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SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND ANTI-MICROBIAL ACTIVITY SOME MONONUCLEAR Ru (II) COMPLEXES

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ABSTRACT

A series of monochloro-ruthenium complexes, [Ru (II)(terpy)(NN)Cl]$^+$ (NN = bidentate nitrogen ligand), containing different electron-donating groups. Surprisingly, [Ru (II)(terpy)(tmephen) Cl]$^+$ inhibited bacterial and fungal growth at less concentrations than cis-[Ru(II)(bpy)$_2$Cl$_2$]. It was suggested on the basis of these results that these ruthenium complexes modified with electron-rich groups may represent a new class of antimicrobial ruthenium drugs. The interactions of a metal complex [Ru (II)(phen)$_2$ PMIP]$^{2+}$ {phen = 1,10-phenanthroline, PMIP = 2-(4-methylphenyl) imidazo[4,5-f] 1,10-phenanthroline} with yeast transfer RNA and calf thymus DNA have been investigated.

The rapid emergence of multidrug resistant pathogenic bacteria has become a serious health threat worldwide. It has been postulated that the development of resistance to known antibiotics could be overcome by development of novel antimicrobial agents. The synthesis and characterization of ruthenium complexes of the type [Ru(M$_1$)$_2$(M$_2$)], (where M$_1$= 1,10-phenanthroline/2,2'-bipyridine and M$_2$=N-BIINH, Thiosemicarbazone ligands, Isonicotinyl hydrazone ligands and N-BIINH= N-Benzyl Isatin Isonicotinyl hydrazone were prepared and characterized by elemental analysis, FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopy. The antibacterial activities of all these complexes were studied against Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi and Bacillus subtilis. The significant antibacterial activity was observed for most of the ruthenium complexes against Escherichia coli, Staphylococcus aureus and Salmonella typhi. Some of the ruthenium complexes have shown mild to moderate activity against some fungal species.

KEY WORDS

monochloro-ruthenium complexes, Escherichia coli

INTRODUCTION

A series of monochloro-ruthenium complexes, [Ru(II)(terpy)(NN)Cl]$^+$ (NN = bidentate nitrogen ligand), containing different electron-donating groups. DNA binding and formation of Ru–DNA adducts were confirmed by gel mobility shift assay. The preferential DNA binding sites of [Ru (II) (terpy) (tmephen) Cl]$^+$ (tmephen = tetramethyl phenanthroline) were purine residues. Surprisingly, [Ru(II) (terpy) (tmephen) Cl]$^+$ inhibited bacterial cell growth (wild type *E. coli*) and fungal growth at less concentrations than *cis*-[Ru(II)(bpy)$_2$Cl$_2$]. It was suggested on the basis of these results that these ruthenium complexes modified with electron-rich groups may represent a new class of antimicrobial ruthenium drugs. The interactions of a metal complex [Ru(II)(phen)$_2$ PMIP]$^{2+}$ {phen = 1,10-phenanthroline, PMIP = 2-(4-methylphenyl) imidazo[4,5-*f*] 1,10-phenanthroline} (with yeast transfer RNA and calf thymus DNA have been investigated. Binding modes of these Ru(II) polypyridyl complex to both nucleic acids involve intercalation.

The rapid emergence of multidrug resistant pathogenic bacteria has become a serious health threat worldwide. It has been postulated that the

development of resistance to known antibiotics could be overcome by development of novel antimicrobial agents. The synthesis and characterization of ruthenium complexes of the type $[Ru(M_1)_2(M_2)]$, (where M_1= 1,10-phenanthroline/2,2'-bipyridine and M_2=N-BIINH, Thiosemicarbazone ligands, Isonicotinyl hydrazone ligands and N-BIINH= N-Benzyl Isatin Isonicotinyl hydrazone were prepared and characterized by elemental analysis, FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopy. The antibacterial activities of all these complexes were studied against *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi and Bacillus subtilis*. The significant antibacterial activity was observed for the most of the ruthenium complexes against Escherichia *coli, Staphylococcus aureus and Salmonella typhi*. Some of the ruthenium complexes have shown mild to moderate activity against some of the fungal species.

EXPERIMENTAL SECTION

Materials:

The chemicals used in the synthetic work were i.e., Hydrated ruthenium trichloride, 1,10-phenanthroline/2,2'-bipyridine, Isatin, Substituted Thiosemicarbazide, Isoniazide, Thiophene-2-aldehyde,Vertraldehyde, salicylaldehyde, 3-Acetylcoumarin, DMF, K_2CO_3, Alcohol, Chloroform & Methanol, Silica gel (60-120 mesh) were purchased from Sd Fine chemicals and Aldrich. All the solvents used were AR grades were obtained from E. Merck, Mumbai and Sd Fine chem., Mumbai. The reagents were obtained from Fluka and E. Merck, Loba Chemie. Hydrated Ruthenium Trichloride was supplied from Mumbai.

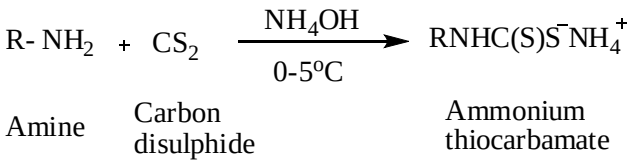
UV/Visible spectra were run on Jasco spectrophotometer. Jasco V410 FT-IR spectrometer is used for the FT-IR spectra by using KBr. ^{1}H- NMR and ^{13}C- NMR spectra and was measured in d_6-DMSO and CD_3OD on a Bruker Ultraspec 500MHz/ AMX400MHz spectrometer. The chemical shifts were reported against TMS. For recording the ES-Mass Spectra JEOL SX 102/Da-600 mass spectrometer with m-NBA matrix.

METHODOLOGY:

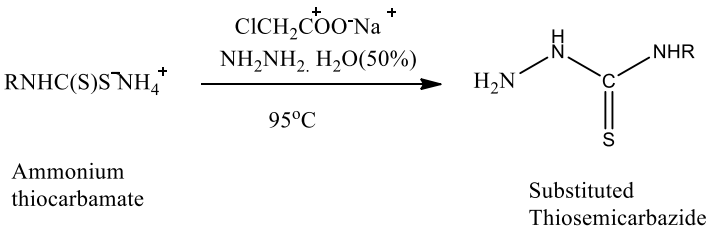
Scheme-I:

(A) Synthesis of substituted thiosemicarbazide:

1) Method of preparation of ammonium thiocarbamate from Amine



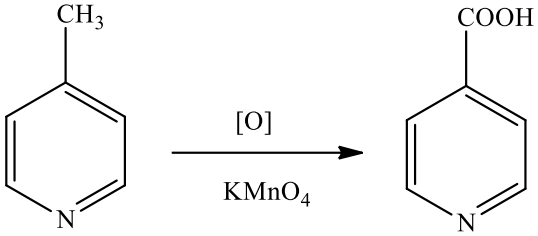
2) Preparation of substituted thiosemicarbazide from Ammonium thiocarbamate



R = H, $-C_6H_4Cl$.

