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Bioorganic & Medicinal Chemistry 13 (2005) 5766–5773
Bioorganic & Medicinal Chemistry

Synthesis and pharmacological activities of some mononuclear Ru(II) complexes

Upal K. Mazumder,[a,*] Malaya Gupta,[a] Subhas S. Karki,[b] Shiladitya Bhattacharya,[a] Suresh Rathinasamy[a] and Thangavel Sivakumar[a]

[a]*Division of Pharmaceutical Chemistry, Department of Pharmaceutical Technology, Jadavpur University, Jadavpur, Kolkata 700 032, India*
[b]*Department of Pharmaceutical Chemistry, KLES College of Pharmacy, P.O. Box 1062, 2nd Block, Rajajinagar, Bangalore 560 010, India*

Received 2 April 2005; revised 28 May 2005; accepted 28 May 2005
Available online 27 June 2005

Abstract—A series of mononuclear Ru(II) complexes of the type $[Ru(M)_2(U)]^{2+}$, where M=2,2′-bipyridine/1,10-phenanthroline and U=tpl (Ru1), 4-Cl-tpl (Ru2), 4-CH_3-tpl (Ru3), 4-CH_3O-tpl (Ru4), and 4-NO_2-tpl (Ru5), -pai (Ru6), where tpl = thiopicolinanilide and pai = 2-phenyl-azo-imidazole, have been prepared and characterized by IR, UV–Vis, ^{1}H NMR, ^{13}C-NMR, FAB-Mass spectrophotometer, and elemental analysis. The complexes display metal–ligand charge transfer (MLCT) transitions in the visible region. The title complexes were subjected to in vivo anticancer activity tests against a transplantable murine tumor cell line, Ehrlich's ascitic carcinoma (EAC) and in vitro antibacterial activity against Gram positive and Gram negative microorganisms. Ru1–Ru6 were found to increase the life span of the tumor hosts by 19–52%, and decreased tumor volume and viable ascitic cell count. The results of the present study clearly demonstrated the tumor inhibitory activity of the ruthenium chelates against transplantable murine tumor cell line. The treatment with ruthenium complexes could be secondary to tumor regression or due to the action of the compounds itself. The significant antibacterial activity was observed for Ru1–Ru4 against microorganisms like *Vibrio cholera* 865, *Staphylococcus aureus* 6571, and *Shigella flexneri* as compared to that of standard drug chloramphenical. Ru5 showed moderate activity against *S. aureus* 8530. However, all the complexes fail to show significant antibacterial activity against *V. cholera* 14033 and *Shigella sonnai*.

1. Introduction

Cisplatin is the most widely used antitumor drug, especially for the treatment of testicular and ovarian cancers.[1,2] However, cisplatin has some major drawbacks: severe toxic side effects, a limited applicability to a relatively small range of tumors, and often occurring resistance. This resistance is either developed or intrinsic.[2] The second-generation platinum drug carboplatin also has wide clinical applications. It has less toxic side effects than cisplatin.[2] In search for other antitumor active metal complexes, several ruthenium complexes have been reported to be promising as anticancer drugs.[3] There are three main properties that make ruthenium complexes well suited for medicinal applications: (i) rate of ligand exchange, (ii) the range of accessible oxidation states, and (iii) the ability of ruthenium to mimic iron in binding to certain biological molecules. There has been considerable interest in ruthenium complexes, in recent years, because of their redox stability, excited state reactivities, and excited state lifetime.[4] Owing to the octahedral structure of Ru(II) and Ru(III) complexes as opposed to the square–planar geometry of Pt(II), ruthenium antitumor complexes probably function in a manner differently than cisplatin, which appears to bind DNA by cross-linking adjacent guanine, thereby causing a class of DNA-binding proteins to adhere to the site.[5–7] Activation by reduction hypothesis, Ru(III) complexes may serve as prodrugs that are activated by reduction in vivo to coordinate more rapidly to biomolecules.[3,8,9] Because tumors rapidly utilize oxygen and other nutrients, and as the development of blood vessels often fails to keep pace with tumor growth, there is usually a lower oxygen content (hypoxia) in tumor cell.[10–14]

Keywords: Ruthenium complexes; Thiopicolinanalide; Anti-cancer; Anti-bacterial.
* Corresponding author. Tel.: +91 33 24404123; fax: +91 80 23425373; e-mail: subhasskarki@hotmail.com

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doi:10.1016/j.bmc.2005.05.047

Since then, complexes such as *cis*-$[Ru(dmso)_4Cl_2]$[15,16] (dmso = dimethyl sulfoxide), *trans*-$[(IndH)Ru(ind)_2Cl_4]$ (Ind = indazole), *mer*-$[Ru(terpy)]Cl_3$ (terpy = 2,2′-terpyridine), and $Ru(ChdH_2)Cl_2$ (Chd = 1,2-cyclohexane diamine tetraacetate) have been reported to be highly active.[17–19] Arene complexes like $[Ru(dmso)_4(arene)]Cl_2$ (where dmso = dimethyl sulfoxide and arene=C_6H_6) were shown to inhibit topoisomerase II (DNA gyrase) activity.[20] Other arene complexes, such as $X(\eta^6\text{-arene})(en)Ru(II)^+$ (where arene = C_6H_6 substituted C_6H_6, en = ethylene diamine, and X = halide), were found to inhibit the growth of the human ovarian cancer cell line A 2780.[21]

