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Ru (III) AZO SCHIFF BASE COMPLEXES: SYNTHESIS, SPECTRAL CHARACTERIZATION, ANTIMICROBIAL AND ANTICANCER STUDIES

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ABSTRACT: The complexes of Ru(III) having general formula $[Ru(L)Cl(PPh_3)$ where L= azo Schiff base derived from 2-aminopyridine, 2-hydroxy naphthaldehyde /vanillin and ethylene diamine have been synthesized. The ligands as well as complexes have been characterized by elemental analysis, molar conductance, magnetic susceptibility, infra-red and electronic spectral data. The azo Schiff base ligand acts as a tetradentate ligand co-ordinating to Ru through azomethine nitrogen and phenolic oxygen atoms. An octahedral geometry has been proposed for the complexes based on electronic spectra and magnetic susceptibility studies. Low molar conductance values revealed the non-electrolytic nature of the complexes. The complexes have been screened for antimicrobial and anticancer activities.

INTRODUCTION: There is a much current interest in the chemistry of ruthenium[1] most of which is due to the fascinating electron transfer and energy transfer properties displayed by complexes of metal. Octahedral ruthenium (III) complexes are the object of great attention in the field of medicinal inorganic chemistry owing to the favorable pharmacological properties of potential antitumor activities manifested by some member of this family of metallodrugs. Transition metal phosphine/arsine complexes of ruthenium show a wide range of applications in catalytic processes such as hydrogenation, isomerisation, decarboxylation, reductive elimination, oxidative addition and in C-C coupling reactions [2].

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Nowadays, metal complexes play a vital role in cancer diagnosis and therapy. The field of metal-based anticancer agents began with the discovery of cisplatin and since then many classes of compounds have been studied including coordination complexes [3] and organometallic compounds. Although cisplatin was used as the anticancer drug, it has several side effects, nowadays it is replaced by ruthenium complexes. In view of the fascinating properties of ruthenium, attempt was made to synthesize azo Schiff base Ru(III) complexes and to evaluate their antimicrobial and anti cancer activities.

MATERIALS AND METHODS:

Chemicals used:

2-hydroxy naphthaldehyde, 2-aminopyridine, vanillin, o-phenylenediamine, ethylene diamine, ethanol and petroleum ether were purchased from commercial sources and used as such without further purification.

Experimental:

Synthesis of Ru(III) complexes- it involves three steps:

Synthesis of diazo compound:

A mixture of 2-aminopyridine (0.1M), water (10 ml) and conc.HCl (0.03M) was stirred until a clear solution was obtained. The mixture was cooled to 0-5ºC and a solution of sodium nitrite (0.76g) in water (5ml) was then added drop wise, maintaining the temperature below 5^{o}C. The resulting mixture was stirred for an additional 1 hour in an ice bath and then a little urea was added and was buffered at pH 6-7 with solid sodium acetate. 2-hydroxy naphthaldehyde/vanillin (0.1M) in 8ml aqueous NaOH solution was cooled to 0-5^{o}C in an ice bath. This solution was then gradually added to the solution of pyridine diazonium chloride and the resulting mixture was continuously stirred at 0-5^{o}C for 2 hours. The resulting crude precipitate was filtered by acidification and washed several times with cold water, dried and recrystallized from ethanol.

Synthesis of azo Schiff base ligands:

An ethanolic solution of o-phenylenediamine (0.1M) was added to a mixture containing an ethanolic solution of the azo compounds (0.2M) and 5 drops of glacial acetic acid. The reaction mixture was heated on a water bath at (40-50^{o}C) for 16 hours in presence of K_2CO_3 after the addition of excess of ethanol (50 ml). A brown solid was formed, filtered, washed with water and then recrystallized from methanol.

Synthesis of Ru(III) Schiff base complexes:

An ethanolic solution of $RuCl_3$ PPh_3 (0.1M in 15ml ethanol) was added drop wise to an ethanolic solution of the Schiff base ligands (0.1M in 20ml ethanol). The resulting mixture was refluxed for 6 hours. The dark brown solid obtained on cooling was filtered, dried, and recrystallised in DMF.

Characterization Techniques:

The percentage of C, H & N of the synthesized Schiff bases and the metal complexes were performed by using Elementer Vario EL III at STIC, CUSAT, Cochin. Conductance of the complexes was measured using the model number EQ-660A conductivity bridge digital conductivity meter using DMF as solvent. The magnetic moment of the complexes has been calculated by determining the magnetic susceptibility using Gouy balance at room temperature. Copper sulphate was used as calibrant.IR spectra of the Schiff bases and their complexes were recorded in the range 4000 to 400cm^{-1} on a Shimadzu FTIR-IR Affinity1 spectrophotometer.

The electronic absorption spectra of the Schiff base ligands and complexes were recorded on Lab India 3000^{+}double beam spectrophotometer (cell length, 1cm) in the 200–800nm range. The thermo grams were recorded in dynamic nitrogen atmosphere with a heating rate of 10^{o}C using a Perkin Elmer (TGS-2 model) thermal analyzer. Antimicrobial studies and anticancer studies were performed for the metal complexes.

Molecular Modelling:

The possible geometries of metal complexes were evaluated using the molecular calculations with Argus lab 4.01 version software. The metal complexes were built and geometry optimization was done using this software.

Antimicrobial studies:

The in vitro biological screening effects of the investigated compounds were tested against the bacteria *Staphylococcus Aureus, Escherichia Coli* and Fungi *Candida Albicans*. Stock solutions were prepared by dissolving the compounds in DMSO and serial dilutions of the compounds were prepared in sterile distilled water to determine the minimum inhibition concentration (MIC).

