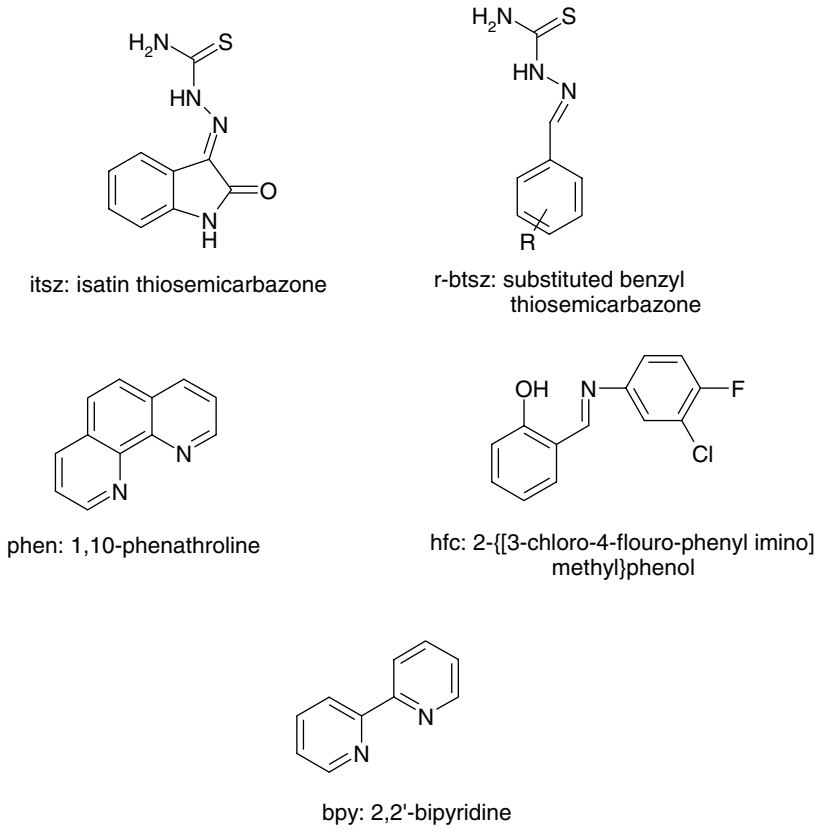


Figure 1. Structures of itsz, r-btsz, hfc, bpy, and phen ligands.

2.2. 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 compounds were tested for their anticancer activity in EAC bearing mice. Ru4 was found to increase the life span of the tumor hosts by 43%. While remaining ruthenium compounds (Ru1–Ru3 and Ru5–Ru12) were able to show the life span in the tumor host by 10–33% only. The results of the present study clearly demonstrated the tumor inhibitory activity of the ruthenium compounds against the transplantable murine tumor cell line.

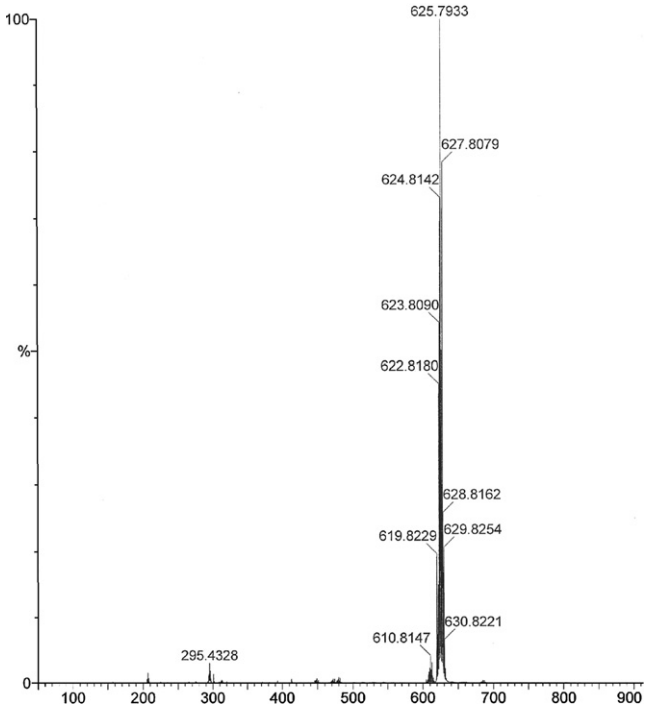


Figure 2. Mass spectrum of Ru8, that is, $[Ru(bpy_2)(2\text{-Cl-btsz})]^{2+}$ shows intense peak at 625.79 (626.5) by the loss of Cl_2.