(B) Synthesis of isonicotinic acid:

1) Preparation of Isonicotinic acid from δ-picoline



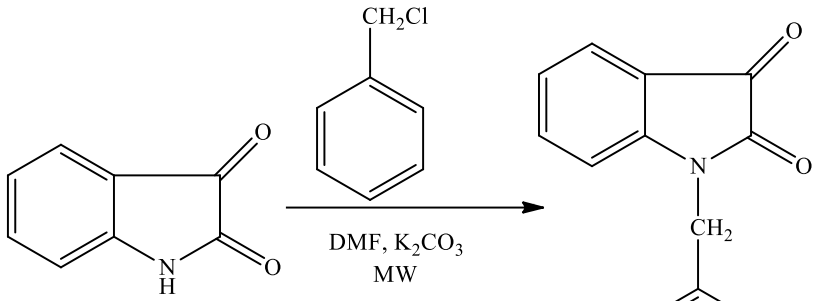
Methyl Pyridine — Isonicotinic acid

Scheme-II

I. Synthesis of various N, S and 'O' ligands:-

1. Synthesis of N-Benzyl Isatin Isonicotinyl Hydrazone:

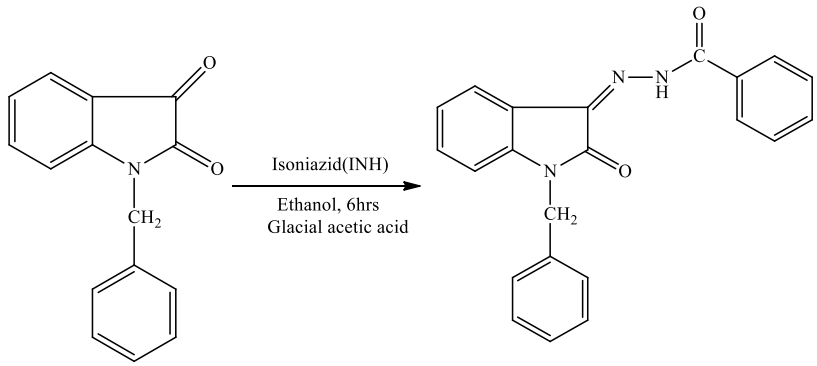
a. Preparation of N-Benzyl Isatin from Isatin:



Isatin (Indole-2,3-dione)

N-Benzyl Isatin (1-benzylindoline-2,3-dione)

b. Preparation of 1-Benzyl-3-(Isonicotinyl hydrazone)-1, 3-dihydro-indol-2-one:



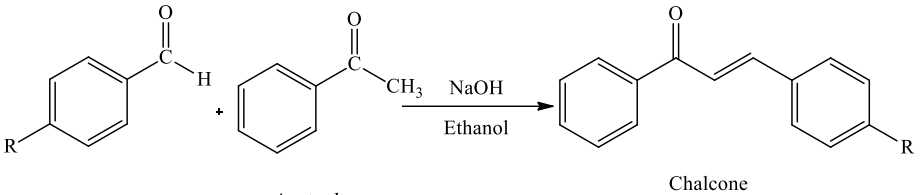
N-Benzyl Isatin (1-benzylindoline-2,3-dione)

1-Benzyl-3-(Isonicotinyl hydrazone) -1, 3-dihydro-indol-2-one

Scheme –III:

1. Synthesis of 3-phenyl-5-(1*H*-pyrrol-2-yl)-4, 5-dihydro-1*H*-pyrazole-1-Carbothioamide:

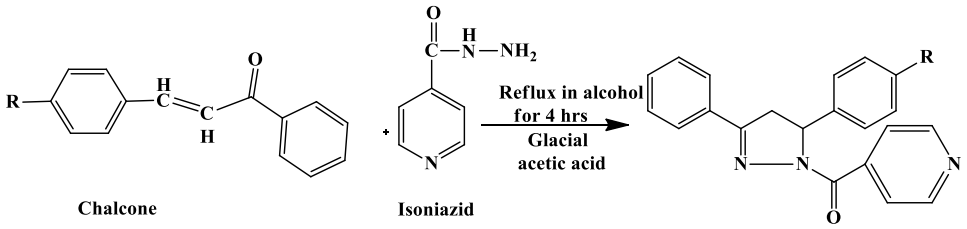
a. Preparation of Chalcones (Benzylidene acetophenone or 1, 3-diphenyl prop-2-en-1--one):



Substituted Benzaldehyde + Acetophenone → Chalcone

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

b. Synthesis of (5-(3-(dimethylamino)phenyl)-3-phenyl-4,5-dihydro-*1H*-pyrazol-1-yl)(phenyl)methanone:

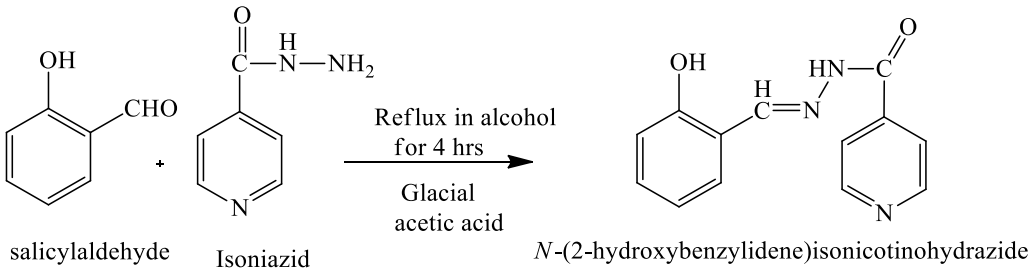


Chalcone + Isoniazid

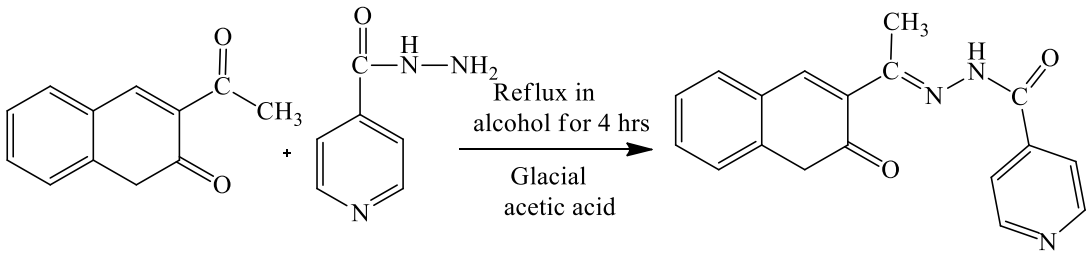
R= N-$(CH_3)_2$, 3,4-di-OCH_3

Scheme-IV:

a. Synthesis of Isonicotinyl hydrazone ligands (Inh):