Ruthenium complexes bearing 2-hydroxy-1-naphthaldehyde thiosemicarbazone as a ligand have been studied for their antimicrobial and antifungal activities.[22] Sulu et al.[23] have evaluated the ruthenium complexes for in vitro antifungal activity with a range of μ values, between 16 and 250 μg/ml. Recently, Reedijk et al.[24] have reported water-soluble complexes of the type α-$[Ru(azpy)_2(L)]$ (azpy = 2-phenyl azo pyridine and L = 1,1-cyclobutane dicarboxylic acid, oxalic acid, and malonic acid) as cytotoxic against A 2780 human ovarian carcinoma and A 2780 *cis*-R, the corresponding cisplatin-resistant cell line. A previous investigation by our group has dealt with the antitumor and antibacterial activities of the type $[Ru(R)_2(L)]^{2+}$ (R = 1,10-phenanthroline/2,2′-bipyridine and L = 5,7-disubstituted-8-hydroxy-quinoline, picolinic acid, 3-hydroxy coumarin, *N*-methyl-isatin-3-thiosemicarbazone, acetazolamide, 4-substituted thiosemicarbazides, etc.).[25,26] Taking into account the pharmacological potential of these class of compounds, we have synthesized a novel range of Ru(II) complexes of the type $[Ru(M)_2(U)]^{2+}$ (M = 1,10-phenanthroline/2,2′-bipyridine and U = tpl, 4-Cl-tpl, 4-CH_3O-tpl, 4-CH_3-tpl, 4-NO_2-tpl, pai, where tpl = thiopicolinanilide and pai = 2-phenyl azo-imidazole) in order to evaluate their anticancer and antibacterial activities.

2. Results and discussion

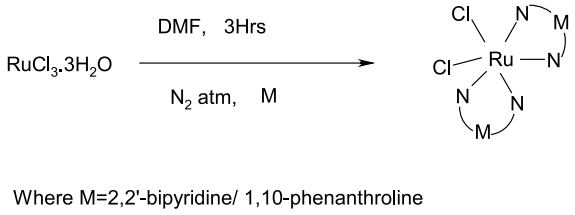
2.1. Chemistry

Results are summarized in Tables 1 and 2, and Schemes 1 and 2 show the details of the synthetic strategy adopted

Table 2. Antibacterial activity of ruthenium complexes at 20 μg/0.1 ml

Complex	V C 865	V C 14033	S A 6571	S A 8530	S F	S S
Ru 1	24	NA	17	20	14	NA
Ru 2	24	NA	16	20	15	NA
Ru 3	25	NA	14	25	15	NA
Ru 4	20	16	18	18	16	NA
Ru 5	NA	NA	NA	12	NA	NA
STD	30	20	26	26	20	19

STD = chloramphenicol 10 μg/ml. Zone inhibitions in mm (including bore size 6 mm). V C 865 = *V. cholera* 865; V C 14033 = *V. cholera* 14033; S A 6571 = *S. aureus* 6571; S A 8530 = *S. aureus* 8530; S F = *S. flexneri*; S S = *S. sonnai*. Ru1–Ru5 = ruthenium complexes.



Scheme 1. Preparation of *cis*-$Ru(phen)_2Cl_2$ and *cis*-$Ru(bpy)_2Cl_2$.

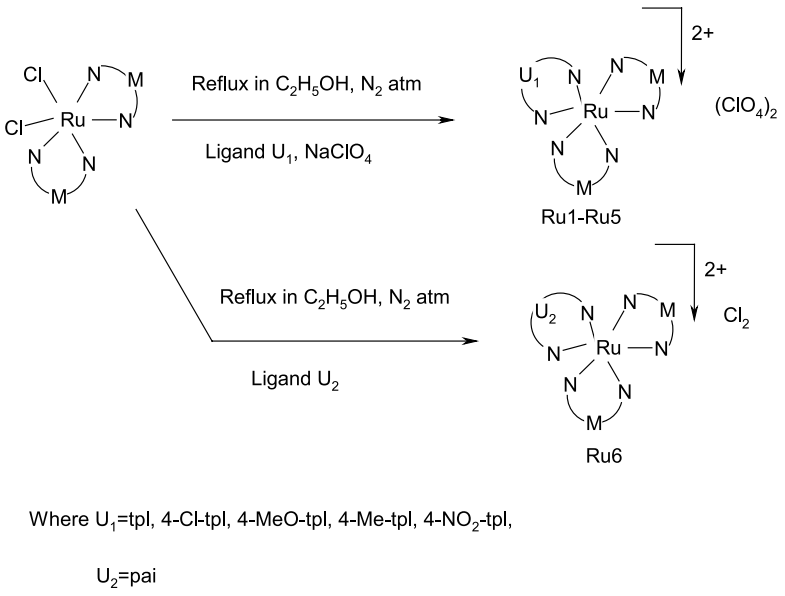
for the synthesis of these homoleptic complexes. Ruthenium trichloride undergoes reduction in a number of organic solvents. The starting material for the synthesis of the complexes was *cis*-bis(1,10-phenanthroline)dichlororuthenium(II)/*cis*-bis(2,2′-bipyridine)dichlororuthenium(II). Ruthenium trichloride 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)[10] (Scheme 1). The third ligand was introduced in the presence of alcohol (Scheme 2).