The in vitro cytotoxic activity was evaluated for all the synthesized ligands and its ruthenium compounds against human Molt 4/C8 and CEM T-lymphocytes as well murine L1210 cells, and its results are summarized in Table 2. The relative potencies between ligands and their ruthenium compounds revealed the importance of ruthenium metal using the Molt 4/C8, CEM assays and murine L1210 assays. These determinations showed that in comparison to ligands, the ruthenium compounds were more potent. Hence, the differences in cytotoxicity between ligands and ruthenium compounds were unlikely to be exception in Ru2 against murine leukemia L1210 (248 ± 91 μM).

The cytotoxicity data in Table 2 revealed that most ruthenium compounds have significance cytotoxic potencies (IC_{50} figures in the 0.28–3.9 μM against CEM, 0.24–3.9 for Molt 4/C8, and 0.94–9.3 μM for L1210). While for ligands, the IC_{50} values were in excess (98–249 μM against CEM, 112–405 μM for Molt 4/C8, and 68–382 μM for L1210). Of the tested ligands and ruthenium compounds, Ru9 showed cytotoxicity against all three cell lines tested in range of 0.24, 0.27, and 0.94 μM for Molt 4/C8, CEM, and L1210, respectively. Whereas another compound Ru7 did show cytotoxicity against cell lines tested 0.33 μM for Molt 4/C8 and CEM and 0.95 for L1210 μM. While Ru11 least being for Molt 4/C8 was 0.38 μM and 1 μM for CEM and 1.8 μM for L1210. While remaining other ruthenium compounds showed selective cytotoxicity for Molt 4/C8 and CEM (low) μM and L1210 (higher) μM. On comparison to ruthenium compounds the ligands displayed the cytotoxicity at higher μM concentration.

3. Conclusion

In conclusion twelve ruthenium (Ru1–Ru12) compounds, bearing 2,2′-bipyridine and 1,10-phenanthroline with itsc, r-btsz, and hfc, were synthesized in alcohol in the presence of nitrogen. The coordination involved for Ru1 and Ru2 compounds is via C=S and imine nitrogen, but not with amide carbonyl functional group of itsc ligand. For ruthenium compounds (Ru3–Ru10) coordination involved is between C=S and imine nitrogen atom, but not with terminal amine group. In case of ruthenium compounds (Ru11 and Ru12), the covalent bonding occurred with oxygen of phenyl group and coordination with imine nitrogen of hfc ligand.

The results in Table 1 of the present study are encouraging as these compounds exhibit significant reduction in the tumor burden and caused prolongation of life span of the tumor hosts (10–43%).

From the results presented in Table 2, it is clear that several ruthenium compounds exhibited a marked inhibitory effect on the proliferation of tumor cells: IC_{50} from as low as 0.24 μM for Molt 4/C8, 0.27 μM for CEM, and 0.94 μM for L1210. Thus, the ruthenium compounds proved inhibitory to tumor growth at submicromolar concentration. Their ligands, however, were not antitumorally active.

4. Experimental

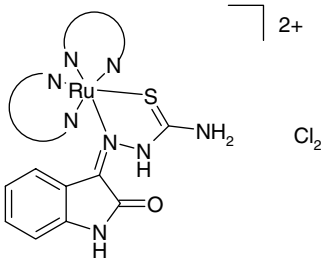
4.1. General methods

The solvents (AR grades) were obtained from Sd Fine Chem., Mumbai, and E. Merck, Mumbai. The reagents (puriss grade) were obtained from Fluka and E. Merck. Hydrated ruthenium trichloride was purchased from Loba Chemie, Mumbai, and used as received. UV–visible spectra were run on a 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 MHz/300 MHz spectrometer. The reported chemical shifts were against that of TMS. Mass spectra were recorded on a Qtof Micro YA263 high resolution mass spectrometer.

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

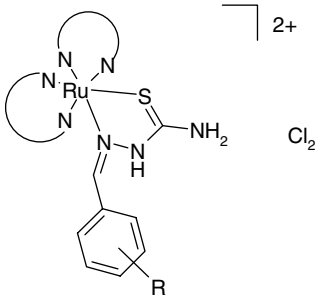
In a round bottom flask fitted with a reflux condenser substituted benzaldehyde (1 mmol), thiosemicarbazide (1 mmol) were added. The mixture was refluxed in alcohol for 3 h and left overnight. The solid that separated was filtered and dried. The crude solid was purified by recrystallization from alcohol to give crystals.

