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Synthesis, Characterization and Biological Studies of Macrocyclic Transition Metal Complexes

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ABSTRACT

A new series of tetraaza macrocyclic complexes [$MLCl_2$] were synthesized via template condensation of 2- [4-methyl-2- (2-oxo-1, 2) -diphenyl-ethylideneamino) -phenylimino] -1,2-diphenyl-ethanone with various diamines and metal salt (Where, M = Zn (II) and Cd (II)). These complexes were identified by basic elemental analysis and spectroscopic techniques (IR, ^{1}HNMR and mass). Based on these studies, an octahedral arrangement is provided around the metal. The ligand and its metal complexes have been tested for antimicrobial activity against bacteria (*B. subtilis, S. aureus*) and fungi (*A. niger, C. albicans*) and the results have shown that the complexes are more active than the free ligands.

Keywords: Tetraaza macrocyclic complexes, Template condensation, Antibacterial activity, Antifungal activity.

INTRODUCTION

Macrocyclic transition metalcomplexes have a focus of scientific attention. The changing macrocyclic metal complexes have received much attention as an active component of metalloenzymes. [1] The widespread use of macrocyclic complexes has been the cause of their rapid growth in the last two decades alone. [2] Macrocyclic ligands and complexes belong to a special category with various atomic donors, exhibiting interesting coordination modes due to their potential use as building blocks in macrocyclic chemistry [3], environmental chemistry [4] and biomedical engineering. [5] These compounds exhibit biological activity, such as antiviral [6], antimicrobial [7,8], anticancer [9], antitumor [10], antibiotics [11], anti-diabetic [12] and DNA-binding cleavage activities. [13, [14] The toxicity of free metal ions is greater than that of the metal complexes due to the reduction in its bioavailability. [15] In the literature, the structures of Zn (II) and Cd (II) have also received much attention due to their biological functions. Zinc deficiency, however, causes the effects of growth and disruption of the central nervous system. The Schiff base macrocyclic complexes can act as interacting electrophilic agents with metals, such as Zn (II), etc. present in the active sites of enzymes to be efficient antibiological agents.[16] Essentially, cadmium is an environmental pollutant and inhibits the activity of RNA polymerase in vivo [17] and responds easily to biological molecules, so the macrocyclic chemistry of cadmium complexes with multi-dentate ligands may have significant potential as opposed to the treatment of heavy metal poisoning is very attractive. [18] Different types of zinc contain ligands of different haptacity especially O, N and S as donor atoms, showing different coordination numbers and geometries, which often create dimeric or polymeric types are reported. [19] Due to the growing interest in macrocyclic complexes, In this paper we report tetraazamacrocyclic complexes of Zn (II) and Cd (II) of the type, [$MLCl_2$] formed in response to template condensation reaction. The coordination behavior of these synthesized complexes was examined via elemental analysis and spectral studies and they were also tested for their in vitro antimicrobial activity against some bacterial and fungal strains.

1. EXPERIMENTAL

All chemicals are purchased from Sigma-Aldrich and used without purification. Used solvents are dried and cleaned in appropriate ways. The nitrogen and chlorine estimation were done by Kjeldahl and Volhard method, respectively. [20] Metals are estimated gravimetrically. [21] The Rast Camphor method has been used to determine the molecular weight of macrocyclic

metal complexes. The FT-IR spectra were taken as KBr discs in the range of 4000-400 cm^{-1} on the Shimadzu FTIR-550 spectrophotometer. The ^{1}H NMR spectra were recorded on the JEOL-DELTA2-NMR 400MHz spectrometer in $CDCl_3$ using TMS as the internal standard.

