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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SOME NOVEL MONONUCLEAR RU(II) COMPLEXES

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ABSTRACT

The synthesis and characterization of ruthenium complexes (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[1]-chloro benzyl)-thiosemicarbazone, 2-F-btsz = 1-(2[1]-fluorobenzyl)-thiosemicarbazone) were prepared and characterized by elemental analysis, FTIR,[1]H-NMR and FAB-MS. The antibacterial activities of all these complexes were studied against Vibrio cholerae 865, Vibrio cholerae 14033, Staphylococcusaureus 6571. All the complexes showed antibacterial activity. Thus, the results suggest that these ruthenium complexes have significant antibacterial activity.

Keywords: Ruthenium complexes; Antibacterial; 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 the researchers[1]. Metal complexes of ruthenium containing nitrogen and oxygen donor ligands are found to be effective catalysts for oxidation, reduction, hydrolysis and other organic transformation[2]. The coordination environment around ruthenium plays the 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 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[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-$[RuCl_2(NH_3)_4]Cl$[6] preceded by the discovery that ruthenium red possesses antitumor properties made in the 1970s[7,8]. Since then compounds such as trans-$(IndH)[Ru(ind)_2Cl_4]$ (Ind = indazole), mer-$[Ru(terpy)\ Cl_3]$(ter = 2,2[1]-terpyridine),[9,10,11] $[Ru(dmso)_4Cl_2]$[12](dmso =dimethyl sulfoxide), $ImH[Ru(im)Cl_5]$,[13]$ImH[Ru(im)_2\text{-}Cl_4]$[14], and ImH [Ru (im) (dmso) Cl_4][15] NAMI-A (im =imidazole) are also well-known antitumor agents.

Although the mechanism of action of ruthenium compounds is not fully understood, it is thought that for certain species, similar to 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. It has a remarkably low general toxicity[19,20] and shows marked efficacy against metastases[21,22].

It does not affect primary tumor growth[23,24] and does not exhibit cytotoxicity against tumor cells in vitro. A related ruthenium(III) compound, indazolium trans[tetrachlorobis (1H-indazole) ruthenate (III)] KP1019[25], has also entered clinical trials, since it was found to exhibit antiproliferative activity in vitro in human colon carcinoma cell lines[26].

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. The higher specificity of these compounds for their targets may also be linked to the selective uptake by the tumor compared with healthy tissue[27,28] and because of a selective activation by reduction to cytotoxic species within the tumor[29].

The ruthenium compounds with bidentate ligands show intercalation properties with DNA[30]. The Ru(II) compounds are kinetically more reactive than Ru(III) [31]. 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[32-35].

In this work, we describe the synthesis and characterization of some ruthenium compounds, as antibacterial activity.

MATERIALS AND METHODS

Reagents

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 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 spectrometer.

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

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

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. Mp 174-175ºC (lit.,[39] 176-177ºC. Anal. Calcd for C9H11N3OS: 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)

Preparation of cis-[bis (S) dichlororuthenium (II)] cis-[Ru $(S)_2$ Cl_2][36] (where S=2,2'-bipyridne/1,10-phenanthroline)

$RuCl_3.H_2O$, 1g (2.5mmol) and Ligand S (5mmol) was refluxed in 50 ml DMF for 3h 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^{o}C. A fine

microcrystalline mass was filtered off. The residue was repeatedly washed with 30% LiCl solution and finally recrystallised 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)$_2$(itsz)Cl2] (where S=1,10-phenanthroline (Ru 1)/2,2'-bipyridne (Ru 2); where itsz= isatin-3-thiosemicarbazone)

To the black microcrystalline cis-bis(S)dichloro ruthenium(II) {cis-Ru(S)$_2$Cl$_2$}(2 mmol) excess of ligand (itsz) (2.5 mmol) was added and refluxed in ethanol under nitrogen atmosphere. The initial coloured 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 distilled off and to this solution add silica gel (60-120 mesh). The product was purified by column chromatography by using silica gel as stationary phase and chloroform-methanol as mobile phase.

Ru 1. Yield 42%. FTIR (KBr) cm^{-1}: 3414-3230 (NH_2 and NH), 3045 (C-H), 1677 (C=O), 161O (N-H), 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=7.4 Hz), 9.21 (1H, d, J=5.0 Hz), 8.80 (1H, d, J=8.8 Hz), 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, 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), 12o.4 (s), 109.1 (s). MS (ESI) (35 eV) m/z%: 681 (100) for [Ru(phen)$_2$(itsz)].