In order to obtain products of high purity, it was necessary to use column chromatography. TLC was attempted in order to determine the efficient supports, eluent compositions, and reaction endpoints. It was carried out using a solvent mixture such as $CHCl_3$–CH_3OH/CH_2Cl_2–isopropanol. The solvent mixture provided sharp spots of the main complex and one or two secondary spots, which had convenient R_f values. Column

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

Parameters	Total body weight (g)	Mean survival time (days)	ILS (%)	Tumor volume (ml)	Viable cells in ascitic fluid (%)
Group I	22.2 ± 0.5	—	—	—	—
Group II	28.3 ± 0.6	21	—	3.4 ± 0.3	95.2 ± 3.5
Group III	18.2 ± 0.7	22	5	—	—
Group IV	22.9 ± 1.2	29	38	0.9 ± 0.05	42.1 ± 1.1
Group V	22.8 ± 1.0	32	52	0.7 ± 0.04	39.2 ± 1.3
Group VI	23.1 ± 0.5	30	43	0.8 ± 0.07	40.5 ± 2.3
Group VII	23.2 ± 0.8	28	33	1.1 ± 0.04	42.9 ± 2.6
Group VIII	25.0 ± 0.9	25	19	1.8 ± 0.05	69.1 ± 2.9
Group IX	24.5 ± 0.9	26	24	1.5 ± 0.06	63.2 ± 1.8

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 VIII: ruthenium complexes (2 mg/kg) + EAC.



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

chromatography was performed using silica gel (230–400 mesh) or neutral alumina as the support and $CHCl_3$–CH_3OH/CH_2Cl_2–isopropanol as the eluate.

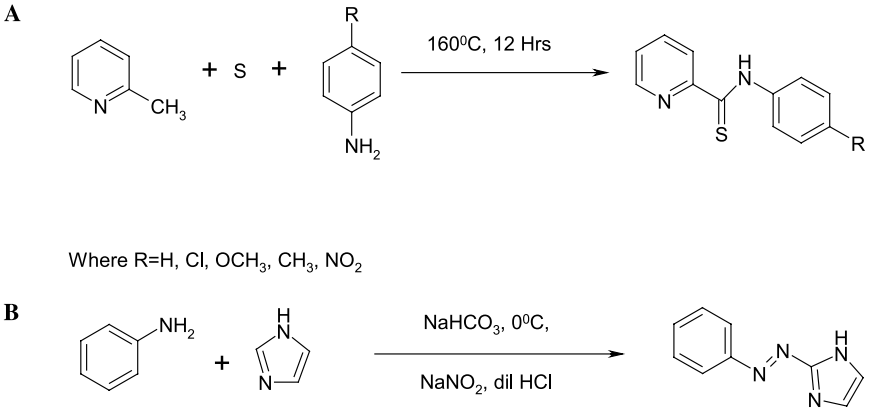
The ligands 4-R-tpl[27] (where R = H, Cl, CH_3, OCH_3, and NO_2) were prepared by heating α-picoline, sulfur, and their respective aromatic amines at 160 °C for 12 h with slight modifications (Scheme 3A). 4-Cl-tpl and 4-NO_2-tpl are new ligands, and they were confirmed for their purity by their mp, IR ^{1}H NMR, EI–MS, and elemental analyses. The other ligand pai[28] (2-phenyl azo imidazole) was prepared according to the literature with little modification (Scheme 3B). All the ligands were confirmed for their purity by their mp, FTIR, ^{1}H NMR, and elemental analyses. Structural models of these ligands are shown in Figure 1. These models are capable of exhibiting bidentate behavior.

There are bands at 3225–3186 cm^{-1} for N–H, at 3100–2900 cm^{-1} for C–H, and at 1375 cm^{-1} (C = S). In 4-Cl-tpl ligand, there are well-resolved resonance peaks at low field at 12.05 (s, br, NH) 8.54–8.53 (d, 1H, py), 8.78–8.76 (d, 1H, py), 8.06–8.04 (d, 2H, Ar) 7.90–7.86 (ddd, 1H, py), and 7.49–7.46 (dddd, 1H, py), and 7.41–7.40 (d, 2H, Ar) δ ppm.