2.1 Synthesis of Ligand

In the preparation of ligand, an estimated amount of 1,2 diphenyl-1,2-ethanedione (benzil) (20 mmol, 4.20 g) was dissolved in 40 mL of ethanol and calculated amount of 3,4-diaminotoluene (10mmol, 1.22 g) added in 2: 1 molar ratio. The reaction mixture was heated under reflux for 4-6 h on the ratio head. The volume of the compound was concentrated in half and kept in a desiccator overnight at room temperature. Colored crystalline products obtained thus purified by the re-crystallizationwith the same solvent and dried in a vacuo. [22]

2.2 Synthesis of macrocyclic metal complexes

Macrocyclic metal complexes were prepared by template condensation of ligand with various diamines namely 1,2-phenylenediamine or 4-chloro-1,2-phenylenediamine or 4-fluoro-1,2-phenylenediamine and 1,8 diaminotoluene in the presence of metal salt in a 1: 1: 1 molar ratio to dry methanol. A methanolic solution of ligand (10 mmol) was taken to around bottom flask of 100 mL and mixed with a methanolic solution of diamine (10 mmol) and metal chloride (10 mmol) at a 1: 1: 1 molar ratio. The reaction mixture was reflux for approximately 7–8 h on the ratio head. The mixture was concentrated to half of its volume and stored in a desiccator at room temperature. Solid products werecollected and washed with methanol and dried under vacuo. [23] The physical characteristics and analytical data of theligand and its metal complexes are given in Table 1. The proposed structure of macrocyclic metal complexes is shown in Fig. 1.

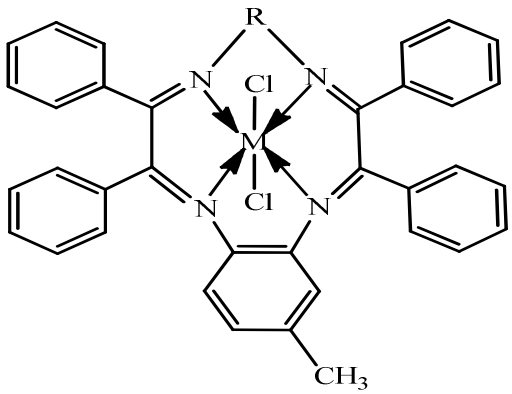

Fig 1: Proposed structure of macrocyclic metal complexes

Where M= Zn(II) and Cd(II) and R= 1,2-phenylenediamine or 4-chloro-1,2-phenylenediamine or 4-fluoro-1,2-phenylenediamine and 1,8 diaminotoluene.

Table-1: The physical and analytical properties of ligand and metal complexes.

S.N.	Compounds	M.W. (calculated)	Colour	M.P. (0°C)	Elemental analysis found (calculated)%				
					C	H	N	Cl	M
1	$C_{35}H_{26}N_2O_2$	505.31 (506.59)	Dark Brown	70	82.80 (82.98)	5.10 (5.17)	5.40 (5.52)	-	-
2	$[Zn(C_{41}H_{30}N_4Cl_2)]$	712.78 (715.00)	Brown	152	68.80 (68.87)	4.10 (4.22)	7.71 (7.83)	9.80 (9.91)	9.03 (9.14)
3	$[Zn(C_{41}H_{29}N_4Cl_3)]$	747.21 (749.44)	Black	165	65.59 (65.70)	3.79 (3.90)	7.38 (7.47)	14.10 (14.19)	8.60 (8.72)
4	$[Zn(C_{41}H_{29}N_4FCl_2)]$	730.09	Black	158	67.06	3.87	7.55	9.58	8.81