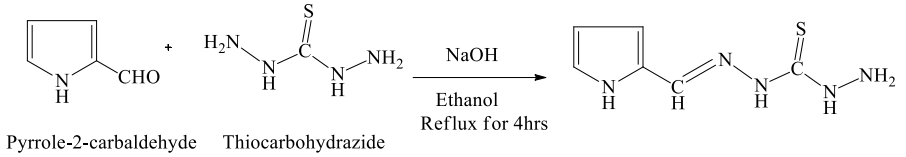


salicylaldehyde + Isoniazid → *N*-(2-hydroxybenzylidene)isonicotinohydrazide



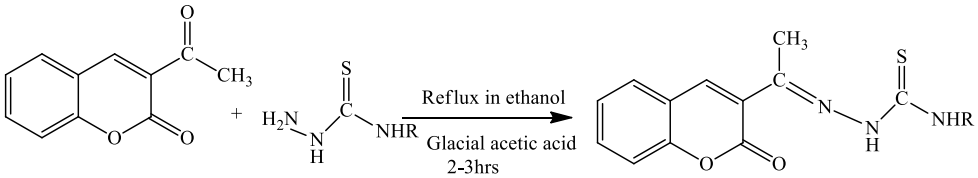
3- Acetyl coumarin + Isoniazid → *N*-(1-(3-oxo-3,4-dihydronaphthalen-2-yl)ethylidene)isonicotinohydrazide

b. Synthesis of Thiocarbohydrazide ligands:

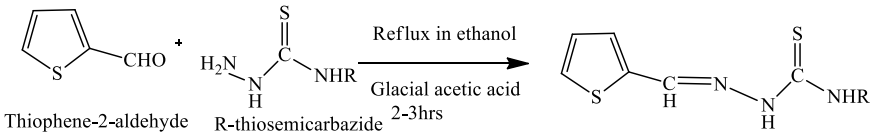


Pyrrole-2-carbaldehyde Thiocarbohydrazide 2-(Pyrrole-2-ylmethylene)hydrazinecarbo-thiohydrazide

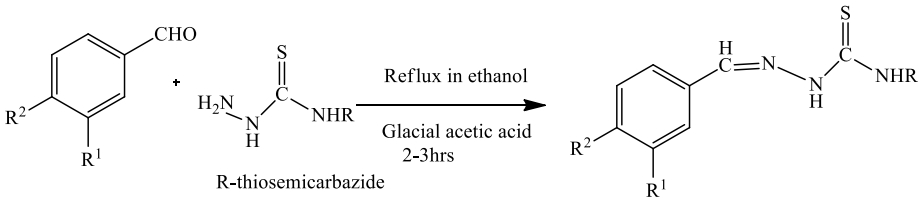
c. Synthesis of Thiosemicarbazone ligands:



3-Acetyl coumarin R-thiosemicarbazide



Thiophene-2-aldehyde R-thiosemicarbazide

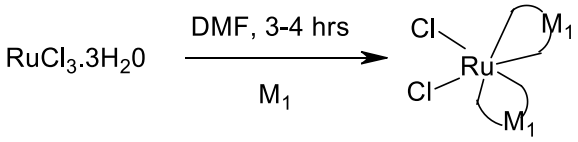


R-thiosemicarbazide

R= -C_6H_4Cl , H; R^1, R^2= -OCH_3,

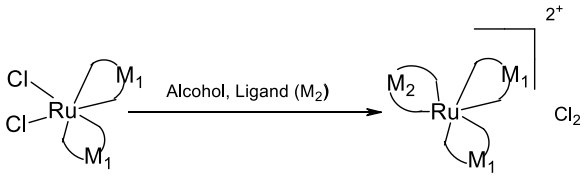
II. Synthesis of Ruthenium complexes:

Scheme-V: Preparation of *cis*-$Ru(phen)_2Cl_2$ and *cis*-$Ru(bpy)_2Cl_2$:



Where M_1= 2, 2'-bipyridine/ 1, 10-phenanthroline

Scheme-VI: Preparation of $[Ru(M_1)_2M_2]Cl_2$: Ru (II) complexes.



Where M_1= 2, 2'-bipyridine/ 1,10-phenanthroline

Ligands: M_2=N-BIINH, Thiosemicarbazone ligands, Isonicotinyl hydrazone ligands ,
Thiocarbohydrazide ligands.
N-BIINH= N-Benzyl Isatin Isonicotinyl hydrazone.

EXPERIMENTAL INVESTIGATIONS:

The experimental work comprises of:

Scheme-I:

(A) Synthesis of substituted thiosemicarbazide:

1) Method of preparation of ammonium thiocarbamate from Amine

One tenth of amine was dissolved in 20 ml of Ammonium hydroxide in a dry conical flask. To this 8 ml of carbon disulphide was slowly added while stirring mixture on ice. Temperature is maintained below 15°C until addition maintain stirring continued for further 30 min. Ethanol was added until all CS_2 went into solution. Mixture is kept at room temperature for 2 hrs. Then white ppt begin to form i.e., Ammonium thiocarbamate.

2) Preparation of substituted thiosemicarbazide from Ammonium thiocarbamate:

10 ml of 50% Hydrazine hydrate was added to 0.1 ml Sodium chloroacetate in successive while mixture is heated on waterbath until it is reduced to half its original volume. Clear solution formed is immediately filtered. The mixture is kept overnight at room temperature and thiosemicarbazide formed is crystallized out.

(B) Synthesis of isonicotinic acid from δ-picoline:

1) Preparation of Isonicotinic acid from δ-picoline:

Place a solution of 10 gm (10.5 ml, 0.0108 M) of methyl pyridine in 100ml of water in flask. Heat to 70°C on a water bath adds 45 gm (0.284 M) of $KMnO_4$ in 10 equal portions through the condenser over a period of 3-4 hours. Maintain the temperature at 70°C for first five additions and 85-90°C for last 5 sec. Make each successive addition of $KMnO_4$ only after preceding amount is decolourised and wash it down with 5ml of water. After the last addition of $KMnO_4$ is decolourised raise the temperature to 95°C. Filter the hot reaction mixture with suction and wash manganese dioxide cake on filter with 25 ml portion of hot water. Allow each portion to soak into cake without application of vacuum and finally soak dry before adding fresh water. Evaporate the combined filtrate and washings to about 150 ml and add HCl until pH is 3.6 where isonicotinic acid precipitates. Heat to 90-95°C and allow mixture to crystallize slowly. Collect the crude isonicotinic acid by filtration, wash well with water and dry at 100°C. Concentrate the mother liquor to about half the volume to obtain second component of acid. Recrystallize from hot water.

