Evaluation of Azamacrocyclic Transition Metal Complexes as Antibacterial and Antifungal Activities

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Abstract - The paper presents antimicrobial activity of the synthesised transition metal complexes. All the synthesized complexes were characterized by elemental analysis, molar conductance, IR, ^{1}H-NMR, mass, electronic spectra and thermal studies. The antimicrobial activity of the complexes has been screened in vitro against bacteria and fungi to access their inhibiting potential. The bioassays indicated that most of the synthesized metal complexes showed potential antibacterial and anti-fungal activity.

Keywords - Azamacrocyclic, Metal Complexes, Antibacterial and Antifungal.

I. INTRODUCTION

Antimicrobial resistance of pathogenic microorganisms has presently emerged both in the community and hospital leading to increased deaths, illness, hospitalization and treatment costs and duration. The increasing incidence of bacterial drug resistance imposes an improvement of the existing antimicrobial drugs and the development of new ones.

Research and development of potent and effective antimicrobial agents represent one of the most important advances in therapeutics; the main aim of these efforts is not only control the serious infections, but also prevention and treatment of some infectious complications of other therapeutic modalities.

Among the new substances exhibiting good antimicrobial activity, a large number of species are bearing either a biocation (Co (II), Ni(II), Cu(II), Cr(III) or Zn(II)) [1-3] or a metallic ion with a proven antimicrobial activity [4-6]. The bio-functional activity of metal based complexes in medicine and chemotherapy has spurring the growth of interest in the scientific world in the past few decades, after the successful clinical use of Cis-platin as an anticancer drug [7-10]. Most of the metals being unnatural to human body, because of having no effective mechanism for its rejection and toxic behavior, there has been rapid expansion in research and development of novel metal based drugs with improved pharmacological properties [11,12].

Transition metal complexes have attracted attentions of inorganic, metallo-organic as well as bio-inorganic chemists because of their extensive applications in wide ranging areas from material to biological sciences [13]. Several metal complexes are known to accelerate the drug action and the efficacy of the organic therapeutic agent. The efficacy of the various organic therapeutic agents can often be enhanced [14] upon coordination with a suitable metal ion. The pharmacological activity of metal complexes is highly dependent on the nature of the metal ions and the donor sequence of the ligands because different ligands exhibit different biological properties [15].

There is a real perceived need for the discovery of new compounds endowed with antimicrobial activities. The newly prepared compounds should be more effective and possibly act through a distinct mechanism from those of well-known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant [16]. Many drugs possess modified pharmacological and toxicological properties when administered in the form of metallic complexes [17]. The family of complexes with aza-macrocyclic ligands has remained a focus of scientific attention for many decades. There is continued interest in synthesizing macrocyclic complexes because of their potential applications in fundamental and applied sciences [18].

Encouraged by these reports, in continuation of our studies for the synthesized compound [19], we report herein antibacterial and antifungal activities of Azamacrocyclic transition metal complexes.

II. EXPERIMENTAL SECTION

Chemistry

Materials and Methods

All the reagents used in the preparation of macrocyclic Ligands and their metal complexes were of reagent grade(Merck).The solvents used for the synthesis of

macrocyclic ligands and metal complexes were distilled before use. All other chemicals were of AR grade and used without further purification. The elemental analysis of the compounds was performed on a Perkin Elmer 2400 Elemental Analyser. The FT-IR spectra were recorded using KBr discs on FT-IR Jasco 4100 infrared spectrophotometer. The 1H NMR spectra were recorded using Bruker DRX 400 spectrometer at 400 MHz with TMS as the internal standard. The magnetic moments were measured out using gouy balance. Purity of the compound checked by TLC.

Preparation of the Macrocyclic ligand and its metal complexes

Preparation of 2,6-diformyl-4-methylphenol

The dialdehyde was prepared by a method as follow. To a solution of p-cresol (10.8g, 10 mmole) in (50mL) acetic acid, hexamethylenetetraamine (28.2g, 20 mmole) and (30g, 100mmole) of paraformaldehyde were added. The mixture was stirred continuously until the light brown viscous solution was obtained then heated to (70-90oC) for two hrs. The solution was cooled to room temperature and concentrated H2SO4 (10mL) carefully added. The resulting solution was refluxed for about 30 min and then on treatment with distilled water (400mL) a light yellow precipitate was formed which was stored overnight at (4oC). The yellow product was isolated by filtration and washed in small amount of cold methanol. More pure product was obtained by means of recrystallisation from toluene [19].