We attempted to prepare 2-(4-carboxy)thiopicolinanilide by the same method; unfortunately; the product was unsubstituted thiopicolinanilide, which was confirmed from its FTIR, NMR, and EI–MS spectra.

In the IR spectra of the ligand pai, the band observed at 3220 cm^{-1} corresponds to N–H stretching. On complexation, there was no change in the vibration frequency of N–H. Therefore, the coordination involved was with both the ring imine nitrogens. In the IR spectra of the complexes, [Ru(phen)$_2$(R-tpl)]$^{2+}$, there were changes in the vibration mode for C = S to 1429. Therefore, the coordination of ruthenium metal with the ligand was via C = S and endocyclic pyridine ring nitrogen but not with the exocyclic N–H, because there is no change in vibration frequency of N–H.

The coordination of 4-Cl-thiopicolinanilide (4-Cl-tpl) with Ru(phen)$_2$Cl$_2$ resulted in a compound, {Ru(phen)$_2$(4-Cl-tpl)}(ClO$_4$)$_2$, that no longer showed a C_2 axis,



Scheme 3. (A) Preparation of 2-(4-substituted)-thiopicolinanilides (4-R-tpl). (B) Preparation of 2-phenyl azo imidazole (pai).

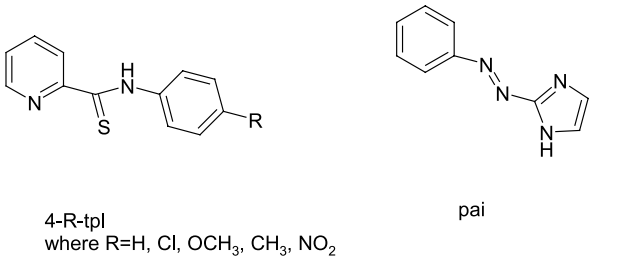
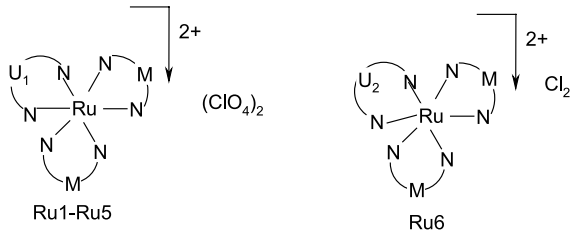


Figure 1. Structure of 4-substituted thiopicolinanalide and 2-phenyl azo imidazole.



Ru1: $[Ru(phen)_2(tpl)](ClO_4)_2$

Ru2: $[Ru(phen)_2(4\text{-}Cl\text{-}tpl)](ClO_4)_2$

Ru3: $[Ru(phen)_2(4\text{-}CH_3\text{-}tpl)](ClO_4)_2$

Ru4: $[Ru(phen)_2(4\text{-}OCH_3\text{-}tpl)](ClO_4)_2$

Ru5: $[Ru(phen)_2(4\text{-}NO_2\text{-}tpl)](ClO_4)_2$

Ru6: $[Ru(bpy)_2(pai)]Cl_2$

Figure 2. Structure of various ruthenium complexes as chlorate salts.

resulting in nonequivalent ligands. Such loss of C_2 axis resulting in nonequivalancy of ligands was observed for $[Ru(phen)_2(nmit)]Cl_2$ and $[Ru(bpy)_2(ihqs)]Cl_2$,[25,26] where phen = 1,10-phenanthroline, nmit = *N*-methyl isatin thiosemicarbazone, bpy = 2,2′-bipyridine, and ihqs = 7-iodo-8-hydroxy quinoline-5-sulfonic acid. Therefore, such NMR spectra will become more complicated. In the aromatic region, 22 well-resolved resonance peaks were obsereved: (9.85–7.10, 24H, aromatic) δ ppm. The complexes bearing R-tpl ligand did not show NH protons in their proton NMR spectra. It might be merged with the baseline, which shows that, there are six different aromatic ring protons of two 1,10-phenanthroline and one 4-Cl-tpl.

In the case of ^{13}C NMR, in aromatic region, 32 well-resolved resonance peaks were observed: (177.304–123.038) δ ppm, which also showed that all the ligands that coordinated with ruthenium were nonequivalent.

The coordination of ligand 2-phenyl azo imidazole (pai) with $Ru(bpy)_2Cl_2$ resulted in a compound, $[Ru(bpy)_2(pai)]Cl_2$, that no longer showed a C_2 axis, resulting in nonequivalent ligands. In the aromatic region, 14 well-resolved resonance peaks were observed: (8.76–6.53, 24H, aromatic) δ ppm.

These complexes showed broad and intense visible bands between 350 and 450 nm due to metal to ligand charge transfer transition.[9] In the UV region, the bands at 290 and 310 nm were assigned to the phenanthroline ligand. The same transition was found in free phenanthroline at 280 nm, so that coordination of the ligand results in a red shift in the transition energy. There were also two shoulders at 390 and 500 nm, which are tentatively attributed to a metal to ligand charge transfer transitions involving phenanthroline ligand.