		(732.98)			(67.18)	(3.98)	(7.64)	(9.67)	(8.92)
5	$[Zn(C_{45}H_{32}N_4Cl_2)]$	762.86 (765.05)	Black	142	70.48 (70.64)	4.10 (4.21)	7.20 (7.32)	9.17 (9.26)	8.42 (8.54)
6	$[Cd(C_{41}H_{30}N_4Cl_2)]$	760.22 (762.01)	Brown	162	64.50 (64.62)	4.83 (3.96)	7.24 (7.35)	9.16 (9.30)	14.61 (14.75)
7	$[Cd(C_{41}H_{29}N_4Cl_3)]$	794.26 (796.46)	Black	176	61.70 (61.82)	3.57 (3.66)	6.92 (7.03)	13.23 (13.35)	14.00 (14.11)
8	$[Cd(C_{41}H_{29}N_4FCl_2)]$	797.76 (780.01)	Black	184	63.01 (63.13)	3.63 (3.74)	7.05 (7.18)	9.00 (9.09)	14.30 (14.41)
9	$[Cd(C_{45}H_{32}N_4Cl_2)]$	810.00 (812.07)	Black	155	66.43 (66.55)	3.84 (3.97)	6.80 (6.89)	8.60 (8.73)	13.75 (13.84)

2.3 Biological assay

Antimicrobial properties of all the complexes were evaluated and compared with the standard drugs. The microorganisms used were *Bacillus subtilis, Staphylococcus aureus, Candida albicans* and *Aspergillus niger*.

2.3.1 In vitro antibacterial activity

All test compounds were dissolved in DMSO and the concentration of the stock solution was made 5 mg / mL.Each well was filled witha 50 µL test compound. Streptomycin was used as positive control (concentration of 5 mg / mL). Agar plates are designed for antibacterial activity. Mueller-Hinton agar medium and Sabouraud dextrose agar medium are susceptibility test medium that has been validated by CLSI for screening the antibacterial activity by disk/well diffusion method. Fresh cultures of test strains of *Staphylococcus aureus and Bacillus subtilis* were placed in peptone water and stored for 30 minutes at 37°C. Bacterial suspension compared to 0.5 McFarland standard turbidity. Bacterial cultures were swabbed onto the Mueller hinton agar surface. Wells were then filled with 50 µL of different dilutions prepared from stock. The *Staphylococcus aureus and Bacillussubtilis* plates were kept for incubation at 37°C for 24-48 h and results were observed.

2.3.2 In vitro antifungal activity

Different concentrations of the test compounds under study were prepared from the stock solution (5 mg/mL and 2.5 mg/mL) and out of which 50 µL was used in each well. Saboraud dextrose agar medium is used for screening the antifungal activity by well diffusion susceptibility testing. *Aspergillus niger* and *Candida albicans* were inoculated in normal saline (0.9%) and kept for 48 h at 28°C. The fungi were swabbed on to the Saboraud dextrose agar surface. 50 µL from different dilutions prepared from stock (5mg/mL and 2.5mg/mL of the compound) was loaded into the respective wells. The antifungal plates were kept for incubation at 28°C for 7 days.

With the same procedures as mentioned above at the same concentration and solvent, the antimicrobial activity of the stsndard antibiotics streptomycin and Itraconazole was also recorded. The medium containing DMSO as a solvent was used as a negative control and the medium of the drug was used as a positive control. The experiments were repeated for three times.

2. RESULTS AND DISSCUSION

Tetraaza Schiff base macrocyclic complexes of the type $[MLCl_2]$ [M = Zn (II) and Cd (II)] are synthesizedby the template condensation reaction. The complexes were recrystallized from methanol and purity of the complexes was checked by thin layer chromatography (TLC) run in 1:1 benzene–methanol. All complexes were stable at room temperature and soluble in DMSO, $CHCl_3$ and DMF. The molar conductance data of the 10^{-3}M solution of the complexes measured in DMSO showed the non-electrolytic nature of all the complexes. The geometry of all complexes was determined from the position of the bands in the FT-IR spectra, resonance signals at ^{1}H NMR and mass spectra.