Evaluation of antibacterial activity[37]

A stock solution of ruthenium complexes were 200 mg/ml was made in sterile water containing 5% DMF under asceptic conditions and further dilutins 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 brothculture 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 activity was expressed in terms of diameters of zone of inhibition. Inoculum was prepared by washing a fresh 5ml medium slant of test organisms with 5ml sterile water and further diluting the 1 ml washing to 10 ml. This suspension was added to 15ml melted medium at temperature 45-50 ºC and plates were prepared. Holes of diameter were 6mm were drug into the agar plates with a sterile borer and filled with the drug. The plates were incubated for at 35 ºC for 24h. The results were compared with that of standard chloramphenicol.

RESULTS AND DISCUSSION

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 (2,2^1-bipyridine) dichlororuthenium(II). Ruthenium trichloride was refluxed in DMF in the presence of 1,10-phenanthroline/2,2^1-bipyridine and in excess of the stoichiometric amount. Which afforded the final product cis-bis (1,10-phenanthroline) dichlororuthenium (II)/cis-bis (2,2^1-bipyridine) dichlororuthenium (II)[38]. (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. There were very few cases in which the thiosemicarbazide acts as monodentate ligand binding to the metal center through the sulfur atom[39,40]. 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_2group 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 hydroxygroup and imine nitrogen, which was confirmed by the absence of O–H vibration peak.

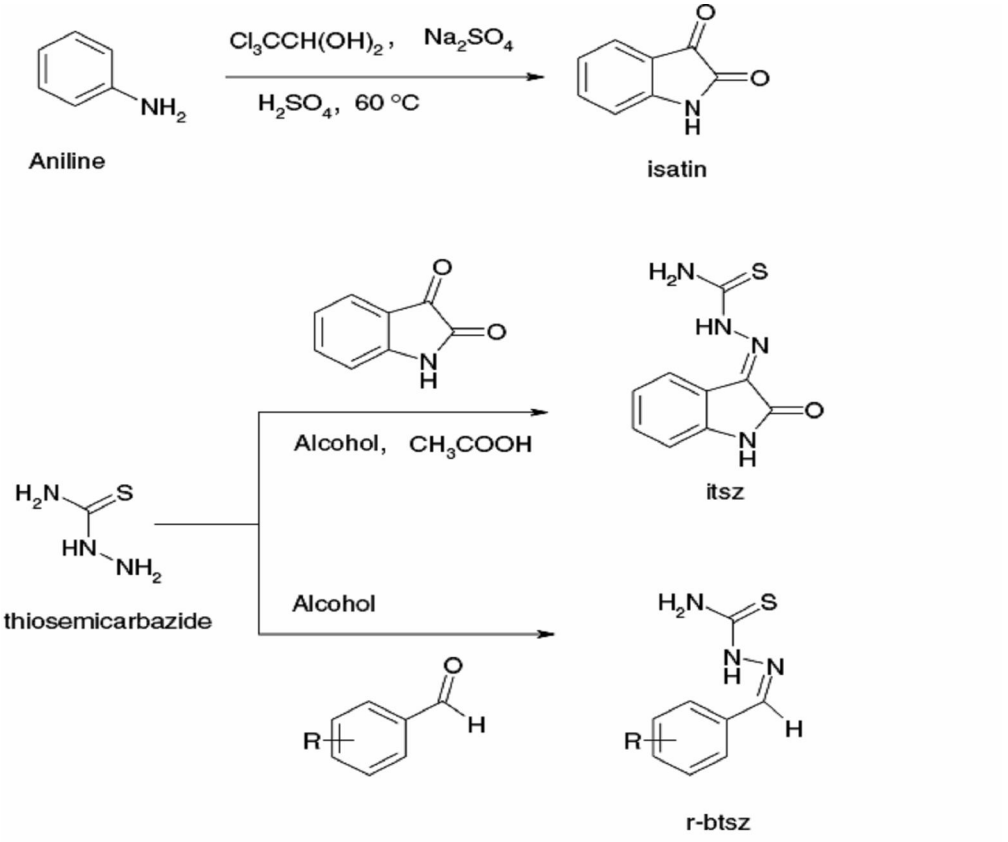
Coordination of ligands (K = itsz, r-btsz, and hfc) to ruthenium results in compounds such as $[Ru\ (S)_2\ (K)]^{2+}\ Cl_2$ (Ru1–Ru10) and $[Ru(S)_2(K)]^{+}Cl$ (Ru11–Ru12), respectively.