Scheme-II

I. Synthesis of various N, S and 'O' ligands:-

2. Synthesis of N-Benzyl Isatin Isonicotinyl Hydrazone:

a. Method for preparation of N-benzyl indole 2,3-dione from indole 2,3-dione (Isatin):

In the round bottomed flask take indole-2,3-dione (Isatin) 0.8 gm (0.00337 M) and equimolar quantity of benzyl chloride i.e., 6.5 ml (0.0037 M), mix with 20 ml of DMF and to this mixture add 2 gm of K_2CO_3. After gentle mixing of this reaction mixture, reflux for 2 hr, cool and pour to 100 ml of ice cold water. The resultant orange red ppt. collected wash with water and dried and recrystallized from acetonitrile.

b. Method for preparation of 1-Benzyl-3-(Isonicotinyl hydrazone)-1, 3-dihydro-indole-2-one:

To the orange coloured 1-benzyl-1,3-dihydro-indol-2,3-dione (1 gm, 0.0042 M) equimolar quantity of isoniazid (0.57 gm, 0.0042 M) and 0.50 ml of glacial acetic acid was added and refluxed in 100 ml of ethanol for two hours in water bath. The initial coloured solution slowly changes into some fluffy solid crystals in the end of reaction, which was verified by TLC plates. Excess ethanol was removed after drying, the compound purified by

combination of ethanol and chloroform (9:1) as a purification solvent.

Scheme –III:

1. Synthesis of 3-phenyl-5-(1*H*-pyrrol-2-yl)-4, 5-dihydro-1*H*-pyrazole-1-Carbothioamide:

a. Methods for preparation of Chalcones (Benzylidene acetophenone or 1, 3-diphenyl prop-2-en-1- one):

Place a solution of 22 gm of sodium hydroxide in 200 ml of water and 100 gm (122.5 ml) rectified spirit in a 500 ml of bolt head flask provided with a mechanical stirrer. Immerse the flask in a bath of a crushed ice, pour in 52 gm (0.43 M) of freshly distilled acetophenone, start the stirrer and then add 46 gm (44 ml, 0.43 M) of pure benzaldehyde. Keep the temperature of the mixture at about 25°C (limits are 15 -30°C) and stirr vigorously until the mixture is so thick that stirring is no longer effective (2-3 hours). Remove the stirrer and leave the reaction mix in an ice-chest or refrigerator overnight. Filter the product with suction on a Buchner funnel or a sintered glass funnel and wash with cold water until the washings are neutral to litmus and then with 20 ml of ice-cold rectified spirit. The crude chalcone, after drying in the air, weigh 88 gm and melts at 50-54°C. Recrystallise from rectified spirit warmed to 50°C (about 5 ml per gram).

b. Synthesis of 3-Phenyl-5-(1*H*-pyrrol-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide:

To the solution of chalcone derivatives (0.01 M) and thiosemicarbazide (0.012 M) in 25 ml of ethanol, a solution of sodium hydroxide (0.025 M) in 5 ml of water was added and refluxed for 8 hour. The products were poured into crushed ice and the solid mass which separated out was filtered dried and recrystallized from appropriate solvents.

Scheme-IV:

a. Method for preparation Thiosemicarbazone and Isonicotinyl Hydrazone ligands

Place a aromatic aldehyde or hetero cyclic aldehyde (1 mM), thiosemicarbazide or (Isoniazide) 0.09 g (1 mM), Alcohol (120 ml) in a round bottomed flask. Reflux the mixture on a water bath at 100°C for 3 h and left overnight. The crude solid that separated out was filtered and dried. The crude product was purified by recrystallisation twice from alcohol to give crystals.

II. Synthesis of Ruthenium complexes:

Scheme-V:

a. Preparation of *cis*-Ru(phen)$_2$Cl$_2$ and *cis*-Ru(bpy)$_2$Cl$_2$:

Where M$_1$=2,2'-bipyridine/ 1,10-phenanthroline.

Place a mixture of $RuCl_3.3H_2O$, 1g (2.5 mM, 1.10-phenanthroline) (5 mmol) and DMF (50 ml) in a round bottomed flask. Reflux the mixture on an oil bath at 160°C for 3 hrs. The colour of the solution, 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 recrystallised from the same. The product was dried and stored in a vacuum desiccator over P_2O_5 for further use (yield- 75%).

Scheme-VI:

A. General method for the preparation of [Ruthenium bis biphenanthroline ligand] [Ru(phen)$_2$M$_2$]Cl$_2$:

Place a mixture of black microcrystalline *cis*-bis(phen)dichloro-ruthenium(II){*cis*-Ru(phen)$_2$Cl$_2$} (106 mg, 2 mmol) ligand (M$_2$) (2.5 mmol) and ethanol (120-150 ml) in a round bottomed flask. Reflux the mixture on a water bath for 4hrs 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. Before adding the silica gel (60-120 mesh) to this solution the excess of ethanol distilled off. Finally the product was purified by column chromatography by using chloroform-methanol as mobile phase and silica gel as stationary phase.

Where phen = 1,10-phenanthroline.

Ligands: - M_2=Thiosemicarbazone ligands (P-TSZ, T-TSZ), Isonicotinyl hydrazone ligands (T-INH, P-INH), Thiocarbohydrazide ligands and Pyrazoline ligands were synthesized.

B) General Method for preparation of [Ruthenium bis bipyridyl ligand] dichloride.

$[Ru(bpy)_2M_2]Cl_2$.

Place a mixture of black microcrystalline *cis*-bis(phen)dichlororuthenium(II) {*cis*-$Ru(bpy)_2Cl_2$} (106 mg, 2 mM) ligand (M_2) (2.5 mM) and ethanol (120-150 ml) in a round bottomed flask. Reflux the mixture on a water bath for 4 hrs 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. Before adding the silica gel (60-120 mesh) to this solution the excess of ethanol distilled off. Finally the product was purified by column chromatography by using chloroform-methanol as mobile phase and silica gel as stationary phase.

Where bpy= 2, 2'-bipyridine.

Ligands: M_2=N-BIINH, Thiosemicarbazone ligands, Isonicotinyl hydrazone ligands , Thiocarbohydrazide ligands and Pyrazoline ligands.

BIOLOGICAL ACTIVITY:

ANTIBACTERIAL ACTIVITY

Stock Culture:

The hot solution of culture medium was transferred into test tubes in 10 ml portions. The tubes were plugged with cotton and sterilized at 121°C & 15 lb/inch^2 for 15 min. The tubes were cooled in a slant position and incubated at 37°C for two days. Afterwards they were observed- if the tubes were contaminated with microorganism, the tubes were rejected and the experiment was repeated until there was no contamination.

The stab culture made in three tubes and incubated at 30-34°C for 18-24 hr and stored in refrigerator. One tube was set aside as stock culture and the others were used for inoculation.

Inoculums:

A volume of 3 ml sterile water was added aseptically into stab culture, shaken for 10 sec and the liquid was decanted aseptically into another sterile test tube. The resulting cell suspension was used as inoculums.

Method of testing:

A stock solution of ruthenium complexes of 200 mg/ mL was made in sterile containing DMSO under aseptic conditions and further dilutions were made with the same solvent in a similar manner. All the dilutions and stock solutions were sterilized by membrane filtration. Solid agar and liquid broth culture media No. 1 were used for all the test organisms and the pH was adjusted to 7.2. Antimicrobial activity of the ruthenium complexes against different strains of bacteria was determined by the cup–plate method and activity was expressed in terms of diameters in mm zones of inhibition. Inoculum was prepared by washing a fresh 5mL medium slant of test organism with 5mL sterile water and further diluting the 1mL washing to 10 mL. The suspension was added to 15mL melted medium at a temperature 45–50^{o}C and plates were prepared. Holes were dug into the agar plates with a sterile borer and filled with the drug. The plates were incubated at 35^{o}C for 24 h. The results were compared with that of standard streptomycin.