3.1 IR spectra

IR spectrum of diamines shows a pair of bands near at 3,250 & 3,390 cm^{-1} corresponding to $\upsilon(NH_2)$ group which were absent in the IR spectra of all the complexes. Further, no strong absorption band was observed near 1675 cm^{-1} indicating the absence of C=O group. The disappearance of these bands and appearance of a new strong absorption band near 1610–1625 cm^{-1} confirms the condensation of carbonyl and amino group and formation of macrocyclic Schiff's base as these bands may be assigned due to $\upsilon(C=N)_{str}$ vibrations. [24] The lower value of υ(C=N) can explained on the basis of a shift of lone pair density of azomethine nitrogen towards the metal atom indicating that coordination takes place through nitrogen of C=N groups.[25] The various absorption bands associated with υ(C=C) aromatic stretching vibrations are present in the region 1,400-1,588 cm^{-1} and bands at 740–780 cm^{-1} may be assigned to C–H out of plane bending of phenyl groups. The phenyl ring absorption appears in the 1462-1494 and 1354-1385 cm^{-1} region are assigned to $\upsilon_{asym}C_6H_5$ and $\upsilon_{sym}C_6H_5$ respectively. The appearance of a new band of medium intensity in the range of 420–445 cm^{-1}, attributed to ν(M-N), which provides strong evidence for the coordination of the imine nitrogen to the metal ion.

3.2 ^{1}H- NMR spectra

The ^{1}H-NMR spectrum of macrocyclic Zn(II) and Cd(II) complexes recorded in DMSO-d_6 (tetramethylsilane (TMS) as internal standard) shows a sharp signal at 2.08 ppm which may reasonably be assigned to the methyl protons (CH_3; 3H). Multiplets observed in the region 7.23–7.96 ppm which are assigned to aromatic protons.The spectra of the ligands and Zn(II) complexes do not show any signals assignable to primary amino protons. This is strong evidence that the proposed macrocyclic complexes are formed by template reactions.

3.3 Mass spectra

Mass spectroscopy is mainly applied in the analysis of complexes has been increasingly used as a powerful structural characterization technique in coordination chemistry. The EI mass spectrum of the $[Cd(C_{41}H_{29}N_4FCl_2)]$ complex (fig- 2.), was studied as a representative case. Peaks of appreciable intensity were observed at m/z values 631.04 for $[CdC_{35}H_{24}N_4F]^+$, 629.05 for $[CdC35H_{22}N_4F]^+$, 477.06 for $[CdC_{23}H_{14}N_4F]^+$ and 286.05 for $[CdC_4N_4Cl_2]^+$. The molecular ion peak for the complex $[Cd(C_{41}H_{29}N_4FCl_2)]$ was observed at m/z 779.03 (calcd m/z = 780.01) and this is in good agreement with the respective theoretical molecular mass of the complex and also these data confirm the mononuclear nature of the complex.

