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Studies on Antibacterial and Antifungal Activities and Minimum Inhibitory Concentrations of Mixed Ligand Transition Metal Complexes of Dibasic Acids as Primary and Heterocyclic Bases as Secondary Ligands

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Abstract

Mixed ligand (diphenic/ adipic as primary Ligand and quinoline / 8 - hydroxyquinoline as secondary) transition metal complexes of Cobalt (II), Cupper (II), Rhodium (III) and Platinum (IV) ions were synthesized and characterized. Their antibacterial activities against ten bacteria had been evaluated by the disc diffusion method,whilst their antifungal activities against two fungi had been also evaluated by the same method. Minimum inhibitory concentrations (MIC) had been evaluated against six bacteria. The MIC of the complexes against *Shigella dysenteriae, Samonella typhi, Streptococcus-β-haemolyticus* and *Bacillus megaterium* were 32 µg/ml; whilst *Escherichia coli and Samonella typhi*-A the MIC of Co (II) and Pt (IV) were 32µg/ml and those of Cu(II) and Rh(III) complexes were 64 µg/ml. It was found that Cobalt (II), Cupper (II) & Rhodium (III) complexes had pronounced antibacterial and antifungal activities. Platinum (IV) complex had moderate antibacterial and antifungal activity. These values indicate that these are active compounds.

Key words: Mixed Ligand, Transition metal, Antimicrobial Activity, Antifungal Activity Minimum Inhibitory Concentrations (MIC)

Introduction

Constituents from natural resources (Alam *et al.*, 2004, Chowdhury *et al.,* 2004, Parvej *et al.,* 2005, and Parihar *et al.,* 2006) as well as synthetic organic and inorganic compounds (Daula *et al.,* 2004, Kamalakannan and Venkappayya 2002) have been receiving increasing attention in biological systems. Many of these compounds are being used as chemotherapeutic agents against infectious diseases. Diphenic acid may be used as bidentate ligand in the formation of complexes with metal ions (Agafonova and Ryazanov, 1969). Heterocyclic bases have a great importance in biological and industrial fields. Most of the heterocyclic bases are used as corrosion inhibitors (Talati and Gandhi 1983) and antibacterial, anticonvulsive, antifungal and antifouling agents (Myer-Rohn and Puschmann 1980). Their activity is generally enhanced when they are allowed to form complexes with metal ions. For example, their complexes with copper exhibit potent antitumor (Doadrio *et al.,* 1979) and antibacterial activities (Heinish *et al.,* 1980). As a matter of fact no such agents till now can be able to destroy effectively pathogenic micro- organisms. It is mainly because these pathogenic organisms develop resistance with time towards these agents. So, it is necessary to find out always new, more safe, effective and inexpensive agents for the purpose. Keeping this fact in mind, Co (II), Cu (II), Pt (IV) and Rh (III) complexes with diphenic/adipic acid as primary and quinoline / 8 - hydroxyquinoline as secondary ligands have been prepared, chareterized and their antibacterial and antifungal activity as well the minimum inhibitory concentrations (MIC) have been determined.

Materials and Methods

General method for synthesis and characterization of the complexes

Metal salts of (0.002 mole) $Cu(NO_3)_2.3H_2O$, (0.001 mole) $H_2Cl_6Pt.6H_2O$, (0.002 mole) $CoCl_26H_2O$ and (0.002 mole) $RhCl_33H_2O$ were dissolved in absolute ethanol (25-50 mL). Each of the solution was heated if necessary and filtered to obtain a clear solution of the metal salt. 25 mL ethanolic solution of dibasic acids (diphenic acid 0.002 mole and adipic acid 0.001 mole) were added with constant stirring. To the clear solution (if any pseudo precipitate formed, it was separated and redissolved in acetone) 25 mL of ethanolic solution of 0.002 M 8-hydroxyl quinoline or quinoline was added and heated on a hot plate with constant magnetic stirring. If immediate precipitation of the complexes did not occur, the volume of the solution was reduced to 50 % by evaporation and allowed to cool. The precipitates formed

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were filtered, washed several times with ethanol and dried in a desiccator over anhydrous $CaCl_2$.The Co (II), Cu (II), Pt (IV) and Rh (III) complexes isolated were abbreviated as complexes 1, 2, 3 and 4 respectively. The obtained complexes are insoluble in ethanol but soluble in dimethyl sulfoxide (DMSO). Melting points of the complexes were measured and found <250°C. The compounds were characterized by elemental analysis, conductrometric analysis, magnetic moment measurement and IR studies.

Measurements

The synthesized mixed ligand complexes were subjected to elemental analysis the microanalysis for carbon; hydrogen and nitrogen were done by using a Perkin Elmer 2400 II Organic Elemental Analyzer. The infrared spectra as KBr disc were recorded with a SHIMADZU FTIR - 8400 infrared spectrophotometer. The molar conductance measurements were carried out in 10-3M solutions using conductivity meter type CG 857 No. 71798 SCHOTT - GREAT Gmbh, Germany. The magnetic moment was achieved by the SHERWOOD SCIENTIFIC magnetic susceptibility balance (M. S. B).