Table-1: Antibacterial activity of Ru(II) complexes

Complexes	E.coli	K.pneumonia	S.aureus	B.subtilis	S.typhi
S_1	5(34.8)	NA	6(40)	4(32)	NA
S_2	6.5(41.9)	4(28.5)	8(53.33)	NA	NA
S_3	4(25.80)	6(42.85)	14(93.33)	7(56)	NA
S_4	7.2(46.45)	11(78.57)	7(46.6)	8(64)	6.2(42.7)
S_5	5(32.25)	NA	4(26.6)	6.5(52)	13(89.6)
S_6	NA	NA	12(80)	11(88)	8(55.11)
S_7	12(77.41)	5(35.7)	6.8(43.33)	10.8(85)	6(41.37)
S_8	15(96.1)	7(50)	14(95)	NA	10.3(71.03)
Standard	15.5(100)	14(100)	15(100)	12.5(100)	14.5(100)

In parenthesis values were % inhibition.

Standard= Stretomycin

NA= No Activity

ZONE OF INHIBITION IN MM (INCLUDING BORE SIZE 6 MM) GRAPH

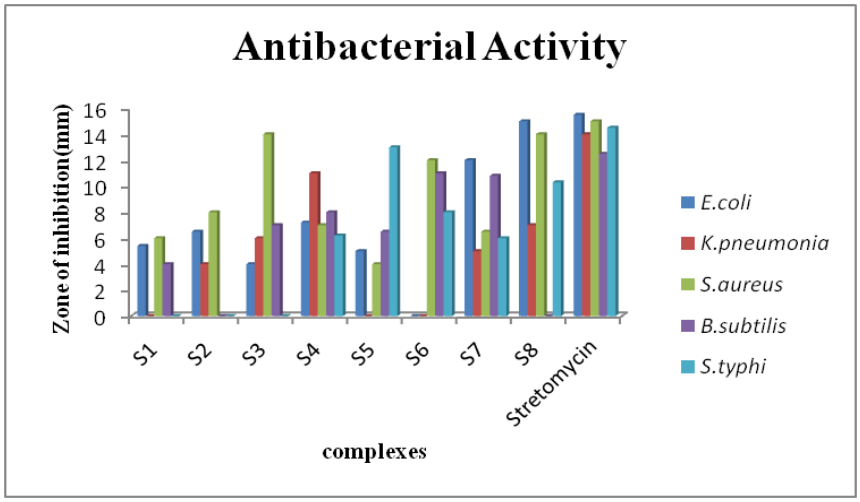


Figure-1: Graphical Representation of Antibacterial Activity

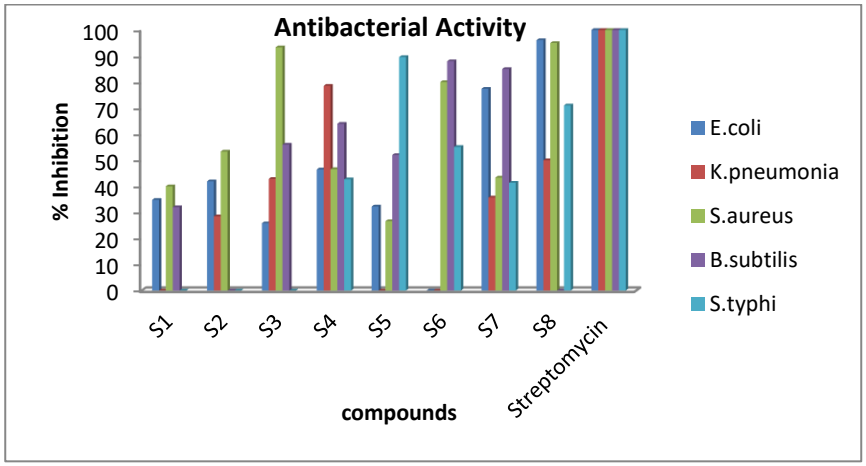


Figure-2: Graphical Representation of Antibacterial Activity (% inhibition)

ANTIFUNGAL ACTIVITY:

Method of testing:

All the compounds have been screened for antifungal activity using cup-plate agar diffusion method by measuring the inhibition zone in mm. Gresiofulvin (50 µg/mL) was used as a standard drug for antifungal activity. The compounds were screened for antifungal activity against *Aspergillus niger, Aspergilus clavatus, Pencillium notatum* and *Collitricum* in nutrient agar medium. These sterilized agar media were cooled and added with bacterial suspension in individual portions and poured into Petri-dishes and allowed to solidify. A stainless steel cylinder of 6 mm diameter (presterilized) was used to bore cavities. All synthesized compounds were placed in serially in the cavities with the help of micropipette and allowed to diffuse for 1.0 hr. DMSO was used as a solvent for all the compounds, and as a control. These plates were incubated at 28°C for 48 hr, for antifungal activity. The zone of inhibition observed around the cups after respective incubation was measured.

Table-2: Antifungal Activity for Ru(II) complexes

complexes	*A.clavatus*	*A.niger*	*C.coffeanum*	*P.notatum*
S_1	NA	NA	NA	NA
S_2	4(22.22)	9(45)	13(50)	NA
S_3	NA	14(70)	NA	14(63.63)
S_4	9(50)	NA	5(19.2)	NA
S_5	NA	NA	22(84.6)	11(50)
S_6	4(22.22)	7(35)	7(26.9)	NA
S_7	8(44.44)	NA	11(42.3)	NA
S_8	12(66.66)	18(90)	24(92.3)	18(81.81)
Griseofulvin	18(100)	20(100)	26(100)	22(100)

Zone of inhibition in mm (Including bore size 5mm)