The FAB-MS of the prepared complexes showed fragmentation in the following manner. The first fragment was due to $[Ru(M)_2(U)]^{2+}(ClO_4)^-$ ion pair. The complex also showed a peak due to the complex cation $[Ru(M)_2(U)]^{2+}$ and others due to $[Ru(M)(U)]^{2+}$ $[Ru(M)_2]^{2+}$. The FAB-MS thus confirms the authenticity of the complex. This type of fragmentaion was reported for $[Ru(phen)_2(nmit)]Cl_2$ and $[Ru(bpy)_2(ihqs)]Cl_2$,[25,26] where phen = 1,10-phenanthroline, nmit = *N*-methyl isatin thiosemicarbazone, bpy = 2,2′-bipyridine, and ihqs = 7-iodo-8-hydroxy quinoline-5-sulfonic acid.

Thus, based on the above observations, the proposed structures for the complexes are shown in Figure 2.

2.2. Biological activity and discussion

The Results are summarized in Tables 1 and 2. The pharmacological data were analyzed statistically by analysis of variance followed by Dunnett's test of significance. The statistical significance were considered only when $p < 0.05$ and $F > F_{critical}$. All the complexes were tested for their anti-cancer activity against EAC bearing mice. Ru1–Ru6 were found to increase the life span of the tumor hosts by 19–52%. The results of the present study clearly demonstrated the tumor inhibitory activity of the ruthenium chelates against transplantable murine tumor cell lines (Table 1). The treatment with ruthenium complexes could be secondary to tumor regression or could be due to the action of the compounds itself. In the EAC bearing mice, the cells were present in the peritoneal cavity and the compounds were administered directly into the peritoneum. Thus, tumor inhibition might be due to the direct effect of these compounds on DNA synthesis or due to some unknown effect but certain structurally related tris-chelates of ruthenium were reported to have DNA-binding property in vitro.[29] Thus, the action of these synthesized complexes could also be mediated via its effect, if any, on the DNA.

Myelosupression is a frequent and major complication of cancer chemotherapy. Cisplatin was known to cause bonemarrow depression and acute nephrotoxicity. Thus, the severe depression of the bonemarrow coupled with acute nephrotoxicity[30–33] probably caused a negative nitrogen balance in the cisplatin-treated animals, which resulted in a loss of body weight. The nephrotoxicity of cisplatin was due to the capability of the compound to generate free radicals and reactive oxygen species within the liver and kidney.[34–41]

The complexes were also evaluated for their antibacterial activity by cup–plate method.[42] The significant antibacterial activity was observed for Ru1–Ru4 against microorganisms such as *Vibrio cholera* 865, *Staphylococcus*

aureus 6571, and *Shigella flexneri* as compared to that of the standard drug chloramphenical. Ru5 showed moderate activity against *S. aureus* 8530, but no activity was found for the remaining microorganisms. However, all the complexes failed to show significant antibacterial activity against *V. cholera* 14033 and *Shigella Sonnai*.

3. Conclusions

In conclusion, five (Ru1–Ru5) complexes, bearing 1,10-phenanrhroline with 4-R-tpl (where R = H, Cl, CH_3, OCH_3, and NO_2), were synthesized; and one (Ru6) complex, bearing 2,2′-bipyridine with pai (where pai = 2-phenyl azo imidazole) was synthesized in alcohol in nitrogen atmosphere. The coordination involved for ruthenium complexes (Ru1–Ru5) is via C = S and endocyclic pyridine ring nitrogen.

The results of the present study clearly demonstrated the tumor inhibitory activity of the ruthenium complexes against transplantable murine tumor cell line, EAC. The ruthenium complex bearing 4-Cl-tpl (Ru2) showed increase in the life span of tumor host by 52% as compared to cisplatin (5%).

The study of in vitro antibacterial activity reveals the significant activity of Ru1–Ru4 against microorganisms such as *V. cholera* 865, *S. aureus* 6571, and *S. flexneri* as compared to the standard drug chloramphenicol.

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–Vis spectra were run on a Beckmann DU 64 spectrophotometer. FTIR spectra were recorded in KBr discs on a Jasco V410/Schimadzu IR spectrometer. ^{1}H NMR spectra were measured in $CDCl_3$ and d_6-DMSO on a Bruker Ultraspec 500 MHz/AMX400 MHz/300 MHz spectrometer. The reported chemical shifts were against that of TMS. FAB mass spectra were recorded on a JEOL JMS600 spectrum with mNBA matrix.