Preparation of the macrocyclic Schiff base ligand

The macrocyclic ligand were synthesized by the condensation of 2,6-diformyl-4-methylphenol with Hexamethylenediamine in 2:2 molar ratios in ethanol and refluxed with stirring with few drops of glacial acetic acid. The completion of reaction was monitored by TLC. The yellow solid precipitate of Schiff base obtained was filtered, washed with distilled water, dried and recrystallized from ethanol [19].

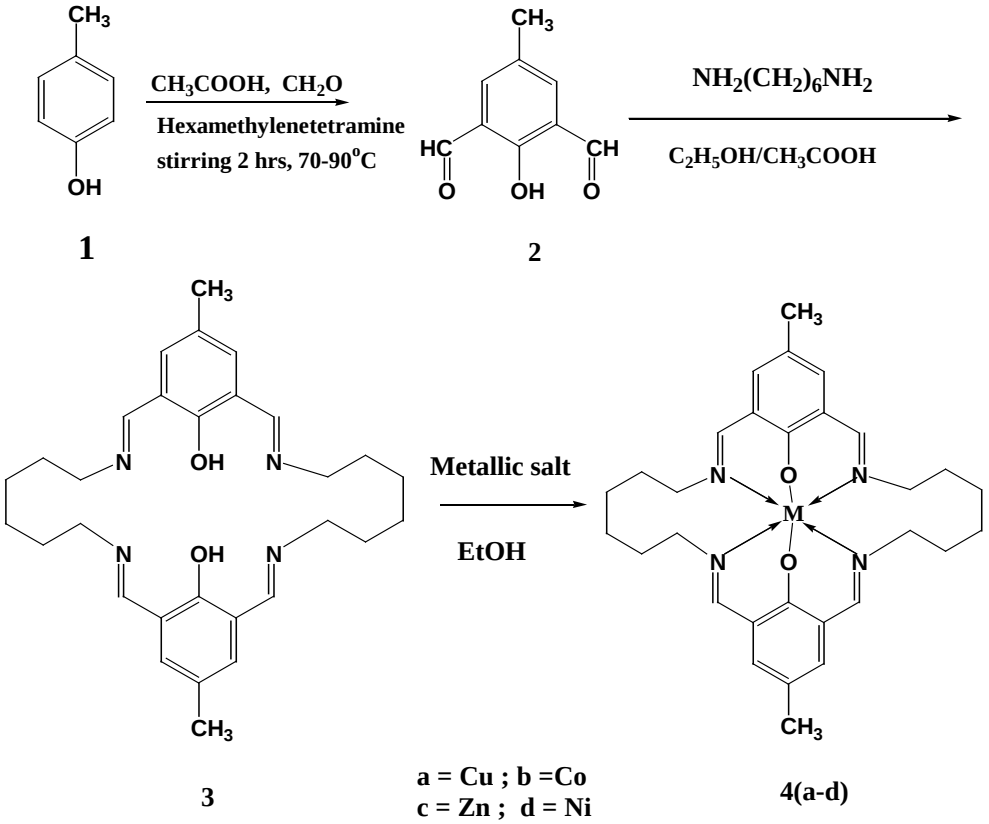
General synthesis of the complexes:

To a boiling solution of metal(II)chloride (2 mmol) and 4-methyl-2,6-diformyl phenol (0.45 g, 4 mmol) in ethanol (20 cm3), an ethanolic solution of diamine (4 mmol) was added slowly with constant stirring and the mixture was boiled under reflux for 4 h when a brown compound separated out. The product was filtered out, washed with ethanol and dried in air [19].