The nutrient agar medium was poured into Petri plates. A suspension of the tested microorganism (0.5 ml) was spread over the solid nutrient agar plates with the help of a spreader. Different dilutions of the stock solutions were applied on the 10 mm diameter sterile disc. After evaporating the solvent, the discs were placed on the inoculated plates.

The Petri plates were placed at low temperature for two hours to allow the diffusion of the chemical and then incubated at a suitable optimum temperature for 30 – 36 hrs. The diameter of the inhibition zones was measured in millimetres [4].

Anti-cancer activity:

***In vitro* cytotoxicity assay:**

Methodology:

The human breast cancer cell line (MCF 7) and murine embryonal fibroblasts cell line (NIH 3T3) were obtained from National Centre for Cell Science (NCCS), Pune. MCF 7 was grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS) where as the NIH 3T3 cells were grown in Dulbeccos Modified Eagles Medium (DMEM) containing 10% FBS. All cells were maintained at 37^0 C, 5% CO2, 95% air and 100% relative humidity. Maintained cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure:

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test sample [$RuL_1Cl(PPh_3)$].

They were initially dissolved in dimethylsulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, and this resulted in the required final sample concentrations. Following drug addition the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium without samples served as control and triplicate was maintained for all concentrations.

MTT assay: 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is an yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells.

After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Non linear regression graph was plotted between % cell inhibition and Log concentration and IC50 was determined using Graph Pad Prism software.

Synthesis of nano ruthenium oxides:

The transition metal complexes were placed in a silica crucible and ignited in a muffle furnace at 800^oC. The dehydrated mixture undergoes a vigorous, exothermic voluminous and foamy powder product occupying the entire reaction container. The exothermic combustion reaction releases a large amount of heat, which can quickly heat up the system to reach a temperature higher than 1600^oC. The combustion method results in uniform and pure powders of high surface to volume ratio. The size of the metal oxide was determined using Atomic Force Microscope.

RESULTS AND DISCUSSION:

Elemental Analysis and Molar Conductance:

The analytical data and physical properties of the ligands and complexes are presented in **Table 1**. The data are consistent with the calculated results from the empirical formula of each compound. The analytical data of the complexes confirm the 1:1 metal to ligand stoichiometry. The very low molar conductance values of the complexes in DMF solvent at 25^oC showed that the complexes were non-electrolytes[5].

IR Spectra:

The significant IR bands for the ligands as well as its metal complexes and their tentative assignments are compiled and presented in **Table 2**.

TABLE 1: ELEMENTAL ANALYSIS, YIELD, MOLAR CONDUCTIVITY, MELTING POINT OF LIGANDS AND THEIR METAL COMPLEXES

Complex	Colour	Empirical formula	Molecular Weight	Yield	Elemental analysis, Found(calculated)%			Conductance ($ohm^{-1}cm^{-1}$ mol^{-1})	Melting point (ºC)
					C	H	N		
L_1	Pale yellow	$C_{34}H_{26}N_8O_2$	578	80	71 (70.5)	4.5 (4)	19.8 (19.3)	0.841	143
L_2	Pale brown	$C_{32}H_{26}N_8O_4$	586	79	68 (65.9)	5 (4.4)	19.7 19.1)	0.865	155
[Ru(L_1)Cl)(PPh_3)]	Brown	$C_{52}H_{39}N_8O_2RuPCl$	975	78	65 (64)	4.8 (4)	12 (11.5)	0.843	78
[Ru(L_2)(Cl)(PPh_3)]	Black	$C_{40}H_{39}N_8O_4RuPCl$	863	80	56 (55.6)	4.7 (4.5)	13.5 (12.9)	0.867	65

TABLE-2 IR SPECTRAL DATA FOR LIGANDS AND THEIR METAL COMPLEXES

Complex	υ(C=N)	υ(OH)	υ(C-O) (phenolic)	υ(N=N)	υ(M-O)	υ(M-N)	Bands due to PPh_3
L_1	1619.31	3357.25	1354.09	1532.51	-	-	-
L_2	1610.00	3335.07	1369.52	1573.9	-	-	-
[Ru(L_1)(Cl)(PPh_3)]	1604.84	-	1344.44	1532.51	541.06	425	686.69,1084.04 ,1442.82
[Ru(L_2)(Cl)(PPh_3)]	1595.16	-	1329.96	1570.23	550.70	470	694.4,1107.191 458.25

The IR spectra of all the free Schiff bases L_1/L_2 show characteristic –CH=N, -OH and –N=N- frequencies around 1619/1610cm^{-1}, 3357 /3335cm^{-1} & 1573/1532cm^{-1} respectively. A strong band observed at 1354/1369cm^{-1} in the ligand has been assigned to phenolic >C-O stretching. On complexation, the band due to –CH=N stretching underwent a negative shift (1604/1595cm^{-1}), suggesting the involvement of azomethine group in co-ordination [6]**.** The phenolic >C-O stretching band has been shifted to 1344/1329cm^{-1} region in the complexes, which indicates that the other coordination site is phenolic oxygen atom [7].

The binding mode of the Schiff base ligands to the ruthenium ion in these complexes is further confirmed by the disappearance of the broad band around 3300 cm^{-1} attributed to –OH, in the complexes. The appearance of new non- ligand bands around 541/550cm^{-1} and425/470cm^{-1}further confirms the (M-O) and (M-N) coordination sites respectively [8]. The ruthenium (III) Schiff base complexes show strong vibrations in the range 655-698, 1084-1107 and 1432-1458cm^{-1} which are attributed to the triphenyl phosphine fragments [9].