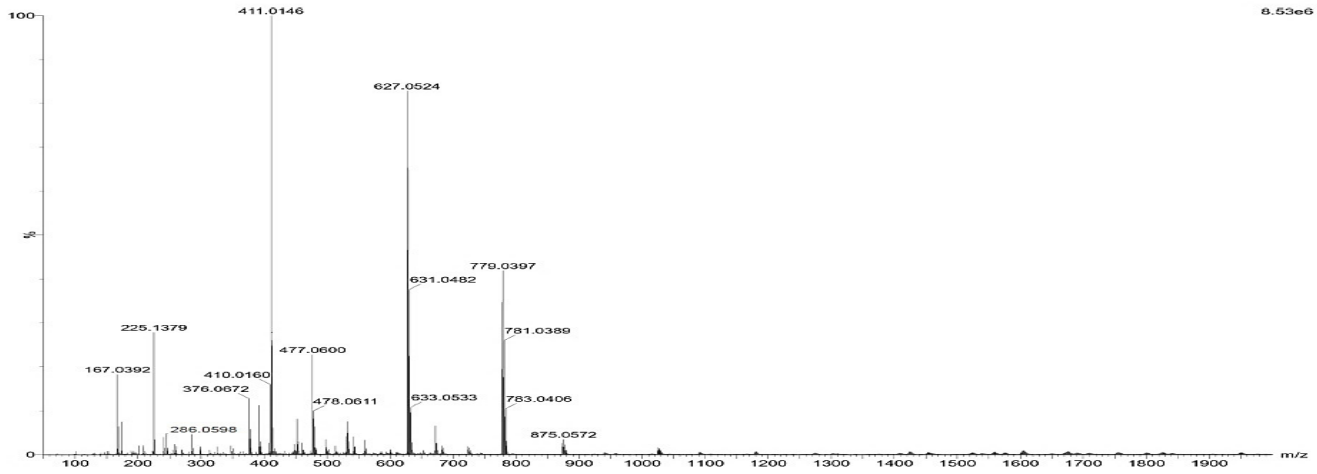

Fig 2: Mass spectrum of the $[Cd(C_{41}H_{29}N_4FCl_2)]$ complex.

2.4 BIOLOGICAL ASSAY

Antimicrobial activity of the ligand and its metal complexes was studied against two bacteria: *Bacillus subtilis* and *Staphylococcus aureus* and two fungi: *Candida albicans* and *Aspergillus niger*. The results were obtained as diameter of inhibition zone (in mm) and compared with the diameter of inhibition zone (in mm) of standard drugs streptomycin for bacteria and itraconazole for fungi. All the complexes were found to be active on all the microorganisms used. The antimicrobial screening data indicate that the metal complexes are more potent antimicrobial agents than the free ligand. The reason for the high antimicrobial activity of metal complexes over their cheating agents can be explained on the basis of the concept of Overtone and the theory of Tweedy's chelation. [26] According to Overtone's concept of cell acquisition, the lipid membrane around the cell prefers the transfer of only lipid-soluble substances because lipophilicity is an important factor that regulates antimicrobial activity. Chelation reduces the polarity of the central metal atom due to the partial sharing of its positive charge by the ligand. In addition, it increases the regeneration of π-electrons over the entire chelate ring and enhances the lipophilicity of the complexes, which favors the permeation of the complexes with the lipid layer of the cell and thus inhibits various metabolic activities of microorganisms. [27] The Zn (II) and Cd (II) complexes show moderate to good activity against all organisms. Therefore, these compounds should be further investigated in order to test their effectiveness as antimicrobial agents.The antimicrobial screening data for the ligands and their complexes are shown in Table- 2 and Fig- 3.

Table 2:Antimicrobial activity of ligand and their corresponding macrocyclic metal complexes

S.No.	Compounds	Diameter of inhibition zone (in mm)			
		Bacillus subtilis	Staphylococcus aureus	Candidaalbicans	Aspergillus niger
1.	$C_{35}H_{26}N_2O_2$	6.8	7.2	8.3	9.6
2.	$[Zn(C_{41}H_{30}N_4Cl_2)]$	12.6	13.7	9	14.7
3.	$[Zn(C_{41}H_{29}N_4Cl_3)]$	14	12	10.2	13.6
4.	$[Zn(C_{45}H_{32}N_4Cl_2)]$	14.5	14.6	9.5	13
5.	$[Cd(C_{41}H_{29}N_4Cl_3)]$	7.5	8.6	8.3	11.2
6.	$[Cd(C_{41}H_{29}N_4FCl_2)]$	8.1	9.5	7.6	9.8
7.	$[Cd(C_{45}H_{32}N_4Cl_2)]$	9.4	11.2	9.4	12.1
8.	Streptomycin	**16**	**18**	-	-
9.	Itraconazole	-	-	**14**	**18**