In parenthesis values of %inhibition

NA= No Activity

ZONE OF INHIBITION IN MM (INCLUDING BORE SIZE 6 MM) GRAPH

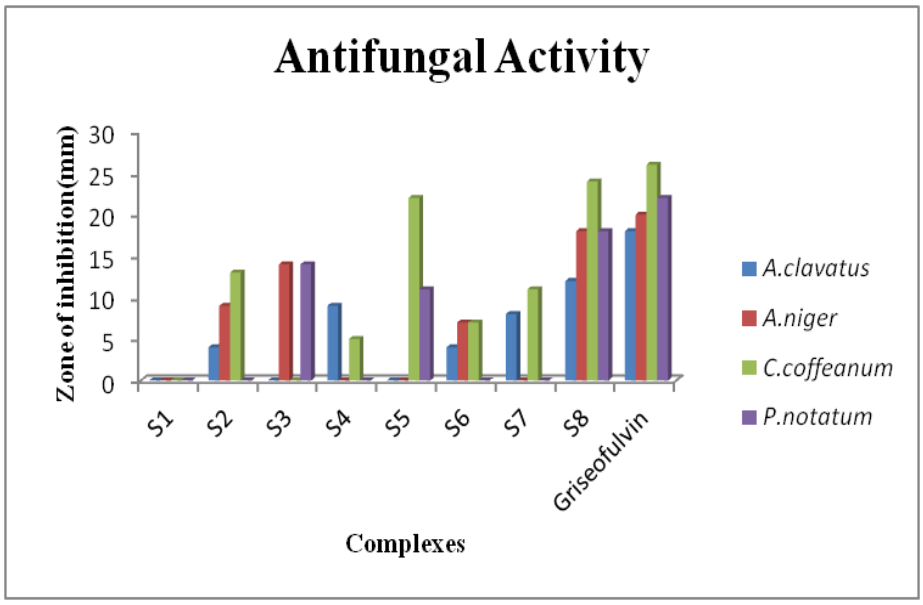


Figure 3: Graphical representation of zone of inhibition of Antifungal Activity

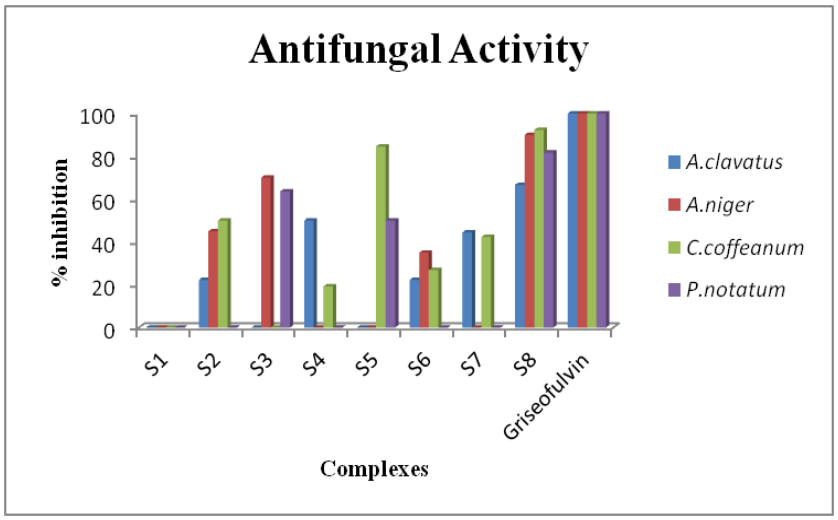


Figure 4: Graphical Representation of Antifungal Activity (% Inhibition)

RESULTS AND DISCUSSION

Chemistry:

Results are summarized in tables and schemes I to VI show the details of the synthetic strategy adopted for the synthesis of ligands and homoleptic Ruthenium compounds. In a number of organic solvents ruthenium trichloride undergoes a reduction. The compound dichlorotetrakis (dimethyl sufoxide) ruthenium(II) is a versatile starting material for a number of organic synthesis. The ease of incorporating the ligands into the aforesaid products lies in the fact that the behavior of the complexing DMSO molecules is predictable. In the final product the four DMSO molecules are not bonded in the same way to the central metal atom. Out of the four, three are bonded via the oxygen atom, when dichlorotetrakis (dimethyl sufoxide) ruthenium (II) refluxed in chloroform two of the DMSO molecules were displaced to make way for the incoming ligand. Further when this product was treated with DMF the remaining two DMSO molecules leaves to facilitate second bidentate ligand to enter the complex. This stepwise assembly of ligands was one of the principal reasons behind the use of this scheme for synthesis. The starting material for the synthesis of complexes was *cis-bis*(2,2'-bipyridine)dichloro-ruthenium(II)/*cis*-bis(1,10-phenanthroline) dichlororuthenium(II).

In the preparation of homoleptic chelate the first two ligands to enter the complex in a stepwise assembly were 2,2'-bipyridine /1,10-phenanthroline molecule respectively. Since both the ligands are similar, so a single step method was used for its synthesis. Ruthenium trichloride was refluxed in DMF in the presence of 1, 10-phenanthroline/2,2'-bipyridine, in excess of the stoichiometric amount, which gives the final product final product *cis*-bis (2,2'-bipyridine) dichlororuthenium (II) / *cis*-bis (1, 10-phenanthroline)dichlororuthenium(II) (Scheme-VI). In the presence of alcohol the third ligand was introduced. The final chelate formed had ionic chloride in the molecule.

In order to obtain pure products, it was necessary to use column chromatography. TLC was carried out in order to determine efficient supports, eluent compositions and reaction end points. Mobile phase used for the column chromatography is $CHCl_3$-CH_3OH solvent mixture. The solvent mixture provided sharp spots of main complex and one or two secondary spots. Which had convenient R_f values. Column chromatography was performed by using silica gel (60-120 mesh)/neutral alumina as

stationary phase and $CHCl_3$-CH_3OH as mobile phase.

The Ligands like N-BIINH, Thiophene- Cl-TSZ and Pyrazoline ligands were prepared by refluxing N-benzyl isatin with isoniazid , Thiophene-2-carbaldehyde with substituted thiosemicarbazide in alcohol, pyrrole-2-carbaldehyde with thiosemicarbazide and Other ligands like N-diMe-INH, 3,4,-di-OMe-TCH, were prepared by heating N-diMethyl benzaldehyde chalcone with thiocarbohydrazide and in alcohol. All these ligands were confirmed for their purity by their melting point and are uncorrected, TLC, FT-IR spectra, ^{1}H-NMR, ^{13}C-NMR and Mass spectra.

SPECTRAL ANALYSIS:

In Salicylaldehyde-PCT ligand, IR bands at 3584 for OH, 3328 for NH, 3018 for CH-Ar, 1328 for C=S.

In ^{1}H-NMR there are 14 well-resolved resonance peaks at (9.58-5.64, 14 Ar-H), δ ppm respectively.

In the IR spectra of the complexes, $[Ru(bpy)_2$ (Salicylaldehyde- Cl-TSZ)$]^{2+}$ $(Cl_2)^-$ there are bands at 3579 for OH, 3339 for NH, 3074 for CH-Ar, 1360 for C=S and 751 for C-Cl. The coordination of Salicylaldehyde- Cl-TSZ to $Ru(bpy)_2Cl_2$ result in a compound $[Ru(bpy)_2$ (Salicylaldehyde- Cl-TSZ)$]^{2+}$ $(Cl_2)^-$ that no longer has a C_2 axis, resulting in non equivalent ligands. Such a loss of C2 axis and resulting to non equivalency of ligands has been observed for Ru $(M_1)_2(M_2)$, where M_1=2,2'-bipyridyl, M_2=Salicylaldehyde- Cl-TSZ. Therefore such ^{1}H-NMR spectra will be more complicated. In the aromatic region showed 28 well resolved resonance peaks (10-3.4, 28 H) δ ppm respectively.