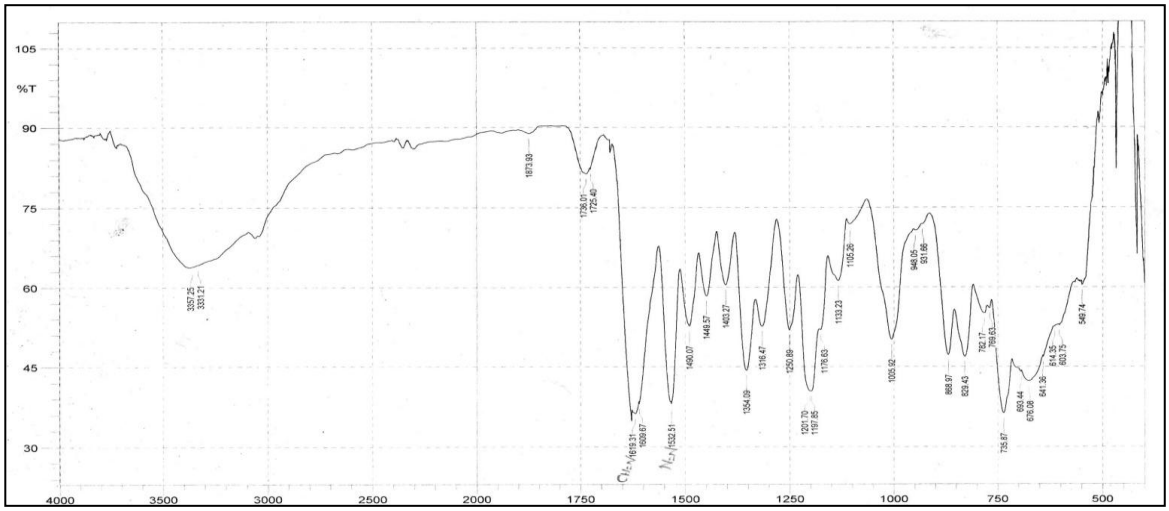


FIG 1 IR SPECTRUM OF AZO SCHIFF BASE L_2

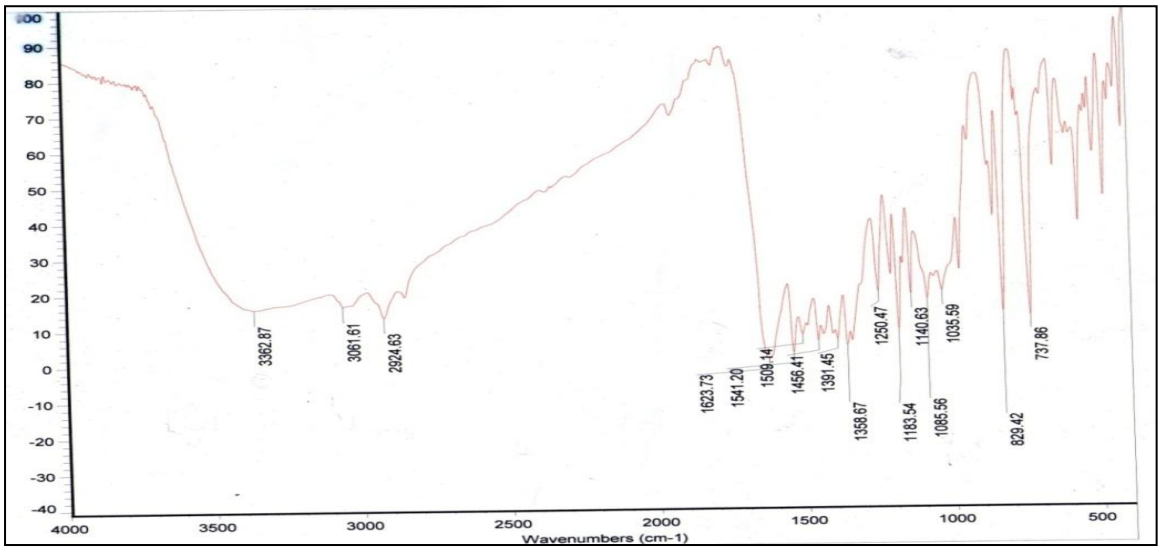


FIG 2: IR SPECTRUM OF [Ru(L_2)(Cl)(PPh_3)]

Electronic spectra and magnetic measurements: Electronic spectral data of the free ligands and their complexes in DMF are listed in **Table 3 (Fig 3 and 4).**

TABLE 3: ELECTRONIC SPECTRAL AND MAGNETIC MOMENT DATA FOR THE LIGANDS AND THEIR COMPLEXES

Ligand/Complex	Absorbance nm	υ/cm^{-1}	Assignment	Geometry	Magnetic moment μ_{eff}(BM)
L_1	300	33333	π-π*	-	-
	431	23202	n- π*		
L_2	314	31847	π- π*	-	-
	450	22222	n- π*		
	304	32895	π - π*/n- π*		
[Ru(L_1)(Cl)(PPh_3)]				Octahedral	1.73
	350	28571	MLCT		
	600	16666	d-d		
	300	33333	π- π*/n- π*		
[Ru(L_2)(Cl)(PPh_3)]	470	212765	MLCT n- π*	Octahedral	1.65

