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Synthesis and antifungal potential of a ruthenium(II) complex

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ABSTRACT

Metal ion dependent processes are found throughout the life science and vary tremendously in their function and complexity. The interaction of ruthenium(II) polypyridyl complexes with DNA has been a topic of major bioinorganic interest. A new mixed ligand ruthenium(II) complex containing benzimidazolyl terpyridine and bipyridine ligands has been synthesized and characterized by elemental analysis, ESI-MS and UV–Visible spectroscopic techniques. The complex has been screened for its in-vitro antifungal activity against some pathogenic fungal strains by using disc diffusion method.

Keywords: Ruthenium(II), benzimidazolyl terpyridine , antifungal, fungal strains.

INTRODUCTION

It is now appreciated that metal ions control a vast range of processes in biology. Many new and exciting developments in the field of biochemistry create interest out of inorganic chemists to court in the new area called "Bioinorganic Chemistry". In recent years considerable attention has focused on the synthesis of polynuclear transition metal complexes and the study of their photochemical, photophysical and electrochemical properties. The transportation of metal complexes into cells plays a key role in the development of metallopharmaceutical agents.[1] Metal ions such as Na^+, K^+, and Ca^{2+} can penetrate the cell through their corresponding carrier proteins and channels.[2-3] Several channels for the transportation of transition metal ions, including Fe(II), Mn(II), Cu(I/II)[4], and Cr(III) [5–8], have been characterized with their uptake pathways. 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.[9] 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.[10-11] Ruthenium is a very good metal to be used in metal complex drugs considering its ability to mimic iron in binding to certain biological molecules. Ruthenium (II) complexes are currently used as antileukaemic and antiviral agents and for the treatment of Crohn's disease. [12] The chemistry of ruthenium complexes with nitrogen containing ligands has been extensively studied in recent years.[13] Polypyridyl ruthenium complexes are an alternative option to platinum drugs in the development of antitumor agents, based on their reaction mechanisms.[14] The cytotoxicity of polypyridyl chlororuthenium complexes has been studied in murine and human tumor cells in vitro, and many bipyridine and terpyridine ligands have been synthesized and reported in the literature.[15-16] Therefore, polypyridyl ruthenium complexes are easily available for a systematic model in which to study the relationships between structure and biorelated reactivity. 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-

$[RuCl_2(NH_3)_4]Cl$ [17] preceded by the discovery that ruthenium red possesses antitumor properties made in the 1970s.[18-19] Since then compounds such as trans-(IndH)$[Ru(ind)_2Cl_4]$ (Ind = indazole), mer-$[Ru(terpy)Cl_3$(terpy = 2,2'-terpyridine),[20-22] $[Ru(dmso)_4Cl_2]$ (dmso =dimethyl sulfoxide) [23], ImH$[Ru(im)Cl_5]$,[24] ImH$[Ru(im)_2$-$Cl_4]$, [25] and ImH[Ru(im) (dmso)Cl_4] NAMI-A (im=imidazole), [26] are also well-known antitumor agents. Antibacterial screening of Ru(II) Schiff base complexes of the type [Ru(CO)(EPh3)(B)(L)] (E = P or As; B= PPh_3, $AsPh_3$, py or pip; L = Schiff bases have been reported by Balasubramanian *et al.* [27]

Herein, we report the synthesis and characterization of $[Ru(bitpy)(bpy)(H_2O)](ClO_4)_2$, where bitpy is 4′-(1H-benzimidazol-2-yl)-2,2':6',2"-terpyridine and bpy is 2,2'- bipyridyl. The antimicrobial activity of the complex was investigated.

MATERIALS AND METHODS

Ruthenium chloride trihydrate was purchased from Aldrich. Benzimidazole-2-carboxaldehyde was prepared by following a reported procedure.[28] 4′-(1H-benzimidazol-2-yl)-2,2′:6′,2″-terpyridine was prepared by adopting the procedure available in the literature.[29] Acetonitrile, dimethyl sulfoxide, dichloromethane and methanol were of chromatographic grade and were used without further purification. Microanalyses were performed at Sophisticated Test and Instrumentation Centre (STIC), Cochin University, Kerala. UV–visible spectra of the complex was recorded on a Perkin–Elmer Lambda 35 double beam spectrophotometer at 25^oC. Positive ion electrospray ionization mass spectra of the complex was obtained by using Thermo Finnigan LCQ 6000 advantage max ion trap mass spectrometer. Antimicrobial studies were carried out in Gene Pool Research Centre, Gudalur.