Pharmocology

Materials and methods for the antimicrobial activity

Streptomycin was used as positive controls against bacteria. ketoconazole (Himedia, Mumbai) were used as positive controls against fungi.



Tested microbes

The following gram positive bacteria were used for the experiments; staphylococcus aureus(MTCC 7443), Staphylococcus aureus (MRSA) (MTCC 84), Enterobacter aerogenes(MTCC 111), Micrococcus luteus(MTCC 1538). The gram negative bacteria included Klebsiella pneumoniae(MTCC 109), Salmonella typhimurium(MTCC 2488), Salmonella paratyphi-B(MTCC 733), Proteus vulgaris(MTCC 321). In addition, fungi Candida albicans(MTCC 227), Botyritis cinerea(MTCC 2880), Candida krusei(MTCC 231), Malassesia pachydermatis, were also used for the experiments. All cultures were obtained from the Department of Microbiology, Manasagangotri, Mysore.

Preparation of inoculums

Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHA) (Himedia) for 24 h at 37oC. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about 104 CFU/ml. The filamentous fungi were grown on sabouraud dextrose agar (SDA) slants at 28oC for 10 days and the spores were collected using sterile doubled distilled water and homogenized.

Disc diffusion assay

Antibacterial activity was carried out using a disc diffusion method [20] Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Himedia, Mumbai). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 mins. The tests were conducted at 1000 µg/disc. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Streptomycin (10 µg/disc) was used as positive control. The plates were incubated for 24 h at 37oC for bacteria and 48 h at 27oC for fungi. Zone of inhibition was recorded in millimeters and the experiment was repeated twice.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration studies of synthesized compounds were performed according to the standard reference method for bacteria [21] and filamentous fungi [22]. Required concentrations (1000 µg/ml, 500µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml and 15.62 µg/ml) of the compound was dissolved in DMSO (2%), and diluted to give serial two-fold dilutions that were added to each medium in 96 well plates. An inoculum of 100 ml from each well was inoculated. The anti-fungal agent's ketoconazole, fluconazole for fungi and streptomycin, ciprofloxacin for bacteria were included in the assays as positive controls. For fungi, the plates were incubated for 48-72 h at 28oC and for bacteria the plates were incubated for 24 h at 37oC. The MIC for fungi was defined as the lowest extract concentration, showing no visible fungal growth after incubation time. 5 ml of tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperatures. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate.

III. Result and Discussion

The template condensation of 2,6-diformyl-4-methylphenol with Hexamethylenediamine in 2:2 molar ratios in the presence of metal chloride in ethanol yields light and dark brown complexes.

The synthesized complex were characterised using different spectroscopic techniques and detailed results along with catalytic activity were clearly discussed earlier [19]. Further in this paper we have reported antifungal and antibacterial activity of the synthesized compounds.

Pharmocology

The antimicrobial activities of synthesized complex compounds were screened against eight bacteria and four fungi using in vitro disc diffusion method. The results revealed that most of the synthesized complex compounds exhibited antimicrobial activities against *Staphylococcus aureus, Staphylococcus aureus (MRSA), Enterobacter aerogenes, Micrococcus luteus, Klebsiella pneumoniae, Salmonella typhimurium, Salmonella paratyphi-B, Proteus vulgaris, Candida albicans, Botyritis cinerea, Malassesia pachydermatis, and Candida krusei* organisms. The results are summarized in Table 1 and 2.

All the synethezied complexes showed good activity when compared with standard drug against S. aureus. Compound b with cobalt has metal ion showed potent activity against both Gram-positive and Gram-negative bacteria among all synthesized compounds compared with the standard. Compound a with copper metal shown good activity against *S. aureus, P. vulgaris and S. typhimurium.* Compound c with zinc metal has shown good activity against *S. aureus, E. erogenes, K.pneumonia, P. vulgaris and S. typhimurium* whereas compound d with nickel has shown poor activity against both Gram-positive and Gram-negative bacteria except S. aureus and E. erogenes.

Compound b showed significant antifungal activity against all the tested fungi compared with standard drug. Compound a has shown potent activity against *C. albicans*

C. Krusei, *M. pachydermatis*. Similarly compound c showed more activity against *C. albicans* and *C. krusei* compared to standard drug. In contrast, compounds d with exhibited poor activity.