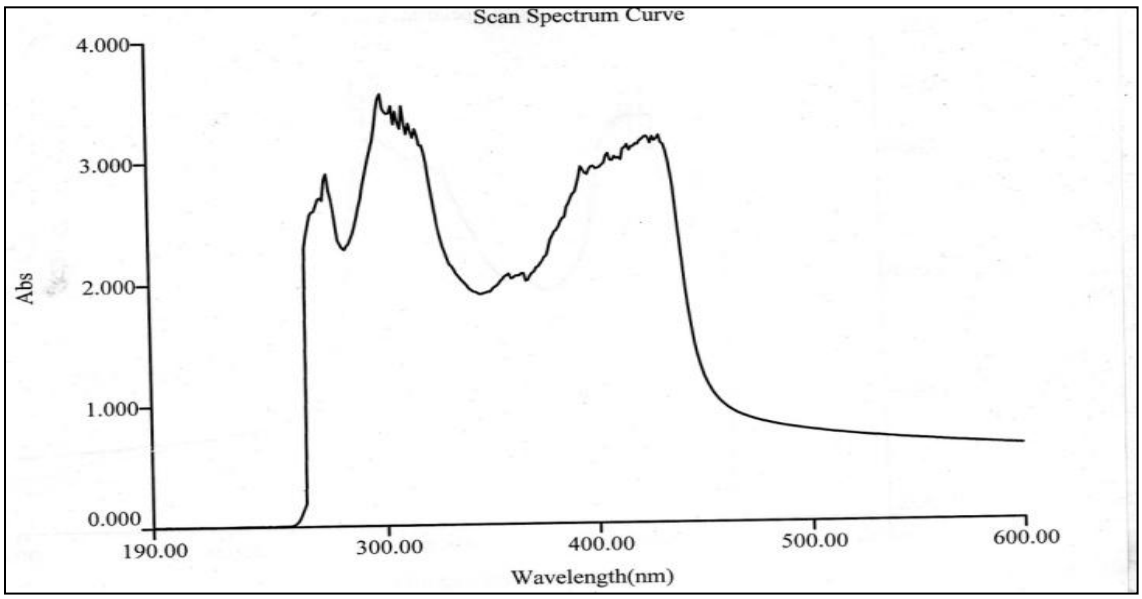


FIG 3: ELECTRONIC SPECTRUM OF AZO SCHIFF BASE L_1

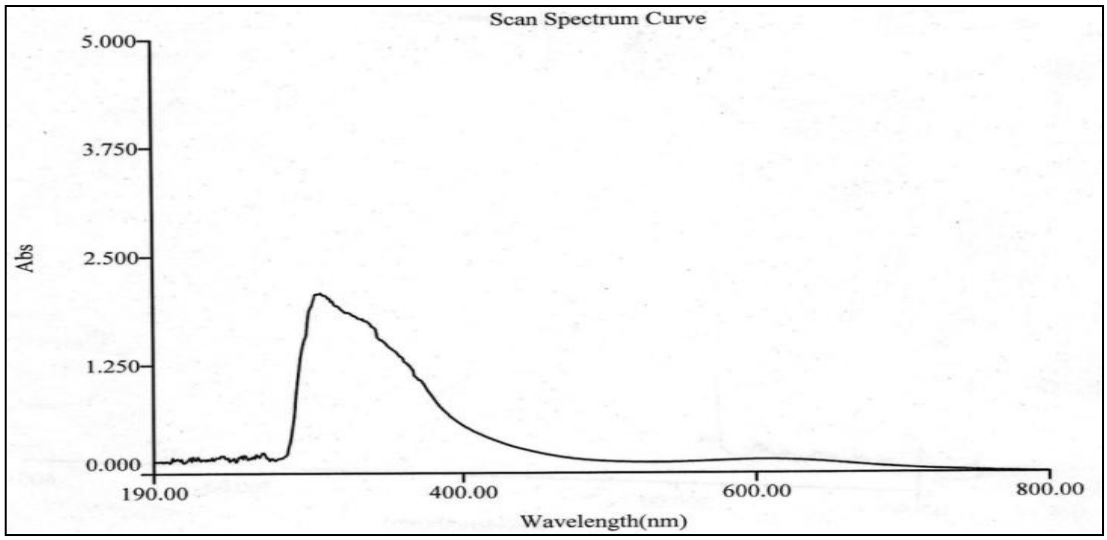


FIG 4: ELECTRONIC SPECTRUM OF $Ru(L_1)(Cl)(PPh_3)$]

Electronic spectra of free ligands show two types of transitions, the first in range 300-339 nm assigned to π-π^* transitions due to molecular orbitals located on phenolic chromophore. These peaks shift in the spectra of the complexes due to donation of a lone pair of electrons from oxygen of the phenoxy group to ruthenium[10]. This reveals that one co-ordination site is phenolic oxygen.

The second type of transition appeared around 300-450 nm assigned to n-π^* transitions due to azomethine groups and benzene of the ligands. These bands also shift in the spectra of the complexes indicating the involvement of imine nitrogen in coordination with ruthenium. The ground state of ruthenium (III) is $^2T_{2g}$ and the first excited doublet levels in the order of increasing energy are $^2A_{2g}$ and $^2A_{1g}$ which arises from the $^4t_{2g}e^1g$ configuration[11].

In most of the ruthenium (III) complexes the UV-Vis spectra show only charge transfer bands [12], since in a d^5 system and especially in ruthenium (III) which has relatively high oxidizing properties, the charge transfer bands of the type $L_{\pi y} \rightarrow t_{2g}$ are prominent in the low energy region which obscure the weaker bands due to d-d transitions. It therefore becomes difficult to assign conclusively the bands of ruthenium (III) complexes which appear in the visible region. The electronic spectra of all the complexes in DMF showed two bands in the region 300-600nm. The extinction coefficients of the bands in this region (33000-16666 cm^{-1}) have been found to be higher than those generally expected for d-d transitions. Hence these bands have been assigned to charge transfer transitions. Similar observations have been made for other ruthenium (III) octahedral complexes [13].