4.2.1. 4-MeO-btsz. Yield 85%. FTIR (KBr) cm^{-1}: 3407–3318 (NH_2 and NH), 3155 (C–H), 2950 (C–H), 1611 (N–H), 1328 (C=S). λ_{max} nm (MeOH): 244, 323 and 399.



Ru 1: N—N = 1,10-phenathroline

Ru 2: N—N = 2,2'-bipyridine



Ru 3: N—N = 1,10-phenanthroline
R= 4-OMe

Ru 4: N—N = 2,2'-bipyridine
R= 4-OMe

Ru 5: N—N = 1,10-phenanthroline
R= 4-Cl

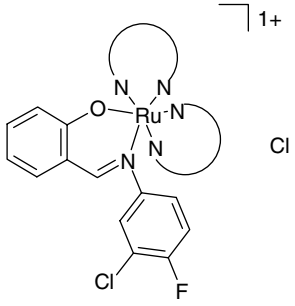
Ru 6: N—N = 2,2'bipyridine
R= 4-Cl

Ru 7: N—N = 1,10-phenanthroline
R= 2-Cl

Ru 8: N—N = 2,2'-bipyridine
R= 2-Cl

Ru 9: N—N = 1,10-phenanthroline
R= 2-F

Ru10: N—N = 2,2'bipyridine
R= 2-F



Ru 11: N—N = 1,10-phenanthroline

Ru 12: N—N = 2,2'-bipyridine

Figure 3.

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

Parameters	Total body weight (g)	Mean survival time (days)	ILS (%)	Tumor volume (ml)	Viable cells in ascitic fluid (%)
Group I	22.1 ± 0.4	—	—	—	—
Group II	28.5 ± 0.7	21	—	3.4 ± 0.3	93.5 ± 4.1
Group III	18.5 ± 0.6	22	5	—	—
Group IV	23.4 ± 1.0	25	19	1.8 ± 0.05	45.4 ± 1.6
Group V	22.9 ± 0.6	23	10	1.9 ± 0.03	48.3 ± 1.4
Group VI	23.5 ± 0.5	24	14	1.6 ± 0.01	46.5 ± 1.6
Group VII	23.3 ± 0.6	30	43	0.9 ± 0.03	34.6 ± 1.8
Group VIII	24.1 ± 0.4	28	33	1.0 ± 0.04	38.5 ± 1.6
Group IX	24.3 ± 0.9	27	29	1.2 ± 0.03	40.4 ± 1.4
Group X	23.8 ± 0.4	25	19	1.4 ± 0.04	44.9 ± 1.7
Group XI	24.9 ± 0.7	24	14	1.3 ± 0.05	46.4 ± 1.8
Group XII	24.7 ± 0.8	24	14	1.2 ± 0.02	46.5 ± 1.4
Group XIII	23.3 ± 0.6	25	19	1.3 ± 0.06	44.4 ± 1.8
Group XIV	24.3 ± 0.5	26	24	1.1 ± 0.04	42.5 ± 1.3
Group XV	25.1 ± 0.7	23	10	2.0 ± 0.06	47.8 ± 1.6

Values are means ± SEM.
Group I, vehicle (5 ml/kg); Group II, EAC (2×10^6 cells/mouse); Group III, cisplatin (2 mg/kg) + EAC; Group IV–Group XV, ruthenium compounds (2 mg/kg) + EAC.

Mp 174–175 °C (lit.,[39] 176–177 °C). Anal. Calcd for $C_9H_{11}N_3OS$: C, 51.7; H, 5.3; N, 20.1. Found: C, 52.0; H, 5.1; N, 19.9.

^{1}H NMR (DMSO-d_6): δ = 11.3 (1H, s), 8.09 (1H, s), 7.98 (1H, s), 7.89 (1H, s), 7.73 (2H, d, J = 8.7 Hz), 6.96 (2H, d, J = 8.7 Hz), 3.78 (3H, s, OCH_3).