Further for proficient molecules MIC values were determined by broth dilution method against selected strains and the MIC values of active compounds (a-d) against bacteria and fungi are given in Table 3 and 4. Significant MIC values were observed against Gram positive and Gram negative bacteria. In particular, it is noticeable that compound a and b has exhibited good MIC result against tested bacteria and fungi. Finally we can conclude that compound b with cobalt has metal ion showed potent activity against both Gram-positive and Gram-negative bacteria among all synthesized compounds compared with the standard.

Table 1: In-vitro antibacterial activity of complex compounds (a-d)

Compounds	Zone of inhibition in mm							
	Gram positive bacteria				Gram negative bacteria			
	S. aureus	S. aureus (MRSA)	E. erogenes	M. luteus	K. pneumonia	P. vulgaris	S. typhimurium	S. Paratyphi-B
a [cu]	21	15	18	11	18	17	23	10
b[co]	22	18	23	20	21	17	23	22
c[Zn]	21	11	20	19	20	15	22	13
d[Ni]	20	18	20	11	10	12	13	15
Streptomycin	24	21	26	23	23	19	25	25

Table 2: In-vitro antifungal activity of complex compounds (a-d)

	Zone of inhibition in mm			
Compounds	B. cinerea	C. albicans	C. krusei	M. pachydermatis
a	11	20	15	20
b	12	22	17	23
c	11	21	16	17
d	10	12	11	15
Ketoconazole	14	23	18	24

Table 3: MIC (□g/ml) of complex compounds (a-d) against tested bacteria

Compounds	Minimum inhibitory concentration (□g/ ml)							
	Gram positive bacteria				Gram negative bacteria			
	S. aureus	S. aureus (MRSA)	E. aerogens	M. luteus	K. pneumonia	P. vulgaris	S. typhimurium	S. Paratyphi-B
a	15.62	126	62.5	62.5	560	125	31.25	500
b	15.32	31.15	62.0	15.32	124	31.15	62.5	500
c	31.15	250	500	250	250	250	125	250
d	31.0	62.5	250	31.25	250	62.5	31.25	250
Streptomycin	6.25	>100	25	6.25	6.25	ni	30	6.25
Ciprofloxacin	<0.78	>100	>100	<0.78	<0.78	6.25	>100	<0.78

ni = no inhibition

Table 4: MIC (□g/ml) of complex compounds (a-d) against tested fungi

Compounds	Minimum inhibitory concentration (□g/ ml)			
	B. cinerea	C. albicans	C. krusei	M. pachydermatis
a	15.32	62.5	250	125
b	15.32	15.32	62.5	125
c	62.5	125	250	250
d	255	125	125	125
Fluconazole	ni	>100	12.5	12.5
Ketoconazole	25	25	15	15

ni = no inhibition.

IV CONCLUSION

All the synthesized complexes were characterized by elemental analysis, molar conductance, IR, 1H-NMR, mass, electronic spectra and thermal studies. An octahedral geometry was proposed for all metal (II) complexes with N, O as donor atoms which was supported by magnetic and electronic spectral studies. Synthesized Cu(II) showed good catalytic activity for the conversion of benzyl alcohol to benzaldehyde.

The antimicrobial activities of synthesized complex compounds were screened against eight bacteria and four fungi using in vitro disc diffusion method. The results revealed that most of the synthesized complex compounds exhibited antimicrobial activities. Compound b with cobalt has metal ion showed potent activity against both Gram-positive and Gram-negative bacteria among all synthesized compounds compared with the standard.

From the present study, we infer that, aza macrocyclic metal complexes could lead to the development of newer therapeutics as antimicrobial agent.

Conflict Of Interest

The authors declare that they have no conflicts of interest with respect to the content of the manuscript.

Acknowledgements

One of the authors Bushra Begum A gratefully acknowledges the financial support provided by the UGC, under the Minor Research Project Scheme [MRP(S)/13-14/KAMY002/UGC-SWRO].

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