The room temperature magnetic moments of all the complexes is in the range 1.63-1.76 BM. This shows that these complexes are paramagnetic corresponding to one unpaired electron, which supports the trivalent state of ruthenium, suggesting a low spin $4d^5$, s = ½ configuration around the Ru (III) in the octahedral environment.

Thermo gravimetric Analysis:

Thermo gravimetric analysis (TGA and DTG) of metal complexes are used to get information about the thermal stability of new complexes decide whether the water molecules are inside or outside the inner co-ordination sphere of the central metal ion. And suggest a general scheme for thermal decomposition of the chelates.

In the present investigation, heating rates were suitably controlled at 10^oCmin^{-1}under nitrogen atmosphere and the weight loss was measured from the ambient temperature up to 1000^oC. The TGA data are presented in **Table 4 (Fig 5)**. From the data obtained from the figures it is evident that all the complexes undergo decomposition around 130-390^oC. The azo Schiff base ligand, PPh_3 and Cl are lost. Above 390^oC metallic oxide alone exist[14].

TABLE 4: THERMAL ANALYSIS DATA FOR METAL COMPLEXES

Complex	Decomposition Temperature (°C)	Lost fragment	Residue	Weight loss %	
				Experimental	Theoretical
$[Ru(L_1)(Cl)(PPh_3)]$	150-300	Azo Schiff base,Cl,PPh_3	RuO_2	86	86
$[Ru(L_2)(Cl)(PPh_3)]$	130-350	Azo Schiff base,Cl,PPh_3	RuO_2	85	84.5

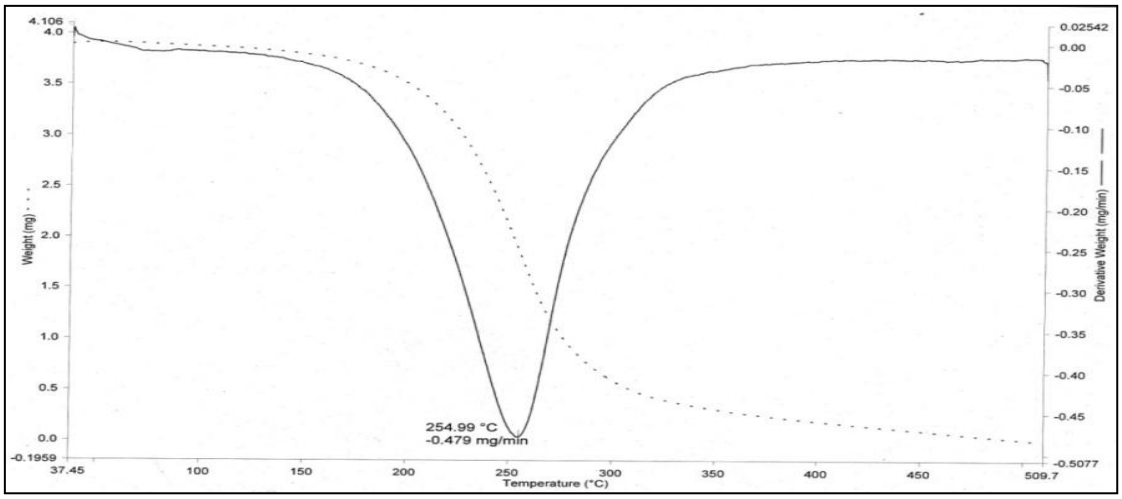
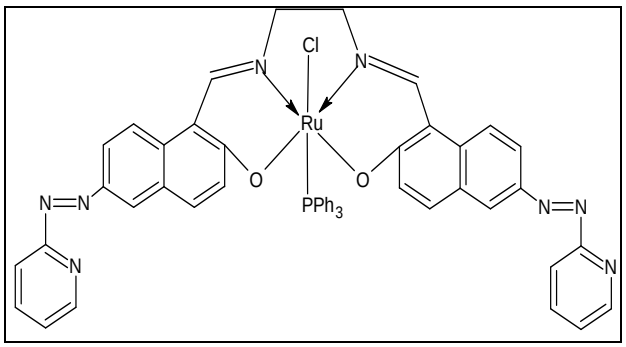


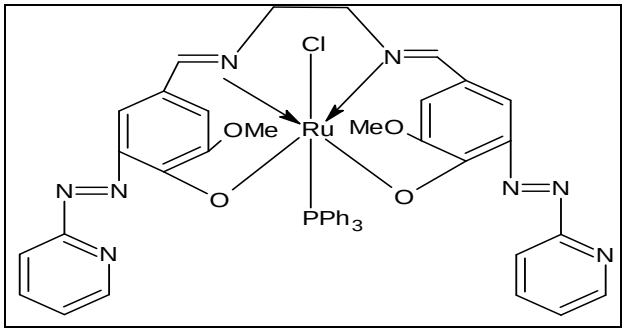
FIG 5: THERMO GRAVIMETRIC ANALYSIS OF $[Ru(L_1)(Cl)(PPh3)]$

Based on the analytic data the following octahedral geometry has been proposed.

Probable Structures:



$[Ru(L_1)(Cl)(PPh_3)]$



$[Ru(L_2)(Cl)(PPh_3)]$

Molecular Modelling:

Molecular modeling of the studied complexes reveals minimum energy values associated with the octahedral geometry. This was in good agreement with the experimental results and confirmed the expected structure. The possible geometries of metal complexes were evaluated using the molecular calculations using the Argus lab 4.0.1 version software. The metal complexes were built and geometry optimization was done using this software.