Screening Method

Antibacterial Screening: The complexes were dissolved separately in DMSO such a manner so that 5µL contained 30 µg of the complexes. Then antimicrobial activity of these complexes was performed by disc diffusion method (Beur *et a.,* 1993). Ten bacteria were used for the study. Dried and sterilized filter paper discs soaked with known amount of test agents were placed on the nutrient agent media solidified in petridishes and inoculated with the test organism. The discs were placed in such a way that the discs were no closer than 15 mm to the edge of the place and for enough apart to prevent over-lapping the zones of inhibition. The plates were then kept in a refrigerator for at 4°C for 24 hours in order to provide sufficient time to diffuse into the medium. They were finally incubated at 37.5°C for 24 hours in an incubator. After incubation, the antibacterial activity of the test sample was determined by measuring the diameter of inhibitory zones in mm with a transparent scale.

Antifungal Screening: The antifungal screening was also performed by the discs diffusion method. Potato dextrose Agar was used as fungicidal media. The two fungi were used in this study. Sterilized filter paper discs were taken and the test material of known concentration was applied on the discs with the help of a micropipette. The solvents from the discs were evaporated by hot air blower. In similar way control discs containing only the solvents were also prepared. The solidify agar plates were seeded with 70 µl of fresh culture with the help of micropipette and spreaded the microorganisms with the help of a sterile spreader in an aseptic condition. The prepared discs of sample were placed gently on the freshly seeded solidify agar plates with a sterile forceps. Control discs were also placed on the test plants to compare the effect of the test samples and to nullify the effect of solvents. The plants were then kept in a refrigerator at 4°C for 24 h, so that the materials had sufficient time to diffuse to a considerable area of the plates. After this, the plates were incubated at 37°C for 2 days. After incubation, the antifungal activity of the test sample was determined by measuring the diameter of inhibitory zone.

Determination of minimum inhibitory concentration (MIC) of the antibiotics: Minimum inhibitory concentration (MIC) may be defined as the lowest concentration of antimicrobial drug to inhibit the growth of organism. The data derived from the test can be corrected with the knowledge of expected or measured antibiotic level *in vivo* to predict the efficacy of antibiotic. There are two methods for determining the MIC. They are Serial tube dilution technique of turbid metric assay & Paper disc plate technique or agar diffusion assay. Here the former method was affilied to determine MIC of antibiotics against the six pathogenic bacteria.

Results and Discussion

The isolated complexes were characterized on the basis of elemental analysis (Table I) physical properties, magnetic moment and IR spectral data (Table II) of the complexes. The conductance's values of the prepared complexes are illustrated in Table I. The conductance value of the complexes (1-4) reveal that those are non electrolyte in nature (Geary 1971).

Table I: Data on elemental analyses of the complexes

Complex No	Molecular weight of Complexes	% Hydrogen		% Carbon		% Nitrogen		% Oxygen	
		Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
1	589	3.73	3.76	65.19	65.06	4.75	4.94	16.29	16.32
2	448.5	3.34	3.43	61.53	61.65	3.12	3.04	17.83	17.20
3	597	3.68	3.48	48.24	48.20	4.69	4.52	10.72	10.50
4	523	3.41	3.56	52.77	52.85	2.67	2.80	21.41	21.24

The elemental mental analysis support that the compositions of the complexes 1, 2, 3 and 4 are [Co(II)(DA)(8-HQ)$_2$], [Cu(II)(DA)(8-HQ)], [Pt(IV)(A)$_2$(Q)$_2$] and [Rh(III)(DA)(8-HQ)(H$_2$O)$_2$], where A, DA, 8-HQ and Q are Adipic Acid ($C_6H_{10}O_4$-), Diphenic Acid ($C_{14}H_{12}O_4$), 8-Hydroxyquinoline (C_9H_7NO) and Quinoline (C_9H7N) respectively.

Table II: Analytical data and physical properties of the complexes

Complex No.	Color	m.p./ d.t. °C	% metal		$\mu_1$ohm^{-1} cm^2 mol^{-1}	μ_{eff}, B.M	No. of unpaired electron	ν_{O-H} cm^{-1}	$\nu_{C=O}$ cm^{-1}	ν_{C-O} cm^{-1}	ν_{M-O} cm^{-1}	ν_{M-N} cm^{-1}
			Theo.	Found								
1	Brown	180	10.06	10.35	15.48	3.90	3	..	1576.1	1376.5	601.4	505.3
2	Brown	240	14.18	14	10.12	1.76	1	..	1582.6	1383.0	609.9	508.1
3	Orange	240	39.2	39.11	11.2	0.53	..	..	1597.2	1383.7	612.8	519.2
4	Brown	250 (d)	28.54	28.3	12.61	0.51	..	3384.8	1596.9	1379.0	621.0	474.5

The measured magnetic moment of complex 1 indicate that the Co (II) complex is paramagnetic and an outer orbital octahedral complex. The complex 2 is paramagnetic with one unpaired electron and is possible square planer structure (Figgis and Lewis 1964). On the other hand, complex 3 and 4 are diamagnetic. And complexes 3 and 4 are identified as an inner orbital octahedral complex.