In case of ^{13}C-NMR, in aromatic region showed 34 well resonance peaks (162.16-64.4) δ ppm, which also shows that the all ligands coordinated to ruthenium are nonequivalent which confirm the authenticity of complex. The Mass Spectra of the complex $[Ru(bpy)_2$ (Salicylaldehyde- Cl-TSZ)$]^{2+}$ $(Cl_2)^-$ showed the base peak at 789.

In Thiophene-PCT ligand, IR bands at 3346 for NH, 3066 for CH-Ar, 1374 for C=S and 754 for C-Cl. In ^{1}H-NMR there are 10 well-resolved resonance peak at (9.98-6.76), δ ppm respectively. Mass Spectra of Thiophene- Cl-TSZ showed intense peak at 296 (M+1).

In the IR spectra of the complexes, $[Ru(bpy)_2$(Thiophene-2- Cl-TSZ$]^{2+}(Cl_2)^-$ there are bands at 3328 for NH, 3052 for CH-Ar, 1366 for C=S, 761 for C-Cl. In case of ^{1}H-NMR spectra in the aromatic region showed 24 well resolved resonance peaks (10.10-4.39) δ ppm respectively.

In case of ^{13}C-NMR, in aromatic region showed 32 well resonance peaks (183.8-108.22) δ ppm, which also shows that the all ligands coordinated to ruthenium are nonequivalent which confirmed the authenticity of complex.

The Mass Spectra of the complex $Ru(bpy)_2$(Thiophene-2- Cl-TSZ$]^{2+}(Cl_2)$ showed the base peak at 742 due to the $[Ru(bpy)_2$(Thiophene-2- Cl-TSZ$]^{2+}(Cl_2)^-$ ion pair. From the above spectral data this confirmed the authenticity of complex.

In (N-diCH_3- chalcone-INH) ligand, IR bands at 3030 for CH-Ar, 2927 for CH-Ar and 1679 for C=O. In ^{1}H-NMR there are 22 well-resolved resonance peak at (8.66-2.26), δ ppm respectively. Mass Spectra of (N-diCH_3- chalcone-INH) showed intense peak at 371(M+1).

In the IR spectra of the complexes, $[Ru(bpy)_2$(N-diCH_3-chalcone-INH$]^{2+}(Cl_2)^-$ there are bands at 3018 for CH-Ar, 2988 for CH-Ar and 1682 for C=O. In case of ^{1}HNMR spectra in the aromatic region showed 38 well resolved resonance peaks (9.96-2.28), 20 H) δ ppm respectively.

In case of ^{13}C-NMR, in aromatic region shows 43 well resonance peaks (169.28-36.28) δ ppm, which also shows that the all ligands coordinated to ruthenium are nonequivalent which confirmed the authenticity of complex.

The Mass Spectra of the complex $[Ru(bpy)_2$(N-diCH_3-chalcone-INH$]^{2+}(Cl_2)$ showed the base peak at 854 due to the $[Ru(bpy)_2$(N-diCH_3-chalcone-

INH]$^{2+}$(Cl$_2$)$^-$ ion pair. From the above spectral data confirmed the authenticity of complex.

In (3,4-di-OCH$_3$-chalcone-TCH) ligand, IR bands at 3482 for NH$_2$, 3246 for NH, 3042 for CH-Ar and 1334 for C=S. In ^{1}H-NMR there are 20 well-resolved resonance peak at (9.24-3.78), δ ppm respectively. Mass Spectra of (3,4-di-OCH$_3$- chalcone-TCH) showed intense peak at 357(M+1).

In the IR spectra of the complexes, [Ru(phen)$_2$(3,4-di-OCH$_3$-chalcone-TCH)]$^{2+}$(Cl$_2$)$^-$ there are bands at 3464 for NH$_2$, 3345 for NH, 3028 for CH-Ar and 1328 for C=S. In case of ^{1}HNMR spectra in the aromatic region shows 36 well resolved resonance peaks (9.81-3.78), 36 H δ ppm respectively.

In case of ^{13}C-NMR spectra in the aromatic region showed 42 well resolved resonance peaks (182.64-42.88) δ ppm respectively. The Mass Spectra of the complex [Ru(phen)$_2$(3,4-di-OCH$_3$-chalcone-TCH)]$^{2+}$(Cl$_2$)$^-$ showed the base peak at 888. From the above spectral data confirmed the authenticity of complex.

In (Pyrrole-2-TCH) ligand, IR bands at 3452 for NH$_2$, 3244 for NH, 2982, 1643 for NH, 1328 for C=S. In ^{1}H-NMR there are 9 well-resolved resonance peak at (9.84-7.18), δppm respectively. Mass Spectra of (Pyrrole-2-TCH) showed intense peak at 184 (M+1).

In the IR spectra of the complexes, [Ru(Phen)$_2$(Pyrrole-2-TCH)]$^{2+}$(Cl$_2$)$^-$ there are bands at 3468 for NH$_2$, 3272 for NH, 3014, 1628 for NH and 1324 for C=S. In case of ^{1}H-NMR spectra in the aromatic region showed 25 well resolved resonance peaks 25H, (10.06-7.06), δ ppm respectively.

In case of ^{13}C-NMR spectra in the aromatic region shows 30 well resolved resonance peaks (184.44-112.38), δ ppm respectively. The Mass Spectra of the complex [Ru(Phen)$_2$(Pyrrole-2-TCH)]$^{2+}$(Cl$_2$) showed the base peak at 715 due to the [Ru(Phen)$_2$(Pyrrole-2-TCH)]$^{2+}$(Cl$_2$) ion pair. From the above spectral data confirmed the authenticity of complex.

In (N-diCH$_3$-chalcone-TSZ) ligand, IR bands at 3414 for NH$_2$, 3270 for NH, 2992 for CH-Ar and 1368 for C=S. In ^{1}H-NMR there are 20 well-resolved resonance peaks at (7.98-2.5) δ ppm respectively. Mass Spectra of (N-diCH$_3$-chalcone-TSZ) showed intense peak at 325 (M+1).

In the IR spectra of the complexes, [Ru(bpy)$_2$(N-diCH$_3$-TSZ]$^{2+}$(Cl$_2$)$^-$ there are bands at 3448 for NH$_2$, 3256 for NH, 3008 for CH-Ar and 1342 for C=S. In case of ^{1}H-NMR spectra in the aromatic region showed 36 well resolved resonance peaks (9.04-2.32, 36H) δ ppm respectively.

In case of ^{13}C-NMR, in aromatic region showed 38 well resonance peaks (178.42-39.84) δ ppm, which also showed that the all ligands coordinated to ruthenium are nonequivalent which confirmed the authenticity of complex.

The Mass Spectra of the complex [Ru(bpy)$_2$(N-di-CH$_3$-TSZ)$^{2+}$]Cl^{2-} showed the base peak at 808 due to the [Ru(bpy)$_2$(N-di-CH$_3$-TSZ)$^{2+}$]Cl^{2-} ion pair. From the above spectral data confirmed the authenticity of complex.