The details of important bond lengths as per 3D structure of ligand and Ru(III) complex **(Fig 6 and 7)** are given in the **Table 5**. These values were obtained as a result of energy minimization of Ru(III) in Argus lab 4.0.1 version software.

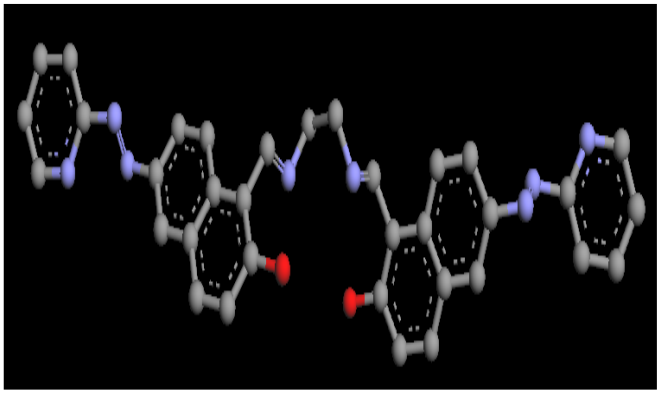


FIG 6: MOLECULAR MODELLING OF AZO SCHIFF BASE L1

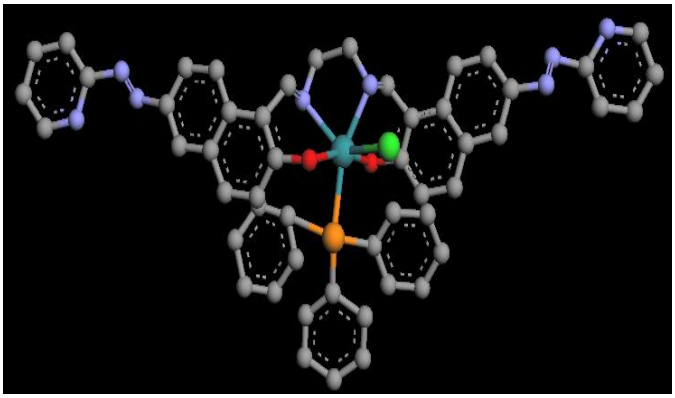


FIG 7: MOLECULAR MODELING OF METAL COMPLEX [Ru(L_1)(Cl)(PPh_3)]

The obtained bond lengths of the ligand L_2 based on the software are 8(C)-37(O) & 16(C)-38(O) [phenolic-OH], 39(C)-41(N) & 40(C)-42(N) [azomethine C=N]. Based on the values from the **Table 5**, it was observed that when these ligand are coordinated with Ru metal ion there is an increase in the bond length between the mentioned atoms, which confirms the coordination of azomethine group through nitrogen and through phenolic oxygen. When the atoms are coordinated with the metal ion by donating a lone pair of electron there is a decrease of electron density on the coordinating atoms, hence bond length increases in metal complexes. This supports the proposed octahedral geometry around the Ru metal ion [15].

TABLE 5: DATA FROM MOLECULAR MODELING OF L_2 AND [Ru(L_2)(Cl)(PPh_3)]

S.No	Ligand/Complex	Bonded atoms	Bond length
1.	L_2	8(C)- 37(O)	1.41017
		39(C)-41(N)	1.33683
		16(C)-38(O)	1.41017
		40(C)-42(N)	1.33719
2.	[Ru(L_2)(Cl)(PPh_3)]	8(C)-37(O)	1.42063
		39(C) - 41(N)	1.33702
		16(C) - 38(O)	2.31253
		40(C) - 42(N)	1.338613

Antimicrobial Studies:

The antimicrobial activity of the metal complexes was studied against two pathogenic bacterial strains (ie) one gram positive (*staphylococcus Aureus*) and one gram negative *(Escherichia coli)* bacteria and one fungal strain *(Candida Albicans)*Antibacterial and antifungal potential of the metal complexes were assessed in terms of zone of inhibition of bacterial and fungal growth. The results of the antifungal and antibacterial activities are presented in **Tables 6 and 7** and represented in **Fig 8 and 9**. The minimum inhibitory concentration (MIC) were calculated as the highest dilution showing complete inhibition of the tested strains and are reported in **Table 8-11**.

TABLE 6: ANTIBACTERIAL ACTIVITY DATA OF SCHIFF BASE METAL COMPLEXES

Bacteria	Samples(100µg/disc)		STD
	[Ru(L_1)(Cl)(PPh_3)]	[Ru(L_2)(Cl)(PPh_3)]	Ciprofloxacin 5µg/disc
S.Aureus	13	16	22
E.Coli	12	21	19

TABLE 7: ANTIFUNGAL ACTIVITY DATA OF AZO SCHIFF BASE METAL COMPLEXES

Microorganism	Samples (100µg/disc)		STD Clotrimazole (100µg/disc)
	[Ru(L_1)(Cl)(PPh_3)]	[Ru(L_2)(Cl)(PPh_3)]	
C.Albicans	11	8	15