The infrared spectra of the complexes confirmed the coordination of metal ion with the ligands. The strong bands obtained at 1700 and 1400 cm^{-1} respectively for ν(C=O) and ν(C-O) vibration in the spectrum of free di-carboxylic acid are shifted to the region 1550-1600 and 1370-1390cm^{-1} respectively; and broad band at 3400-3600 cm^{-1} due to ν (O-H) in the spectrum of free di-carboxylic acid disappears in the spectra of complexes. In the spectrum of the complexes indicating the di-carboxylic acid is dinegative bidentate ligand coordinating through the carboxylate anions. The presence of M-O bonding is evident from the appearance of v (M-O) modes with in 600-670 cm^{-1} and (M-N) modes with in 470-550 cm^{-1} in the spectra of the complexes (Nakamoto1963). The complex 4 display broad v (O-H) bands at 3385 cm^{-1} indicating the presence of coordinated water.

The antifungal activities of the complexes are shown in Tables III against two fungi. The zone of inhibition values show that Cu (DA) (8-HQ) has significant activity towards *Colleto trichum gloespaoides penz and Botryodiplodia theobrame.*

The antibacterial activities of the purified complexes has been determined at 100 µg/disc against a series of gram positive and gram negative pathogenic bacteria; which kanamycin 30 µg/disc has been used as standard. The results are shown in Table IV. It is found that all complexes show activity against all the bacteria. Complex 3 shows the highest antibacterial activity against all bacteria. Whilst, the complex 4 shows the least antibacterial activity.

Table III: Antifungal activity of the complexes against *Botryodiplodia theobrome and Colletotricum gloesparioides penz.*

Complexes	Zone of inhibition of mycelia growth, mm					
	Botryodiplodia theobrome			*Colletotricum gloesparioides*		
	80 µg/disc	50 µg/disc	30 µg/disc	80 µg/disc	50 µg/disc	30 µg/disc
Co(II)(DA)(8-HQ)$_2$	15	13	12	12	10	8
Cu(II)(DA)(8-HQ)	16	13	12	13	11	10
Pt(IV)(A)$_2$(Q)$_2$	12	10	8	11	9	8
Rh(III)(DA)(8- HQ)(H$_2$ O)$_2$	11	9	7	10	8	7

Results of Minimum Inhibitory concentration (MIC) of Complexes 1- 4:

The MICs of the complexes 1 and 3 against *Shigella dysenteriae, Samonella typhi, Escherichia coli, Sarcina lutea, Streptococcus-ß-haemolyticus and Bacillus megaterium* were 32 µg/ml.

MICs of complexes 2 and 4 against *Shigella dysenteriae, Bacillus megaterium, Sarcina lutea and Streptococcus-ß-haemolyticus were 32µg/ml;* where as, against *Escherichia coli and Salmonilla typhi*-A are 64µg/ml.

Conclusion

The compositions of the complexes 1, 2, 3 and 4 are [Co(II)(DA)(8-HQ)$_2$],[Cu(II)(DA)(8-HQ)], [Pt(IV)(A)$_2$(Q)$_2$] and [Rh(III)(DA)(8-HQ)(H$_2$O)$_2$]. The elemental analysis

Table IV: Antibacterial activity of the complexes "1- 4" and kanamycin standar

Test Organism	Strain No	Diameter of zone of inhibition (mm)				
		Kanamycin 30 µg/disc	Complex 1, 100 µg/disc	Complex 2, 100 µg/disc	Complex 3, 100 µg/disc	Complex 4, 100 µg/disc
i) Gram negative						
Shigella dysenteriae	AL-35587	27	21	22	22	8
Shigella bodyii	AL-17313	32	20	30	30	10
Shigella flexneri	AL-30372	18	17	24	28	16
Escherichia coli	FPFC-1407	26	20	25	30	10
Pseudomonas aeruginosa	CRL	18	22	30	18	9
Kebsiella spp		19	18	20	28	10
Salmonilla typhi-A		26	18	24	34	12
ii) Gram Positive						
Bacillus megaterium	QL-38	22	20	22	30	12
Sarcina lutea	Ql-166	19	16	19	28	12
Streptococcus-β-haemolyticus	CRL	21	27	21	33	11

supports that the compositions of the complexes. From the above discussion it can be conclude that all complexes show activity against all bacteria. Complex 3 shows the highest antibacterial activity against all bacteria. Whilst, the complex 4 shows the least antibacterial activity. If different ligand modifies the antimicrobial activity of the complexes so, proper ligand selection may reveal metal complex to be potent antifungal and antibacterial agents. However further studies in advanced level are required to explore these complexes as antitumor and anticancer agents.

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