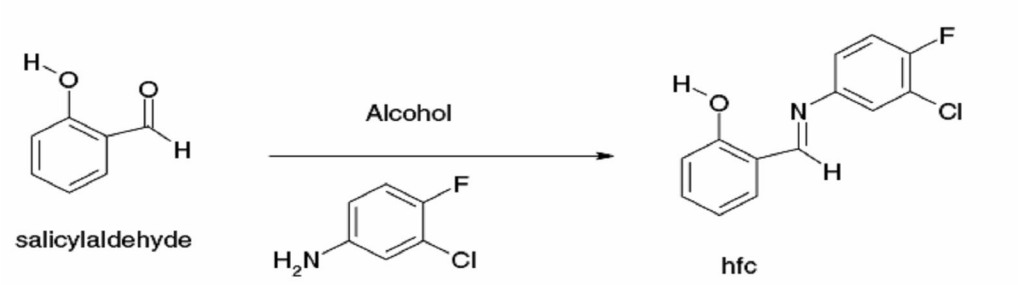
All these compounds do not possess any C2 axes of symmetry. Such a loss of C2 axis of symmetry was seen for [Ru $(L)_2$ (R)][32-35] (where L = 2,2[1]-bipyridine/1,10-phenanthroline and R = acetazolamide, 7-iodo-8-hydroxy-quinoline,4-substituted thiopicolinanalide, etc.). All compounds had well-resolved resonance peaks, which correspond to four di□erent aromatic ring protons of the two 2,2[1]-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 p–p* 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[1]-bipyridine, 1,10-phenanthroline, and the third ligand.

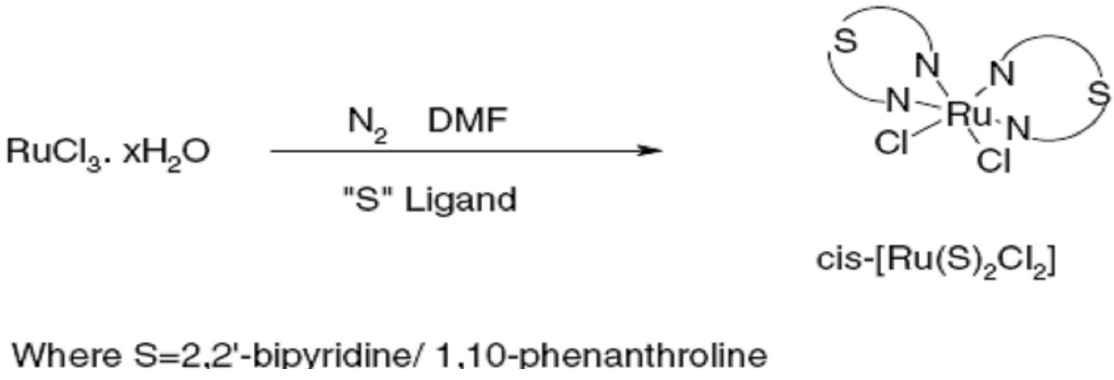
The most significant m/z peaks in TOF mass spectra of the free ligands and their respective metal compounds were recorded on a Qtof Micro YA263 high-resolution mass spectrometer. The MS analyses of the free ligands show the molecular peak of each compound. The ruthenium compounds' (Ru1–Ru12) mass spectra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

Scheme 1 : Synthesis of various ligands.

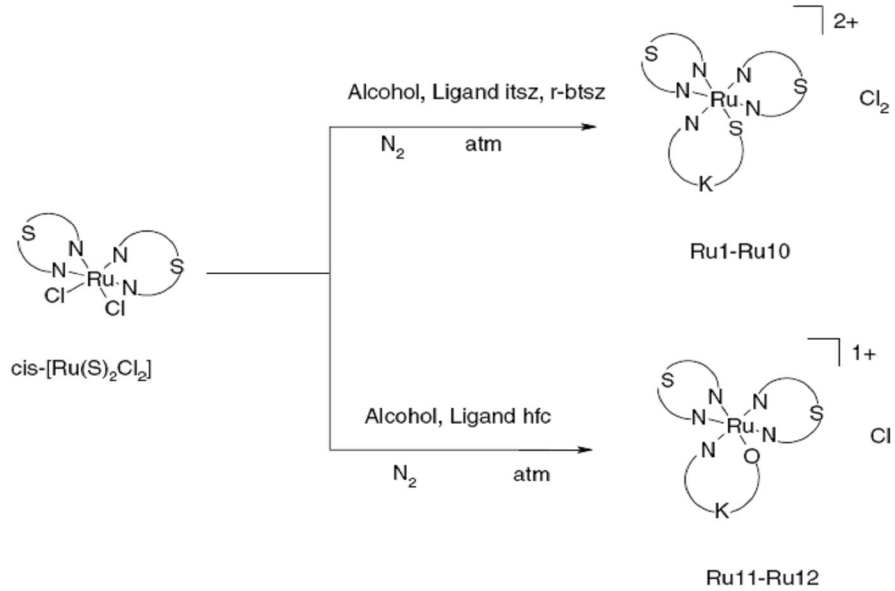




Scheme 2 : Preparation of ligands (itsz, r-btsz, and hfc).