4.2. General procedure for preparing 4-substituted thiopicolinanilide[27]

A suspension of 4.7 g (0.15 g of atom) of sulfur, 5.2 ml (0.05 mol) of α-picoline, and amine (0.055 mol) were heated in an oil bath, maintained at 160 °C for 12 h; unreacted α-picoline and amine were then removed by distillation in vacuo. The residue in the still pot was then extracted by heating ethanol to remove sulfur completely. Distillation of the ethanol extract produced 2.5–3.8 g of crude thiopicolinanilides. The crude product was then separated by column chromatography on efficient supports then separately choosen for each ligand.

4.2.1. tpl. Silica gel as stationary phase, petroleum ether and benzene as mobile phase. 60%, yellow, FTIR (KBr) cm^{-1}: 3220 (N–H), 3054 (C–H), 1535 (C = C), 1375 (C = S). ^{1}H NMR ($CDCl_3$): δ 12.06 (1H, s, S–H), 8.82 (1H, d, J = 8.03 Hz), 8.57 (1H, d, J = 5.29 Hz), 8.09 (2H, d, J = 7.75 Hz), 7.92–7.87 (1H, m), 7.50–7.40 (3H, m), and 7.32–7.26 (1H, m). Mp 45 °C (46.5 °C). Anal. calcd for $C_{12}H_{10}N_2S$: C, 67.26; H, 4.70; N, 13.07. Found C, 67.02; H, 4.67; N, 12.55.

4.2.2. 4-CH_3-tpl. Silica gel as stationary phase, petroleum ether and benzene as mobile phase. 45%, orange, FTIR (KBr) cm^{-1}: 3225 (N–H), 3039 (C–H), 1540 (C = C), 1380 (C = S). Mp = 96 °C (98.5 °C). Anal. calcd for $C_{13}H_{12}N_2OS$: C, 63.91; H, 4.95; N, 11.47. Found C, 63.70; H, 5.01; N, 11.05.

4.2.3. 4-Cl-tpl. Silica gel as stationary phase, and petroleum ether as mobile phase. 58%, yellow, FTIR (KBr) cm^{-1}: 3186 (N–H), 3050 (C–H), 1531 (C = C), 1370 (C = S). ^{1}H NMR ($CDCl_3$): δ 12.06 (1H, s, S–H), 8.77 (1H, d, J = 4.80 Hz), 8.55 (1H, d, J = 2.50 Hz), 8.05 (2H, d, J = 5.26 Hz), 7.90–7.87 (1H, m), 7.49–7.47 (1H, m), and 7.41 (2H, d, J = 5.27 Hz). Mp = 73–75 °C. Anal. calcd for $C_{12}H_9Cl\,N_2S$: C, 57.95; H, 3.65; N, 11.26. Found C, 57.31; H, 3.41; N, 10.82.

4.2.4. 4-CH_3O-tpl. Silica gel as stationary phase, and benzene as mobile phase. 54%, orange, FTIR (KBr) cm^{-1}: 3200 (N–H), 3055 (C–H), 1529 (C = C), 1378 (C = S). Mp = 98.5 °C (100.5 °C). Anal. calcd for $C_{12}H_9Cl\,N_2S$: C, 57.95; H, 3.65; N, 11.26. Found C, 57.32; H, 3.26; N, 10.24.

4.2.5. 4-NO_2-tpl. Silica gel as stationary phase, petroleum ether and benzene as mobile phase. 32%, yellowish orange, FTIR (KBr) cm^{-1}: 3180 (N–H), 3029 (C–H), 1520 (C = C), 1372 (C = S). Mp = 178–184 °C. Anal. calcd for $C_{12}H_9ClN_2S$: C, 57.95; H, 3.65; N, 11.26. Found C, 57.99; H, 3.18; N, 10.34.

4.2.6. General procedure for preparing 2-phenyl azo imidazole (pai).[28] To an ice-cold solution of imidazole, sodium bicarbonate, and sodium nitrite dil. HCl was added with stirring. The orange colored azo product precipitated almost instantaneously. Stirring was continued for 3 h after all the acid had been added. The product was kept at 0 °C overnight and filtered off. It was recrystallized from hot aqueous ethanol.

65%, red, FTIR (KBr) cm^{-1}: 3220 (N–H), 3005 (C–H), 1520 (C = C). Mp = 187 °C. Anal. calcd for $C_9H_8N_4$: C, 62.78; H, 4.68; N, 32.54. Found C, 62.01; H, 4.11; N, 31.05.

4.3. General procedure for preparing *cis*-[Ru(phen)$_2$ (4-R-tpl)](ClO$_4$)$_2$

Where phen = 1,10-phenanthroline; R = H (Ru 1), Cl (Ru 2), CH_3 (Ru 3), OCH_3 (Ru 4), and NO_2 (Ru 5); and tpl = thiopicolinanilide.