Table 2. Cytotoxic studies of ligands and ruthenium compounds

Compound	IC_{50}[a] (μM)		
	L1210	Molt 4/C8	CEM
Itsz	382 ± 166	405 ± 134	249 ± 54
4-MeO-btsz	232 ± 75	196 ± 44	158 ± 46
Hfc	68 ± 4	112 ± 8	100 ± 18
4-Cl-btsz	284 ± 4	302 ± 35	194 ± 60
2-Cl-btsz	84 ± 30	200 ± 13	98 ± 33
2-F-btsz	242 ± 32	140 ± 125	166 ± 5
Ru-1	49 ± 4	7.4 ± 1.5	3.9 ± 0.9
Ru-2	248 ± 91	27 ± 17	48 ± 3
Ru-3	2.0 ± 0.0	0.28 ± 0.16	0.30 ± 0.20
Ru-4	9.3 ± 1.6	0.74 ± 0.05	1.7 ± 0.2
Ru-5	0.94 ± 0.09	0.64 ± 0.27	0.67 ± 0.28
Ru-6	2.5 ± 0.8	1.0 ± 0.6	0.28 ± 0.10
Ru-7	0.95 ± 0.05	0.33 ± 0.02	0.33 ± 0.02
Ru-8	8.5 ± 0.9	1.6 ± 0.3	1.6 ± 0.5
Ru-9	0.94 ± 0.06	0.24 ± 0.01	0.27 ± 0.04
Ru-10	9.3 ± 0.5	0.84 ± 0.67	1.2 ± 0.3
Ru-11	1.8 ± 0.1	0.38 ± 0.00	1.0 ± 0.7
Ru-12	44 ± 4	50 ± 18	36 ± 0

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

4.2.2. 4-Cl-btsz. Yield 71%. FTIR (KBr) cm^{-1}: 3400–3125 (NH_2 and N–H), 1600 (N–H), 1325 (C═S) and 821 (C–Cl). λ_{max} nm (MeOH): 206, 314. Mp 190–192 °C (lit.,[40] 193 °C). Anal. Calcd for $C_8H_8ClN_3S$: C, 45.0; H, 3.8; N, 19.7. Found: C, 44.7; H, 3.9; N, 19.1.

4.2.3. 2-Cl-btsz. Yield 68%. FTIR (KBr) cm^{-1}: 3401–3128 (NH_2 and N–H), 1601 (N–H), 1323 (C═S), 820 (C–Cl). λ_{max} nm (MeOH): 208, 230 and 352. Mp 183–185 °C (lit.,[41] 185 °C). Anal. Calcd for $C_8H_8ClN_3S$: C, 45.0; H, 3.8; N, 19.7. Found: C, 45.6; H, 3.2; N, 18.9.

4.2.4. 2-F-btsz. Yield 63%. FTIR (KBr) cm^{-1}: 3411–3125 (NH_2 and N–H), 1598 (N–H), 1320 (C═S). λ_{max} nm (MeOH): 210, 313. Mp 194–197 °C (lit.,[42] 190 °C). Anal. Calcd for $C_8H_8FN_3S$: C, 48.7; H, 4.1; N, 21.3. Found: C, 47.9; H, 4.5; N, 20.8. ESI MS: *m/z* 198 (100).

4.2.5. General procedure for preparing isatin thiosemicarbazone (itsz). In a round bottom flask fitted with a reflux condenser isatin (1 mmol), thiosemicarbazide (1 mmol) were added. The mixture was refluxed in alcohol for 3 h and left overnight. The solid that separated out was filtered and dried. The crude solid was purified by recrystallization twice from alcohol to give yellow crystals (68%). FTIR (KBr) cm^{-1}: 3422 (NH_2) 3238 (N–H), 3143 (C–H), 1682 (C═O), 1611 (N–H), 1342 (C═S). λ_{max} nm (MeOH): 208, 244, 270 and 358. Mp 239–240 °C (lit.,[43] 247–249 °C). Anal. Calcd for $C_9H_8N_4OS$: C, 49.1; H, 3.7; N, 25.5. Found: C, 48.5; H, 3.2; N, 25.0.

^{1}H NMR (DMSO-d_6): δ = 12.45 (1H, s, S–H), 11.18 (1H, s), 9.02 (1H, s), 8.67 (1H, s), 7.65 (1H, d, J = 7.4 Hz), 7.35 (1H, t, J = 14.7 Hz,), 7.08 (1H, t, J = 11.2 Hz), 6.91 (1H, d, J = 7.8 Hz).

4.2.6. General procedure for preparing chloro fluoro phenylimino methyl phenol (hfc). In a round bottom flask fitted with a reflux condenser salicylaldehyde (1 mmol), 3-chloro-4-fluoro-aniline (1 mmol) were added. The mixture was refluxed in alcohol 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 yellow crystals (70%). FTIR (KBr) cm^{-1}: 3200–2520 (O–H bonded) 3062 (C–H), 752 (C–Cl). λ_{max} nm (MeOH): 212, 240 and 352. Mp 128–130 C°. Anal. Calcd for $C_{13}H_9ClFNO$: C, 62.5; H, 3.6; N, 5.6. Found: C, 62.7; H, 3.2; N, 4.9.