Scheme 3 : Preparation of cis-$[Ru(S)_2Cl_2]$.



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

Antibacterial activity

Results are summarized in Table. The ruthenium complexes were evaluated for its antibacterial activity by cup plate method. Significant antibacterial activity was observed for Ru 7, Ru 8, Ru 9 aginst the microorganisms *Vibrio cholerae* 865, *Vibrio cholerae* 14033 and *Staphylococcus aureus* 6571 as compared that of chloramphenicol. A moderate activity was observed for Ru 1- Ru 6 and Ru 10- Ru 12

against the microorganisms. The enhanced antibacterial was observed for complexes with alkyl substituted thiosemicarbazides and $Ru(phen)_2Cl_2$. This increase in activity may be associated with larger ring size of phenanthroline moiety and the presence of alkyl substituents which together make the complexes more lipophillic.[32]

Table 1 : Antibacterial activity of ruthenium complexes at 200 mg/ml

Treatment	*Vibrio cholerae* 865	*Vibrio cholerae* 14033	*Staphylococcus aureus* 6571
Ru1	15±0.2	11±0.2	12±0.2
Ru2	15±0.1	13±0.2	12±0.2
Ru3	13±0.2	12±0.2	10±0.2
Ru4	12±0.2	9±0.2	8±0.2
Ru5	8±0.3	8±0.2	11±0.2
Ru6	10±0.2	9±0.2	10±0.2
Ru7	17±0.2	14±0.2	21±0.2
Ru8	21±0.1	15±0.2	22±0.2
Ru9	21±0.2	16±0.2	23±0.2
Ru10	16±0.2	12±0.2	19±0.2
Ru11	14±0.1	11±0.2	19±0.2
Ru12	15±0.1	10±0.2	18±0.2
STD	30±0.2	20±0.2	26±0.2

Values are mean± S.E.M STD=Chloramphenicol 10µg/ml. Zone of inhibition in mm
Ru1= $[Ru(phen)_2(itsz)]Cl_2$, Ru7= $[Ru(bpy)_2(itsz)]Cl_2$, Ru2= $[Ru(phen)_2(4\text{-MeO-btsz})]Cl_2$, Ru8= $[Ru(bpy)_2(4\text{-MeO-btsz})]Cl_2$, Ru3= $[Ru(phen)_2(hfc)]Cl_2$, Ru9= $[Ru(bpy)_2(hfc)]Cl_2$, Ru4= $[Ru(phen)_2(4\text{-Cl-btsz})]Cl_2$, Ru10= $[Ru(bpy)_2(4\text{-Cl-btsz})]Cl_2$, Ru5= $[Ru(phen)_2(2\text{-Cl-ptsz})]Cl_2$, Ru11= $[Ru(bpy)_2(2\text{-Cl-btsz})]Cl_2$, Ru6= $[Ru(phen)_2(2\text{-fl-ptsz})]Cl_2$
Ru12= $[Ru(bpy)_2(2\text{-fl-ptsz})]Cl_2$

Conclusion

In conclusion, twelve (Ru1–Ru12) complexes, bearing 1,10-phenanrhroline and 2,2'-bipyridine with itsz, MeO-btsz, 4-Cl-btsz, 2-Cl-btsz, 2-Fl-btsz and hfc, were synthesized 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 itsz ligand. For ruthenium compounds (Ru3–Ru10) coordination involved is between C=S and imine nitrogen atom, but not with the terminal amine group. In case of ruthenium compounds (Ru11–Ru12), the covalent bond occurred with oxygen of phenyl group and coordination with imine nitrogen of hfc ligand. The results of the present study clearly demonstrated the antibacterial activity of the ruthenium complexes against *Vibrio cholerae* 865, *Vibrio cholerae* 14033, Staphylococcus *aureus* 6571. The study of in vitro antibacterial activity reveals the significant activity of Ru1–Ru12 against microorganisms such as *Vibrio cholerae* 865, *Vibrio cholerae* 14033, *Staphylococcus aureus* 6571.

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