To the black microcrystalline *cis*-bis(phen)dichlororuthenium(II) *cis*-$Ru(phen)_2Cl_2$/ (106 mg, 2 mmol), excess of 4-substituted thiopicolinanilide (4-R-tpl) (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. Then, the excess of ethanol was distilled off, the saturated aqueous solution of sodium perchlorate was added to the mixture, and the resultant solution was cooled at 0 °C overnight. A microcrystalline precipitate was obtained. The crystals were filtered and the solid was washed with very little amount of water and dried over $CaCl_2$ in vacuum (yield 45–50%). Finally, they were purified on column chromatography by using neutral alumina as stationary phase and dichloromethane–isopropanol as eluent.

4.3.1. Ru 1. 48%, black crystals, FTIR (KBr) cm^{-1}: 3215 (N–H), 3058 (C–H), 1533 (C = C), 1429 (C = S). λ_{max} nm (methanol): 245, 260, 355, and 490. Anal. calcd for $RuC_{36}H_{26}N_6Cl_2O_8S$: C, 49.43; H, 2.97; N, 9.61. Found C, 49.12; H, 2.15; N, 9.99.

FAB-MS *m*/*z*: 775 $[Ru(phen)_2(tpl)]^{2+}(ClO_4)^-$; 675 $[Ru(phen)_2(tpl)]^{2+}$; 495 $[Ru(phen)(tpl)]^{2+}$.

4.3.2. Ru 2. 45%, Black crystals, FTIR (KBr) cm^{-1}: 3190 (N–H), 3058 (C–H), 1523 (C = C), 1426 (C = S). λ_{max} nm (methanol) : 240, 265, 340, and 485. Anal. calcd for $RuC_{36}H_{25}N_6O_8Cl_3S$: C, 47.52; H, 2.75; N, 9.24. Found C, 47.45; H, 2.66; N, 9.41.

^1H NMR (DMSO-d_6): δ 9.85 (1H, d, J = 4.34 Hz), 8.82 (2H, dd, J = 11.47, 8.54 Hz), 8.70 (1H, d, J = 8.14 Hz), 8.50 (1H, d, J = 8.09 Hz), 8.40–8.32 (5H, m), 8.25 (1H, d, J = 8.91 Hz), 8.21 (1H, dd, J = 8.17, 5.31 Hz), 8.06 (1H, dd, J = 8.17, 5.60 Hz), 8.00 (1H, d, J = 5.11 Hz), 7.89 (1H, t, J = 14.23 Hz), 7.76 (1H, d, J = 4.49 Hz), 7.72 (1H, dd, J = 8.12, 5.23 Hz), 7.51 (1H, t, J = 11.55 Hz), 7.47 (1H, d, J = 5.35 Hz), 7.31 (2H, d, J = 8.62 Hz), 7.22 (1H, t, J = 11.67 Hz), and 7.13 (2H, d, J = 8.61 Hz).

^{13}C NMR (DMSO-d_6): δ 177.3 (s), 162.5 (s), 154.3 (s), 151.8 (s), 151.6 (s), 151.5 (s), 151.3 (s), 150.9 (s), 148.2 (s), 147.7 (s), 147.4 (s), 147.1 (s), 136.4 (s), 136.1 (s), 135.7 (s), 135.0 (s), 134.9 (s), 130.4 (s), 130.2 (s), 129.7 (s), 128.4 (s), 128.1 (s), 127.8 (d), 127.6 (s), 127.0 (s), 126.8 (s), 126.3 (s), 126.0 (s), 125.8 (s), 125.2 (s), 123.8 (s), and 123.0 (s).

FAB-MS *m*/*z*: 809 $[Ru(phen)_2(Cl\text{- }tpl)]^{2+}(ClO_4)^-$; 709 $[Ru(phen)_2(Cl\text{-}tpl)]^{2+}$; 529$[Ru(phen)(Cl\text{-}tpl)]^{2+}$.

4.3.3. Ru 3. 45%, black crystals, FTIR (KBr) cm^{-1}: 3215 (N–H), 3052 (C–H), 1539 (C = C), 1435 (C = S). λ_{max} nm (methanol): 245, 270, 360, and 485. Anal. calcd for $RuC_{37}H_{28}N_6O_9Cl_2S$: C, 50.00; H, 3.15; N, 9.46. Found C, 49.05; H, 2.89; N, 9.35.

FAB-MS *m*/*z*: 789 $[Ru(phen)_2(Me\text{-}tpl)]^{2+}(ClO_4)^-$; 689 $[Ru(phen)_2(Me\text{-}tpl)]^{2+}$; 509 $[Ru(phen)(Me\text{-}tpl)]^{2+}$.

4.3.4. Ru 4. 48%, black crystals, FTIR (KBr) cm^{-1}: 3210 (N–H), 3041 (C–H), 1532 (C = C), 1438 (C = S). λ_{max} nm (methanol): 246, 268, 365, and 490. Anal. calcd for $RuC_{37}H_{28}N_6O_9Cl_2S$: C, 49.12; H, 3.10; N, 9.29. Found C, 49.24; H, 2.89; N, 9.35.