^{1}H NMR (DMSO-d_6): δ = 12.6 (1H, s), 8.96 (1H, s), 7.75–7.73 (m, 1H), 7.66–7.64 (1H, m), 7.52–7.41 (3H, m), 7.00–6.95 (2H, m). ESI MS: *m/z* 249 (100).

4.3. Preparation of *cis*-[bis(S)dichlororuthenium(II)]*cis*-[Ru(S)$_2$Cl$_2$][44] (where S = 2,2′-bipyridine/1,10-phenanthroline)

$RuCl_3{\cdot}XH_2O$ 1.15 g (2.5 mmol) and ligand S (5 mmol) were refluxed in 50 ml DMF for 3 h under 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. The product was dried and stored in a vacuum desiccator over P_2O_5 for further use (75%).

4.4. General procedure for preparing [Ru(S)$_2$(itsz)]Cl$_2$ (where S = 1,10-phenanthroline (Ru1)/2,2′-bipyridine (Ru2); where itsz = isatin-3-thiosemicarbazone)

To the black microcrystalline *cis*-bis(S)dichloro ruthenium(II) *cis*-$Ru(S)_2Cl_2$/(2 mmol), excess of ligand itsz (2.5 mmol) was added and refluxed in ethanol under nitrogen atmosphere. The initial colored solution slowly changed to a brownish orange at the end of the reaction, which was verified by TLC on silica plates. Finally, they were purified by column chromatography using silica gel as stationary phase and chloroform–methanol as mobile phase.

4.4.1. Ru1. Yield 42%. FTIR (KBr) cm^{-1}: 3414 (NH_2), 3230 (N–H), 3045 (C–H), 1677 (C═O), 1610 (N–H) and 1325 (C═S). λ_{max} nm (MeOH): 220, 265, 399, and 472. Anal. Calcd for $RuC_{33}H_{24}N_8SOCl_2$: C, 52.7; H, 3.2; N, 14.9. Found: C, 52.9; H, 3.5; N, 14.05.

^{1}H NMR (DMSO-d_6): δ = 9.93 (1H, s), 9.64 (1H, d, J = 4.9 Hz), 9.21 (1H, d, J = 5.0 Hz), 8.80 (1H, d, J = 8.8Hz), 8.74 (1H, d, J = 8.0 Hz), 8.51–8.46 (2H, m), 8.38–8.35 (2H, m), 8.22–8.14 (4H, m), 8.11–8.09 (1H, m), 8.07–8.04 (1H, m) 7.93 (2H, s, br, NH_2), 7.84 (1H, d, J = 5.0 Hz), 7.55 (1H, d, J = 4.9 Hz), 7.44 (1H, dd, J = 8.1, 8.1 Hz), 7.36 (1H, dd, J = 8.1, 8.1 Hz), 7.06 (1H, t, J = 15.1 Hz), 6.79 (1H, t, J = 15.2 Hz), 6.49 (1H, d, J = 7.7 Hz).

^{13}C NMR (DMSO-d_6): δ = 185.6 (s), 164.3 (s), 155.2 (s), 153.1(s), 152.9 (s), 151.5 (s), 148.9 (s), 147.5 (s), 147.4 (s),

147.2 (s), 146.9 (s), 138.6 (s), 135.8 (s), 135.4 (s), 135.1 (s), 130.3 (s), 129.9 (s), 129.7 (s), 129.5 (s), 129.0 (s), 127.9 (s), 127.5 (s), 127.3 (s), 127.2 (s), 125.2 (s), 124.9 (s), 123.5 (s), 121.5 (s), 120.4 (s), 109.1 (s). MS (ESI) (35 eV) *m*/*z*%: 681 (100) for [Ru(phen)$_2$(itsz)].

4.4.2. Ru2. Yield 40%. FTIR (KBr) cm^{-1}: 3410 (NH_2), 3227 (N–H), 3040 (C–H), 1680 (C=O), 1611 (N–H) and 1324 (C=S). λ_{max} nm (MeOH): 219, 263, 398, and 470. Anal. Calcd for $RuC_{29}H_{24}N_8SOCl_2$: C, 49.4; H, 3.4; N, 15.9. Found: C, 49.7; H, 3.1; N, 15.2.