2.1 Synthesis of $Ru^{III}(bitpy)Cl_3$

To synthesize $Ru^{III}(bitpy)Cl_3$, we have adopted the literature procedure. $RuCl_3.3H_2O$ (0.437 g, 1.67 mmol) and 4′-(1H-benzimidazol-2-yl)-2,2':6',2"-terpyridine (bitpy) (0.58 g, 1.67 mmol) were suspended in ethanol (50 mL) and the mixture was heated to reflux for 3 h while vigorous magnetic stirring was maintained. Subsequently the mixture was allowed to cool. The resulting dark brown powder was collected by filtration. The product was washed with ethanol (3 X 10 mL) followed by diethyl ether (3 X 10 mL) and air dried. Yield: 0.80 g (89 %);

2.2 Synthesis of $[Ru(bitpy)(bpy)(H_2O)](ClO_4)_2$, 1

$Ru^{III}(bitpy)Cl_3$ (0.557 g, 1 mmol), 2,2'-bipyridyl (0.156 g, 1mmol) and LiCl (0.5 g, 12.5 mmol) were suspended in EtOH-H_2O solvent mixture (3:1 v/v). Et_3N (0.3 mL) was added as reductant and the mixture was refluxed under inert atmosphere for 4 h while vigorous stirring was maintained. The reaction mixture was cooled to room temperature; the solvent was removed under vacuum to one-third of its initial volume. Saturated aqueous solution of $NaClO_4$ was added to precipitate $[Ru(bitpy)(bpy)Cl]^+$ as perchlorate salt. The product was filtered, washed with water (3 x 10 mL) and dried. The product obtained (0.450 g, 0.5 mmol) and silver perchlorate (0.104 g, 0.5 mmol) were suspended in acetone-water solvent mixture (1:1 v/v)and the mixture was refluxed under nitrogen atmosphere for 2 h The reaction mixture was cooled to room temperature; the solvent was removed under vacuum to one-third of its initial volume. Saturated aqueous solution of $NaClO_4$ was added to precipitate $[Ru(bitpy)(bpy)(H_2O]^{2+}$ as perchlorate salt. The product was filtered, washed with water (3 x 10 mL) and dried. Yield: 0.409 g (72 %); ESI-MS: m/z 323.93 $(M-(ClO_4)_2)^{2+}$.

2.3 Antifungal screening

Preparation of a Disc:

The compound (30 µg) in DMSO (0.01 ml) was applied on a paper disc, [prepared from blotting paper (3 mm diameter)] with the help of a micropipette. The discs were left in an incubator for 48 h at 37°C and then applied on the bacteria grown agar plates.

Preparation of Agar Plate:

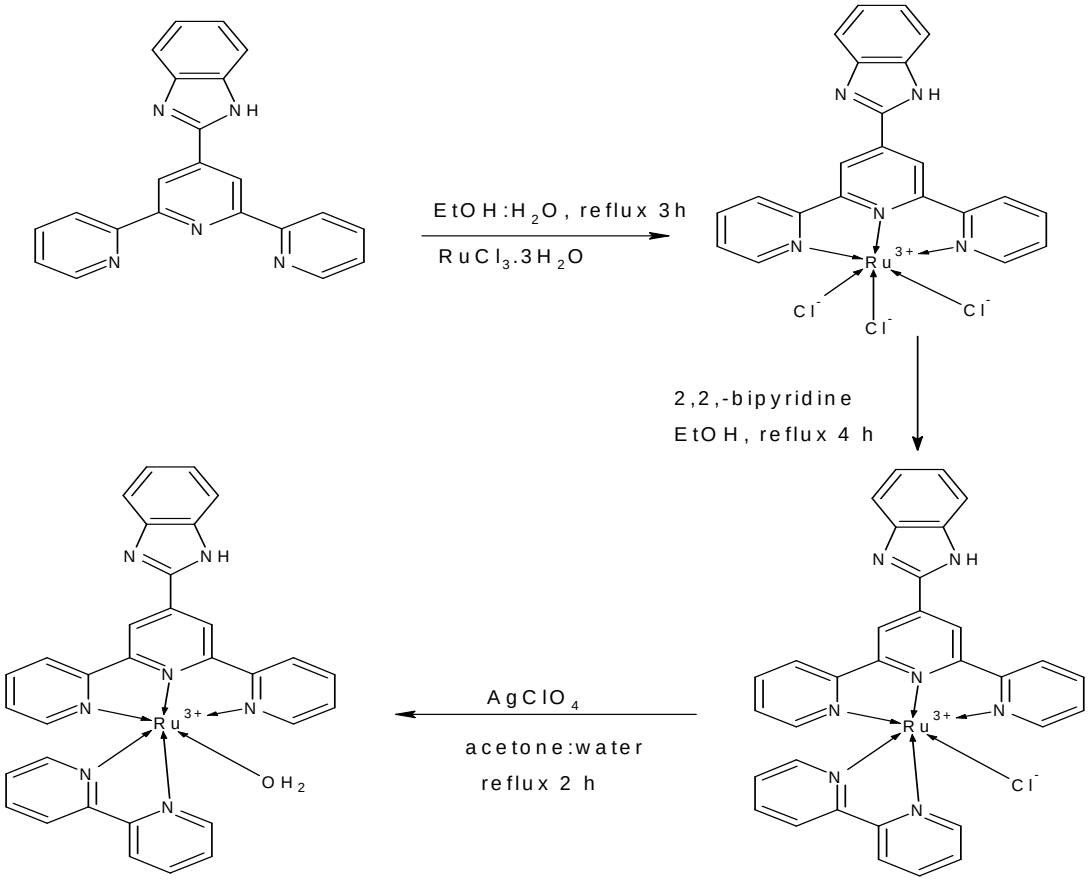
Minimal agar was used for the growth of specific fungal species. It was allowed to soak for 15 minutes and then boiled on a water bath until the agar was completely dissolved. The mixture was autoclaved for 15 min at 120°C and then poured into previously washed and sterilized Petri dishes and stored at 40°C for inoculation.

Procedure of Inoculation:

Inoculation was done with the help of a platinum wire loop which was made red hot in a flame, cooled and then used for the application of bacterial strains.

Application of Disc:
A sterilized forceps was used for the application of the paper disc on the already inoculated agar plates. When the discs were applied, they were incubated at 37°C for 24 h. The zone of inhibition was then measured (in diameter) around the disc.

Scheme. 1 Synthetic scheme for mixed ligand Ru(II) complex 1



RESULTS AND DISCUSSION

3.1 Synthesis and spectral characterization

The complex [Ru(bitpy)(bpy)(H_2O)](ClO_4)$_2$, where bitpy is the tridentate ligand 4′-(1H-benzimidazol-2-yl)-2,2':6',2''-terpyridine and bpy is the bidentate ligand 2,2'-bipyridine, have been isolated from ethanloic solution containing Ru^{III}(bitpy)Cl_3 as the starting material.

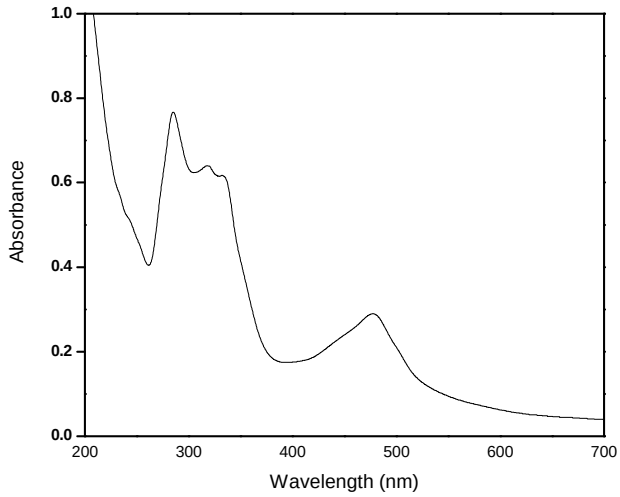
Table. 3.1. Microanalytical data of ligand and Ru(II) complex