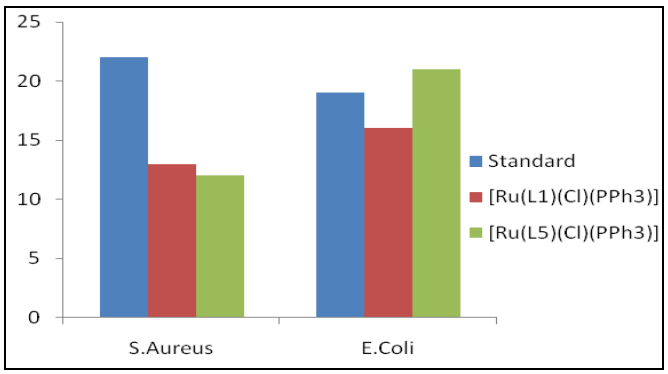


FIG 8: ANTIMICROBIAL ACTIVITY OF AZO SCHIFF BASE COMPLEXES AGAINST BACTERIAL PATHOGENS

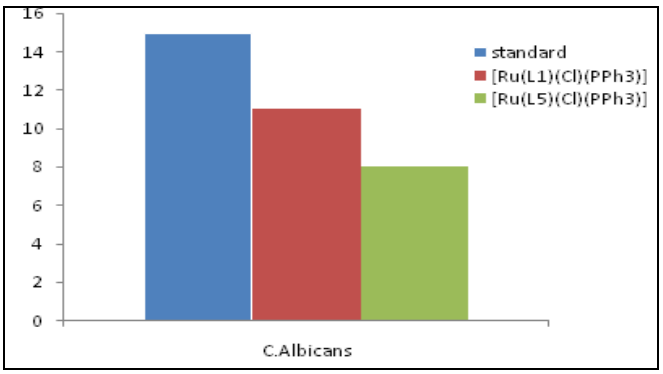


FIG 9: ANTIMICROBIAL ACTIVITY OF AZO SCHIFF BASE COMPLEXES AGAINST FUNGAL PATHOGEN

The results of the antibacterial and antifungal activities of the compounds compared with that of ciprofloxacin a standard broad-spectrum antibiotic for bacterial strains and clotrimazole for fungal strain indicate that the complex $[RuL_5Cl(PPh_3)]$ were active but activity was less compared with the standard drug.

TABLE 8: MIC FOR ANTIBACTERIAL ACTIVITY

Sample	Organism	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.5 µ/ml	15.625 µg/ml
$[Ru(L_1)(Cl)(PPh_3)]$	*S.Aureus*	-	-	-	+	+	+	+
	E.coli	-	-	-	+	+	+	+
$[Ru(L_2)(Cl)(PPh_3)]$	*S.Aureus*	-	-	-	-	+	+	+
	E.coli	-	-	-	+	+	+	+

Minus (-) indicates the absence of growth Plus (+) indicates presence of growth

TABLE 9: ANTIBACTERIAL ACTIVITY: MIC VALUES

Sample	Organisms	MIC values
$[Ru(L_1)(Cl)(PPh_3)]$	*S.Aureus*	250µg/ml
	E.coli	250/µml
$[Ru(L_2)(Cl)(PPh_3)]$	*S.Aureus*	125µ/ml
	E.coli	250µ/ml

TABLE10: MIC FOR ANTIFUNGAL ACTIVITY

Sample	Organism	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.5 µg/ml	15.625 µg/ml
$[Ru(L_1)(Cl)(PPh_3)]$	*C.Albicans*	-	-	-	-	-	-	+
$[Ru(L_2)(Cl)(PPh_3)]$	*C.Albicans*	-	-	-	-	-	-	+

TABLE11: ANTIFUNGAL ACTIVITY: MIC VALUES

Sample	Organisms	MIC values
$[Ru(L_1)(Cl)(PPh_3)]$	*C.Albicans*	31.25µg/ml
$[Ru(L_2)(Cl)(PPh_3)]$	*C.Albicans*	31.25µg/ml

Growth of bacterial pathogens on each concentration was checked to determine the minimum concentration that inhibits growth of the organism. It is evident from the table that the MIC values for both the complexes are 250 µg/ml for both *staphylococcusAureus and E-coli.*

Likewise the MIC value for fungal pathogen *C. Albicans* is 31.25 µg/ml for $[RuL_2Cl(PPh_3)]$ i.e the complexes $[RuL_2Cl(PPh_3)]$ show better inhibition for growth of fungi than bacteria **(Tables10 and 11)**.

The activity of the complexes can be explained with respect to overtones concept and Tweedy's chelation theory. According to overtones concept of cell, permeability, the lipid membrane that surrounds the cell favours the passage of only the lipid soluble materials whose lipo solubility is an important factor which controls the antimicrobial activity. On chelation, the polarity of the metal ion is reduced to a great extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of π- electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organisms. Furthermore, the mode of action of the compounds may involve formation of a hydrogen bond through the azomethine group with the active centres of cell constituents, resulting in interference with the normal cell process[16].

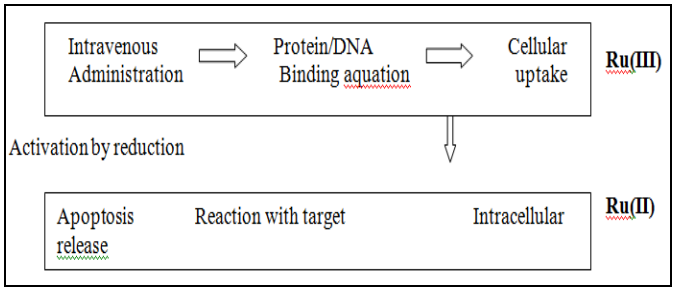
Anticancer activity:

Cytotoxic activity evaluation by MTT assay:

Kinetic liability/inertness toward ligand substitution is a major determinant that controls the

covalent interactions of a metal complex with biological target molecules. Ru(III) complexes probably act as pro drugs that are relatively inert toward ligand substitution and therefore their anticancer activity depends on the ease of reduction to more labile plus kinetically more reactive Ru(II) complex. The resulting Ru(II) species generally less inert, have a high propensity for ligand exchange reactions and may therefore interact with target molecules more rapidly [17].