^{1}H NMR (DMSO-d_6): δ = 9.77 (1H, s), 9.41 (1H, d), 8.83 (1H, d), 8.53 (1H, d), 8.48 (1H, d), 8.38–8.36 (2H, d), 8.26 (1H, d), 8.09–7.98 (2H, tt), 7.89–7.78 (3H, m), 7.74 (1H, d), 7.63 (1H, t), 7.53 (1H, t), 7.49 (1H, d), 7.36 (2H, s, NH_2), 7.13 (1H, t), 7.08–7.05 (2H, m), 6.81 (1H, t), 6.64 (1H, d). MS (ESI) (35 eV) *m*/*z*%: 633 (100) for [Ru(bpy)$_2$(itsz)].

4.5. General procedure for preparing [Ru(S)$_2$(r-btsz)]Cl$_2$ (where S = 2,2′-bipyridine/1,10-phenanthroline and r = 4-OCH$_3$ (Ru3), (Ru4); 4-Cl (Ru5), (Ru6); 2-Cl (Ru7), (Ru8); 2-F (Ru9), (Ru10) and btsz = 1-benzyl-thiosemicarbazone)

The compounds were prepared in a similar manner to Ru1 with microcrystalline *cis*-bis(S)dichlororuthenium(II) [*cis*-Ru(S)$_2$Cl$_2$] and ligand (r-btsz), finally, they were purified by column chromatography using silica gel as stationary phase and chloroform–methanol as eluent.

4.5.1. Ru3. Yield 47%. FTIR (KBr) cm^{-1}: 3401–3313 (NH_2 and N–H), 1601 (N–H), 1305 (C=S). λ_{max} nm (MeOH): 205, 222, 265, 315, 396 and 440. Anal. Calcd for $RuC_{33}H_{27}N_7SOCl_2$: C, 53.4; H, 3.6; N, 13.2. Found: C, 53.2; H, 3.3; N, 13.0.

^{1}H NMR (DMSO-d_6): δ = 10.01 (1H, d), 8.90 (1H, d), 8.83 (2H, t), 8.65 (1H, d), 8.48 (1H, d), 8.36–8.18 (6H, m), 8.15–8.09 (2H, m), 7.92 (1H, d), 7.80–7.74 (2H, m) 7.67–7.63 (1H, m), 7.48–7045 (1H, m), 6.91 (2H, s, br, NH_2), 6.74 (2H, d), 6.14 (1H, s), 3.69 (3H, s, OCH_3). MS (ESI) (35 eV) *m*/*z*%: 670 (100) for [Ru(phen)$_2$(4-MeO-btsz)].

4.5.2. Ru4. Yield 43%. FTIR (KBr) cm^{-1}: 3418–3310 (NH_2 and N–H), 1603 (N–H), 1303 (C=S). λ_{max} nm (MeOH): 208, 241, 294, 315, 397 and 460. Anal. Calcd for $RuC_{29}H_{27}N_7SOCl_2$: C, 50.2; H, 3.9; N, 14.1. Found: C, 50.7; H, 3.5; N, 14.9.

^{1}H NMR (DMSO-d_6): δ = 10 (1H, d), 8.91 (1H, d), 8.73–8.40 (5H, m), 8.13–7.97 (2H, m), 7.83–7.52 (3H, m), 7.45–7.33 (2H, m), 7.22–7.16 (1H, m), 7.10–6.98 (2H, m), 6.93–6.71 (3H, m), 6.60 (2H, s, br, NH_2), 6.35–6.12 (2H, m), 3.73 (3H, s, OCH_3). MS (ESI) (35 eV) *m*/*z*%: 622 (100) for [Ru(bpy)$_2$(4-MeO-btsz)].

4.5.3. Ru5. Yield 40%. FTIR (KBr) cm^{-1}: 3398–3312 (NH_2 and N–H), 1601 (N–H), 1305 (C=S). λ_{max} nm (MeOH): 221, 266, 399 and 467. Anal. Calcd for $RuC_{32}H_{24}N_7SCl_2$: C, 51.5; H, 3.2; N, 13.15. Found: C, 51.7; H, 3.2; N, 14.9.