FAB-MS *m*/*z*: 805 $[Ru(phen)_2(MeO\text{-}tpl)]^{2+}(ClO_4)^-$; 705 $[Ru(phen)_2(MeO\text{-}tpl)]^{2+}$; 525 $[Ru(phen)(MeO\text{-}tpl)]^{2+}$.

4.3.5. Ru 5. 40%, black crystals, FTIR (KBr) cm^{-1}: 3195 (N–H), 3025 (C = H), 1525 (C = C), 1425 (C = S). λ_{max} nm (methanol): 255, 275, 380, and 498. Anal. calcd for $RuC_{37}H_{25}N_7O_{10}Cl_2S$: C, 47.00; H, 2.72; N, 10.66. Found C, 46.29; H, 2.22; N, 11.01.

FAB-MS *m*/*z*: 811 $[Ru(phen)_2(NO_2\text{-}tpl)]^{2+}(ClO_4)^-$; 711 $[Ru(phen)_2(NO_2\text{-}tpl)]^{2+}$; 531 $[Ru(phen)(NO_2\text{-}tpl)]^{2+}$.

4.3.6. General procedure for preparing *cis*-$[Ru(bpy)_2(pai)]Cl_2$ Ru 6. Where bpy = 2,2′-bipyridine, pai = 2-phenyl azo imidazole.

The complex was prepared in a similar manner to Ru 1 with microcrystalline *cis*-bis(bpy)dichlororuthenium(II) {*cis*-$Ru(bpy)_2Cl_2$} and ligand (pai). Finally, they were purified on column chromatography by using silica gel as stationary phase and chloroform–methanol as eluent.

41%, black crystals, FTIR (KBr) cm^{-1}: 3205 (N–H), 1520 (C = C). Anal. calcd for $RuC_{29}H_{24}N_8Cl_2$: C, 53.05; H, 3.66; N, 17.07. Found C, 52.29; H, 3.17; N, 17.29.

^1H NMR (DMSO-d_6) δ ppm: 8.78–8.75 (2H, m), 8.57 (1H, d, J = 8.07 Hz), 8.50 (1H, d, J = 8.03 Hz), 8.29 (1H, d, J = 5.27 Hz), 8.07–7.99 (3H, tt, J = 15.25 Hz, 15.38 Hz), 7.91–7.88 (1H, t, J = 15.31 Hz), 7.72 (1H, br, NH), 7.70 (1H, d, J = 5.26 Hz), 7.60 (1H, d, J = 5.26 Hz), 7.44 (1H, d, J = 5.15 Hz), 7.41–7.34 (4H, m), 7.12 (1H, m), 7.01–6.98 (3H, m), and 6.90 (2H, d, J = 7.61 Hz).

FAB-MS *m*/*z*: 669 $[Ru(phen)_2(pai)]^{2+}Cl_2$; 633 $[Ru(phen)_2(pai)]^{2+}$; 453 $[Ru(phen)(pai)]^{2+}$.

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, Bombay, India) and water ad libitum. LD_{50} values of the synthesized complexes were obtained from the literature.[44] The animals were divided into nine groups each containing 12 mice. Group I acted as a vehicle control (5 ml/kg bodyweight, i.p.) and group II as Ehrlich ascites Carcinoma control (EAC; 2×10^6 EAC cells/mouse, i.p.). Group III was treated with the standard drug cisplatin (2 mg/kg body weight). All the complexes were administered (i.p.) at a dose of 2 mg/kg body weight in groups IV–IX. All the complexes (Ru1–Ru6) and cisplatin were administered daily for nine days starting 24 h after tumor transplantation. Six animals from each group were sacrificed 24 h after the last dose

and 18 h after fasting. The ascitic fluid volume, and ascitic cell counts were noted. Mean survival time (MST) for the remaining six mice of each group was noted.

5.1. Tumour volume and viable count

The volume of ascites was noted by taking it in a graduated centrifuge tube and the packed cell volume was determined by centrifuging at 1000*g* for 5 min, Viablity of the ascitic cells were 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 the percentage increase in life span (%ILS) was calculated by the following formula:

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

5.2. Evaluation of antibacterial activity[43]

A stock solution (2000 μg/ml) of ruthenium complexes was made in sterile water 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 cup–plate method, and the activity was expressed in terms of diameters of zone of inhibition. Innoculum was prepared by washing a fresh 5 ml medium slant of test organisms with 5 ml sterile water and further diluting the 1 ml washing to 10 ml. This suspension (0.15 ml) was added to 15 ml of melted medium at 45–50 °C and plates were prepared. A different drug concentration was applied in the 6 mm diameter holes created in the agar plate with a sterile borer. The plates were incubated at 35 °C for 24 h. The results were compared with that of standard chloramphenicol.

Acknowledgments

The authors are thankful to All India Council of Technical Education (AICTE), New Delhi, for providing the funds for carrying out research. Dr. Subhas S. Karki expresses his thanks to Dr. R. Mukharjee and Dr. V.S. Giri, Indian Institute of Chemical Biology, Kolkata, India, for their valuable help in performing spectral studies.

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