To verify this bioreductive activation mechanism **(scheme 1)** under *in vitro* conditions, cytotoxicity studies were carried out. Moreover, as the balance between the therapeutic potential and toxic side effects of a compound is very important when evaluating its usefulness as a pharmacological drug, experiments were designed to investigate the *in vitro* cytotoxicity of the synthesized ruthenium complexes against the human breast cancer cell line MCF 7 and the normal cell line NIH 3T3.



SCHEME 1: PROPOSED MODE OF ACTION OF RUTHENIUM ANTICANCER AGENTS

Cytotoxcity was determined by means of a colorimetric microculture MTT assay, which measures mitochondrial dehydrogenase activity as an indication of cell viability. It is evident from the graphs **(Figs 9 and 10)** that the number of cells decreased with an increase in the concentration of the Ru complex **(Tables 12** and**13)**. The complex showed higher potential antineoplastic activity which is evidenced by low IC_{50} values (50% inhibitory concentration after exposure for 48 hours in MTT assay) of 25.85µg/ml. Despite this potency the Ru complex was much less toxic toward normal cells (NIH 3T3) with IC_{50} values of 102.2 µg/ml (figs53and54). Breast cancer cells have been shown to be very sensitive to additional oxidative stress produced by the complex due to their down regulated antioxidant defence enzymes leading to apoptotic death[18].

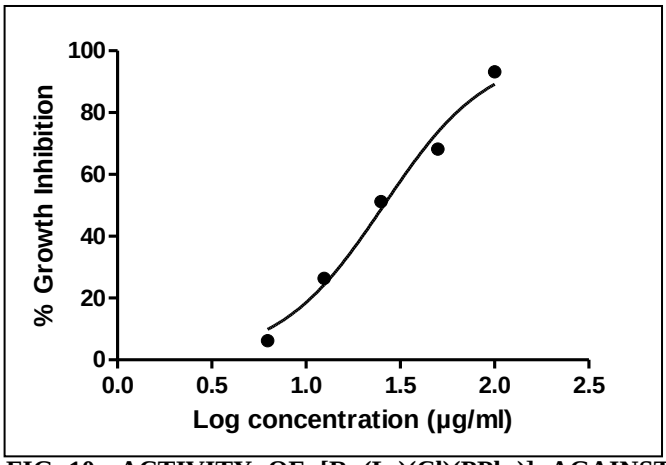


FIG 10: ACTIVITY OF [Ru(L_1)(Cl)(PPh_3)] AGAINST MCF BREAST CANCER CELLS

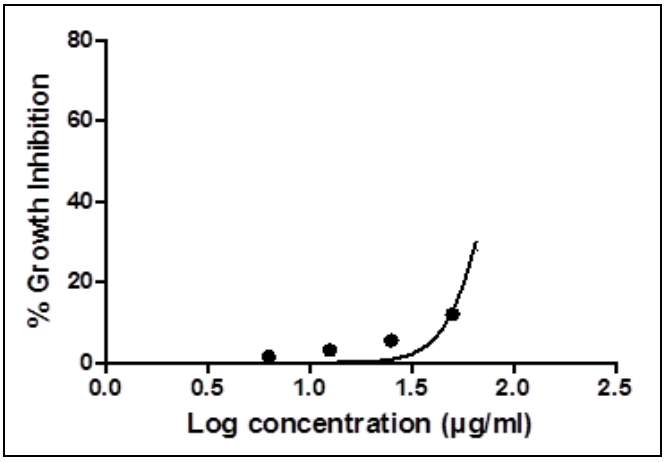


FIG 11: ACTIVITY OF [Ru(L_1)(Cl)(PPh_3)] AGAINST MCF BREAST CANCER CELL

TABLE 12: EFFECT OF RUTHENIUM COMPLEX ON CYTOTOXICITY AGAINST MCF 7 BREAST CANCER CELLS

Complex	Conc. in µg/ml	Triplicates			Average	µg/ml % cell inhibition	IC_{50}
	6.25	0.369	0.358	0.351	0.359	6.18	
	12.5	0.258	0.278	0.283	0.282	26.37	
	25	0.196	0.185	0.18	0.187	51.17	
[Ru(L_1)(Cl)(PPh_3)]	50	0.127	0.125	0.113	0.121	68.23	25.85
	100	0.028	0.026	0.024	0.026	93.21	

TABLE13: EFFECT OF RUTHENIUM COMPLEX ON CYTOTOXICITY AGAINST NIH 3T3 NORMAL CELLS

Complex	Conc. In µg/ml	Triplicates			Average	µg/ml % cell inhibition	IC_{50}
	6.25	0.313	0.317	0.32	0.316	1.55	
	12.5	0.311	0.308	0.316	0.312	3.11	
$[Ru(L_1)(Cl)(PPh_3)]$	25	0.308	0.301	0.302	0.303	5.59	79.77
	50	0.282	0.289	0.277	0.282	12.12	
	100	0.092	0.085	0.095	0.091	21.81	

Determination of size of nano metal oxide:

The size of the metal oxide was determined by Atomic force Microscopy. AFM provides a 3D profile of the surface on a nanoscale by measuring forces between a sharp probe (<10 nm) and the surface at very short distance (0.2-10 nm probe-sample separation). The probe is supported on a flexible cantilever. The AFM tip gently touches the surface and records the small force between the probe and the surface. Measurements are made in three dimensions x, y and z. The 2D AFM images of the metal oxide and the 3D images are shown in fig 55-58. From the figures it is evident that the size of the synthesized metal oxide is in the range (~10-25 nm).

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