^{1}H NMR (DMSO-d_6): δ = 10.19–10.08 (2H, m), 9.41 (1H, s), 8.87–8.83 (2H, m), 8.70 (1H, s, br), 8.54–8.52 (1H, m), 8.47–8.35 (3H, m), 8.30–8.23 (4H, m), 8.01 (2H, s, br, NH_2), 7.91–7.86 (3H, m), 7.58 (1H, dd, J = 8.2, 8.1 Hz), 7.51–7.46 (2H, m), 7.23 (1H, d, J = 8.6 Hz), 6.95 (1H, d, J = 8.5 Hz). MS (ESI) (35 eV) *m*/*z*%: 674 (100) for [Ru(phen)$_2$(4-Cl-btsz)].

4.5.4. Ru6. Yield 38%. FTIR (KBr) cm^{-1}: 3395–3317 (NH_2 and N–H), 1605 (N–H), 1300 (C=S). λ_{max} nm (MeOH): 205, 244, 294 397 and 458. Anal. Calcd for $RuC_{28}H_{24}N_7SCl_3$: C, 48.2; H, 3.4; N, 14.05. Found: C, 48.3; H, 3.2; N, 14.9.

^{1}H NMR (DMSO-d_6): δ = 9.98 (1H, m), 8.82–8.78 (2H, m), 8.71 (1H, d, J = 5.6 Hz), 8.60 (1H, d, J = 8.0 Hz), 8.44 (1H, d, J = 8.0 Hz), 8.08–8.02 (3H, m), 7.78–7.72 (2H, m), 7.66–7.58 (2H, m), 7.48 (1H, d, J = 5.6 Hz), 7.32–7.23 (3H, m), 7.18–7.17 (3H, mt, J = 12.00 Hz), 6.97 (2H, d, J = 8.00 Hz) 6.22 (2H, s). MS (ESI) (35 eV) *m*/*z*%: 626 (100) for [Ru(bpy)$_2$(4-Cl-btsz)].

4.5.5. Ru7. Yield 37%. FTIR (KBr) cm^{-1}: 3392–3312 (NH_2 and N–H), 1608 (N–H), 1309 (C=S). λ_{max} nm (MeOH): 206, 223, 265, 402 and 455. Anal. Calcd for $RuC_{32}H_{24}N_7SCl_3$: C, 51.5; H, 3.2; N, 13.15. Found: C, 51.7; H, 3.2; N, 13.1.

^{1}H NMR (DMSO-d_6): δ = 9.88 (1H, d, J = 5.39 Hz), 8.79–8.73 (4H, m), 8.65–8.61 (3H, m), 8.17–8.13 (3H, m), 7.98–7.83 (5H, m), 7.78 (1H, d, J = 5.2 Hz), 7.52 (1H, d, J = 5.0 Hz), 7.39 (2H, t, J = 13.0 Hz), 7.26–7.21 (3H, m), 6.83 (1H, s).

^{13}C NMR (DMSO-d_6): δ = 181.95 (s), 154.87 (s), 153.63 (s), 152.73 (d), 148.99 (s), 148.88 (s), 148.74 (s), 148.31 (s), 139.70 (s), 134.57 (d), 134.37 (s), 134.19 (s), 133.17 (s), 130.05 (s), 129.79 (s), 128.81 (s), 128.48 (s), 126.30 (s), 125.95 (s), 125.36 (s), 125.06 (s), 62.86 (s). MS (ESI) (35 eV) *m*/*z*%: 674 (100) for [Ru(phen)$_2$(2-Cl-btsz)].

4.5.6. Ru8. Yield 40%. FTIR (KBr) cm^{-1}: 3397–3319 (NH_2 and N–H), 1611 (N–H), 1308 (C=S). λ_{max} nm (MeOH): 211, 244, 294, 340, 400 and 485. Anal. Calcd for $RuC_{28}H_{24}N_7SCl_3$: C, 48.2; H, 3.4; N, 14.05. Found: C, 48.7; H, 3.3; N, 14.9.

^{1}H NMR (DMSO-d_6): δ = 10.02 (1H, d), 8.73–8.72 (1H, d), 8.64–8.39 (5H, m), 8.10–8.04 (3H, m), 8.01–7.97 (1H, m), 7.86–7.68 (6H, tm), 7.47 (2H, d), 7.39–6.89 (5H, m). MS (ESI) (35 eV) *m*/*z*%: 626 (100) for [Ru(bpy)$_2$(2-Cl-btsz)].