Compound	Colour	Empirical formula	Molecular weight	Elemental analysis Calculated (found) (%)		
				C	H	N
Ru^{III}(bitpy)Cl_3	Reddish brown	$C_{22}H_{15}Cl_3N_5Ru$	556.82	47.45 (47.46)	2.72 (2.68)	12.57 (12.54)
[Ru(bitpy)(bpy)(H_2O)](ClO_4)$_2$	Reddish brown	$C_{32}H_{25}Cl_2N_7O_9Ru$	823.56	46.67 (46.68)	3.06 (3.04)	11.90 (11.88)

The complex was obtained in good yield and characterized by using elemental analysis, ESI-MS and UV-Vis spectral techniques. The synthetic route for the present complex is shown in **Scheme.1.** Microanalytical data for the present complex is given in **Table. 3.1**. The stoichiometry of the complex was determined as [Ru(bitpy)(bpy)(H2O)](ClO4)2 based on elemental analysis. The ESI-MS data revealed that the complex retains its

identity in solution. The ESI mass spectra of the complex shows base peak assignable to the parent ion $(M-(ClO4)_2)^{2+}$. FT-IR spectrum of the present compound shows peak in the region of 1085 cm-1 corresponding to ClO_4- stretching frequency. **Figure.1** shows the electronic absorption spectrum of the complex. The absorption spectrum of the present complex is typical of the ruthenium polypyridyl complexes with intense UV bands assignable to ligand-centered π-π* transitions at 284.8. 318.4 and 332.8 nm. Metal to ligand charge transfer (MLCT) transition is observed as a slightly broad band in the visible region at 476.8 nm for complex **1**.

Figure.1. UV-Visible Spectrum of Ru(II) Complex



3.2 Antifungal screening of Ru(II) Complex

Metal ions are adsorbed on the cell walls of the microorganisms, disturbing the respiration processes of the cells and thus blocking the protein synthesis that is required for further growth of the organisms. Hence, metal ions are essential for the growth-inhibitory effects. [29] Antifungal activity of the present complex was explored by determining zone of inhibition (Disc Diffusion Tests) using nystatin as reference standard (**Table**. **3.2**) at 0.25 %, 0.50 % and 1 % concentration. The effectiveness of an antimicrobial agent in sensitivity is based on the zones of inhibition. The diameter of the zone is measured to the nearest millimeter (mm). The observed order of zone of inhibition was Candida *albicans* > Aspergillus *niger* > Alternaria *alternata*. The ruthenium chelates are more toxic compared to their parent ligands against the same microorganisms under identical conditions. [27] The data reveal that the antifungal activity of the present ruthenium(II) complex is superior to that of the free ligands (bitpy and bpy) and standard reference against same microbes under identical experimental conditions. The toxicity of ruthenium chelates increases on increasing the concentration.

Table. 3.2. Antifungal activity (Zone of Inhibition) of ligands and Ru(II) complex

Test compound	Antifungal activity								
	C. albicans			*A. alternata*			*A. niger*		
	0.25%	0.5%	1%	0.25%	0.5%	1%	0.25%	0.5%	1%
$[Ru(bitpy)(bpy)(H_2O)](ClO_4)_2$	20	22	25	16	18	19	18	20	22
bitpy	12	10	10	9	6	8	8	10	10
bpy	8	7	8	6	4	3	6	7	7
Standard (nystatin)	19			14			17		

Values of zone of inhibition [mm, including the diameter of the disk (6 mm)]

The increased activity of metal chelates can be explained on the basis of Tweedy's chelation theory.[30] On chelation the polarity of the metal ion will be reduced to a greater extent due to overlap of 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 complexes. It is likely that the increased liposolubility of the ligand upon metal complexation may contribute to its facile transport into the bacterial cell

which blocks the metal binding sites in 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 organism.

The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or on differences in ribosome of microbial cells. The enhanced activity of the complexes can also be explained on the basis of their high solubility (as a result, water-soluble complexes are readily accumulated in bacterial and fungal cells, resulting in the activation of enzymes), fineness of the particles, size of the metal ion and the presence of bulkier organic moieties.

CONCLUSION

In the present work a mixed ligand mononuclear ruthenium(II) complex has been isolated and characterized by various physico-chemical techniques and a six coordinated distorted octahedral environment has been proposed for the complex. This complex was subjected to find out their antifungal activity and exhibits greater activity than the respective standards.

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