In (3,4-diOCH$_3$- Cl-TSZ) ligand, IR bands at 3288 for NH, 3042 for CH-Ar, 1378 for C=S and 745 for C-Cl. In ^{1}H-NMR there are 16 well-resolved resonance peaks at (9.64-3.78) δ ppm respectively. Mass Spectra of (3,4-diOCH$_3$- Cl-TSZ) showed intense peak at 350 (M+1).

In the IR spectra of the complexes, [Ru(bpy)$_2$(3,4-diOCH$_3$- Cl-TSZ]$^{2+}$(Cl$_2$) there are bands at 3324 for NH, 3064 for CH-Ar, 1328 for C=S and 761 for C-Cl. In case of ^{1}H-NMR spectra in the aromatic region showed 32 well resolved resonance peaks (10.12-3.78) δ ppm respectively.

In case of ^{13}C-NMR, in aromatic region showed 36 well resonance peaks (183.42-57.2) δ ppm, which also shows that the all ligands coordinated to ruthenium are nonequivalent which confirmed the authenticity of complex.

The Mass Spectra of the complex [Ru(bpy)$_2$(3,4-diOCH$_3$- Cl-TSZ]$^{2+}$(Cl$_2$)$^-$ shows the base peak at 834 due to the [Ru(bpy)$_2$(3,4-diOCH$_3$- Cl-TSZ]$^{2+}$(Cl$_2$)$^-$ ion

pair. From the above spectral data confirmed the authenticity of complex.

In (N-BIINH) ligand, IR bands at 3288 for NH, 3042 for CH-Ar, 1378 for C=S and 745 for C-Cl. In ^{1}H-NMR there are 16 well-resolved resonance peaks at (9.64-3.78) δ ppm respectively. Mass Spectra of (3,4-diOCH_3-PCT) showed intense peak at 350 (M+1).

In the IR spectra of the complexes, $[Ru(bpy)_2$(N-BIINH)$]^{2+}(Cl_2)^-$ there are bands at 3224 for NH, 3016 for CH-Ar and 1679 for C=O. In case of ^{1}H-NMR spectra in the aromatic region shows 32 well resolved resonance peaks (10.14-4.82) δ ppm respectively.

In case of ^{13}C-NMR, in aromatic region showed 41 well resonance peaks (164.8-48.4) δ ppm, which also shows that the all ligands coordinated to ruthenium are nonequivalent which confirmed the authenticity of complex.

The Mass Spectra of the complex $[Ru(bpy)_2$(N-BIINH)$]^{2+}(Cl_2)^-$ shows the base peak at 840 due to the $[Ru(bpy)_2$(N-BIINH)$]^{2+}(Cl_2)^-$ ion pair. From the above spectral data confirmed confirms the authenticity of complex.

In (3-Acetyl Coumarin-Tsc) ligand, IR bands at 3428 for NH_2, 2998 for CH-Ar, 1681 for C=O and 1328 for C=S. In ^{1}H-NMR there are 11 well-resolved resonance peaks at (9.82-2.34) δ ppm respectively. Mass Spectra of (3-Acetyl Coumarin-Tsc) shows intense peak at 262 (M+1).

In the IR spectra of the complexes, $[Ru(bpy)_2$(3-Acetyl Coumarin-Tsc)$]^{2+}(Cl_2)^-$ there are bands at 3408 for NH_2, 2985 for CH-Ar, 1678 for C=O and 1334 for C=S. In case of ^{1}H-NMR spectra in the aromatic region shows 27 well resolved resonance peaks (10.22-2.28) δ ppm respectively.

The Mass Spectra of the complex $[Ru(bpy)_2$(3-Acetyl Coumarin-Tsc)$]^{2+}(Cl_2)^-$ showed the base peak at 745 due to the $[Ru(bpy)_2$(3-Acetyl Coumarin-Tsc)$]^{2+}(Cl_2)^-$ ion pair. From the above spectral data confirmed the authenticity of complex.

In (3-Acetyl Coumarin-INH) ligand, IR bands at 3262 for NH, 3044-2976 for CH-Ar and 1682 for C=O. In ^{1}H-NMR there are 13 well-resolved resonance peaks at (9.64-2.26) δ ppm respectively. Mass Spectra of (3-Acetyl Coumarin-Tsc) showed intense peak at 308.

In the IR spectra of the complexes, $[Ru(bpy)_2$(3-Acetyl Coumarin-INH)$]^{2+}(Cl_2)^-$ there are bands at 3342 for NH, 3015 for CH-Ar and 1676 for C=O. In case of ^{1}H-NMR spectra in the aromatic region showed 29 well resolved resonance peaks (9.98-2.34) δ ppm respectively.

The Mass Spectra of the complex $[Ru(bpy)_2$(3-Acetyl Coumarin-Tsc)$]^{2+}(Cl_2)^-$ showed the base peak at 791 due to the $[Ru(bpy)_2$(3-Acetyl Coumarin-Tsc)$]^{2+}(Cl_2)^-$ ion pair. From the above spectral data confirmed the authenticity of complex.

BIOLOGICAL ACTIVITY AND DISCUSSION:

***In-Vitro* antibacterial activity:**

The antibacterial activities of all these complexes were studied against *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi* and *Bacillus subtilis*. The significant antibacterial activity was observed for the most of the ruthenium complexes against *Escherichia coli, Staphylococcus aureus and Salmonella typhi*. Some of the ruthenium complexes have shown mild to moderate activity against Bacillus subtilis and Klebsiella pneumonia. The compound S_8 $[Ru(bpy)_2$(N-BIINH)$]^{2+}$(Cl2)$^-$ showed significant antibacterial activity. The results were summarized in **table-1**

Antifungal activity:

The complexes were also evaluated for its antifungal activity by cup- plate method.

Results are summarized in **table-2** respectively, 8 complexes were tested on various strains of fungal spores like *Penicillin notatum, Aspergillus clavatus, A.nigrum, Colliobacterium*.

Significant antifungal activity was observed S_8, against all fungal spores likes *A.teavatus,*

Colibacterium, A.nigrum and *P. notatum*. The complex S_5 showing potent antifungal activity against *Colibacterium*. The complexes names are $[Ru(bpy)_2(N\text{-}BIINH)]^{2+}(Cl_2)^-$ and $[Ru(Phen)_2(Pyrrole\text{-}2\text{-}TCH)]^{2+}(Cl_2)^-$ as compared to that of the standard drug Greseofulvin. However S_1 complex failed to show significant antifungal activity against all fungal spores likes *A.clavatus, Colibacterium, A.nigrum* and *P. notatum*.

The results of the antibacterial and antifungal screening clearly demonstrate the antibacterial and antifungal activity of the ruthenium complexes against the different microorganisms and fungal spores.

CONCLUSION

- This work describes the necessary to synthesize Ru(II) complexes.
- In this study, a detailed method of synthesis of various N, S, O bearing ligands and Ru(II) complexes along with their purification, physical constants have been given. All the compounds were synthesized in good yields and high purity. The complexes were characterized by subjecting to various special studies such as FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectra has been compiled.
- Finally *In vitro* antibacterial and antifungal activities of the synthesized Ru(II) complexes have been studied.

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