4.5.7. Ru9. Yield 42%. FTIR (KBr) cm^{-1}: 3397–3311 (NH_2 and N–H), 1611 (N–H), 1308 (C=S). λ_{max} nm (MeOH): 211, 244, 294 340, 400 and 485. Anal. Calcd for $RuC_{32}H_{24}N_7SFCl_2$: C, 52.7; H, 3.3; N, 13.4. Found: C, 52.5; H, 3.1; N, 13.1. MS (ESI) (35 eV) *m*/*z*%: 659 (100) for [Ru(phen)$_2$(2-F-btsz)].

4.5.8. Ru10. Yield 38%. FTIR (KBr) cm^{-1}: 3396–3318 (NH_2 and N–H), 1609 (N–H), 1307 (C=S). λ_{max} nm (MeOH): 223, 265, 402 and 455. Anal. Calcd for $RuC_{28}H_{24}N_7SFCl_2$: C, 49.3; H, 3.5; N, 14.4. Found: C, 49.0; H, 3.4; N, 14.7. MS (ESI) (35 eV) *m*/*z*%: 611 (100) for $[Ru(bpy)_2(2\text{-F-btsz})]^{2+}$.

4.6. General procedure for preparing $[Ru(S)_2(hfc)]Cl_2$ (where S = 1,10-phenanthroline/2,2′-bipyridine and where hfc = 2-{[3-chloro-4-fluoro-phenylimino]methyl}phenol)

The complexes were prepared in a similar manner to Ru1 with microcrystalline *cis*-bis(S)dichlororuthenium(II) {*cis*-$Ru(S)_2Cl_2$} and ligand hfc.

4.6.1. Ru11. Yield 35%. FTIR (KBr) cm^{-1}: 3069 (C–H), 755 (C–Cl). λ_{max} nm (MeOH): 470, 395, 298, 244 and 210. Anal. Calcd for $RuC_{37}H_{24}N_5OFCl_2$: C, 59.60; H, 3.08; N, 9.39. Found C, 59.1; H, 3.18; N, 9.05.

^1H NMR (DMSO-d_6): δ = 9.17 (2H, m), 9.08 (1H, s), 8.75–8.71 (3H, m), 8.57–7.93 (8H, m), 7.48–7.47 (2H, m), 7.34–7.26 (4H, m), 7.20 (1H, t), 6.55-6.36 (3H, m). MS (ESI) (35 eV) *m*/*z*%: 710 (100) for $[Ru(phen)_2(hfc)]$.

4.6.2. Ru12. Yield 38%. FTIR (KBr) cm^{-1}: 3070 (C–H), 758 (C–Cl). λ_{max} nm (MeOH): 469, 398, 293, 241 and 212. Anal. Calcd for $RuC_{33}H_{24}N_5OFCl_2$: C, 56.81; H, 3.3; N, 10.04. Found C, 56.90; H, 3.2; N, 9.9.

^1H NMR (DMSO-d_6): δ = 9.15 (1H, s), 8.83–8.65 (6H, tm, J = 15.0 Hz), 8.36–8.12 (3H, m), 7.98–7.89 (2H, m), 7.84–7.60 (5H, mt, J = 12.2 Hz), 7.31–7.20 (4H, m), 7.09–7.04 (1H, m), 6.57–6.38 (2H, m). MS (ESI) (35 eV) *m*/*z*%: 662 (100) for $[Ru(bpy)_2(hfc)]$.

5. Evaluation of therapeutic effect in vivo

Albino Swiss mice (18–20 g body weight) were maintained in identical laboratory conditions and given standard food pellets (Hindustan Lever Ltd, Bombay, India) and water *ad libitum*. LD_{50} value of the synthesized compounds was determined according to the literature.[47] 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 bodyweight ip) and group II as Ehrlich Ascites Carcinoma control (EAC; 2×10^6 EAC cells/mouse ip). Group III was treated with standard drug cisplatin (2 mg/kg body weight). All the compounds were administered (ip) at a dose of 2 mg/kg body weight in groups IV–XV, respectively. All the compounds (Ru1–Ru12) 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 ascitic fluid volume and ascitic cell count parameters were noted. Mean survival time (MST) for remaining 6 mice of each group was noted.

5.1. Tumor volume and viable count

Ascites volume was noted by taking it in a graduated centrifuge tube and packed cell volume determined by centrifuging at 1000*g* for 5 min. Viability of ascitic cells 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 compounds on tumor growth was monitored by recording the mortality daily and percentage increase in life span (% ILS) was calculated by the following formula:

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

5.2. Cytotoxic evaluation

The compounds prepared in laboratory were evaluated against Molt 4/C8, CEM, and L1210 cells by a literature procedure.[48]

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