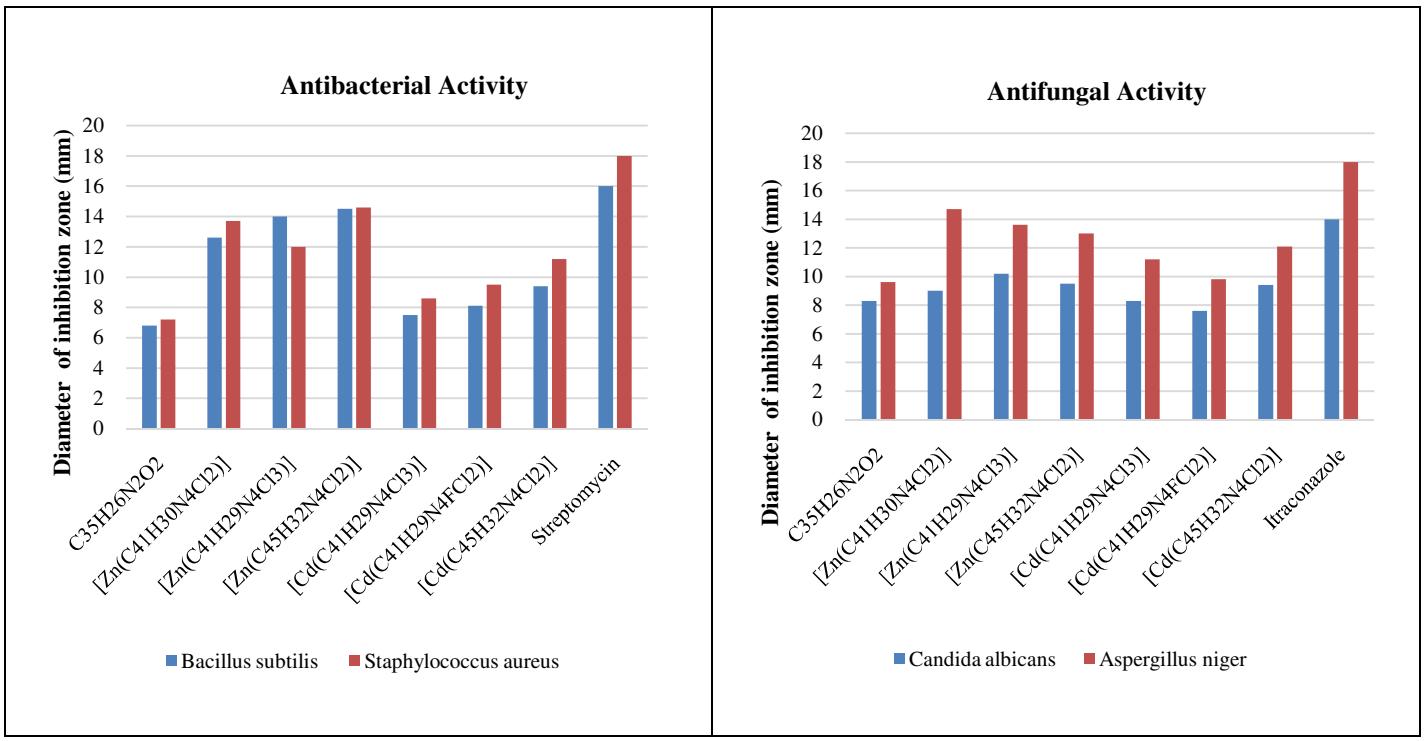

Fig 3: Antimicrobial activity of ligand and their metal complexes.

3. CONCLUSIONS

The new Zn (II) and Cd (II) macrocycliccomplexes were prepared using template condensation for optimal yield. The ligand and its metal complexes were identified using elemental analysis, molecular weight determination, IR, ^{1}HNMR and EI mass spectroscopy. On the basis of spectral data, an octahedral geometry has been suggested in all macrocyclic complexes. The results also reveal the formation of monomeric macrocyclic metal complexes. Antimicrobial data have shown that these metal complexes are superior to the free ligand in the inhibition of growth of the tested microorganisms. Improved function of macrocyclic complexes is defined on the basis of chelation theory.

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