



Synthesis, Characterization and Antibacterial Activity of Zn (II) Tetraaza Macrocylic Complexes

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ABSTRACT

The objective of present study was to synthesize a new series of metal based therapeutic agents with good antimicrobial and antioxidant properties and their structural characterization. Synthesis of Zn complexes of type $[M(C_{16}H_{10}N_4O_2)X_2]$, where $M=Zn(II)$, $X = Cl^-$, NO_3^- and CH_3COO^- derived from template condensation of 1,3-dicarbonyl-phenyl-dihydrazide and 1H-indole-2,3-dione (isatin) and their characterization by UV-Vis, FT-IR, H-NMR and mass spectral studies. Antimicrobial activity was determined by using agar well diffusion method against two bacterial strains *S.aureus* and *B.Subtilis*. All test compounds posses varied but significant antibacterial activity against the bacterial strains.

KEYWORDS : Antibacterial, macrocyclic complex, *S.aureus*, *B. subtilis*

1. INTRODUCTION

The design and study of metal containing macrocyclic molecules have attracted the attention of researchers for a number of reasons: their pharmacological [1-2], physiological activities, and their role in molecular processes [3-4], drug development, and usefulness in many catalytic applications [5-6]. Macrocyclic compounds and their derivatives have been proven to be excellent host system for a guest metal ion [7-10].

Prompted by above facts the synthesis and characterization of the chemical structure derived from the template condensation of 1, 3-dicarbonyl-phenyl-dihydrazide and isatin (1H-indole-2,3-dione) with Zn(II) salts have been discussed. Besides characterization of synthesized species by modern instrumentation techniques i.e. molar conductance, UV-Vis, IR, NMR and mass spectroscopy, compounds were also evaluated for their antibacterial potential against various pathogenic strains of bacteria.

2. EXPERIMENTAL

2.1 Materials

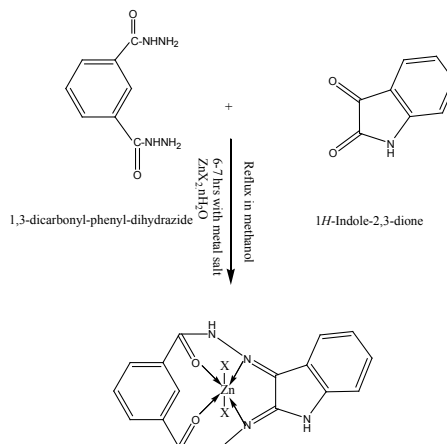
All the reagents and solvents used in this study were of AR grade. Diethylbenzene-1, 3-dicarboxylate, isatin (1H-indole-2,3-dione) and 98% hydrazine hydrate were purchased from Sigma(USA) and Aldrich(Germany).

The metal salts were purchased from S.D. fine, Mumbai India, Merck, Ranbaxy, India, and were

used as received. Solvents like methanol, ethanol ether, DMSO and DMF were used as such without any distillation.

2.3 Synthesis of complexes

The solution of Zn(II) of Cl^- , NO_3^- , or CH_3COO^- (0.01mol) salt dissolved in the minimum quantity of methanol (\approx 20ml) was added to a hot stirring solution of 1,3-dicarbonyl-phenyl-dihydrazide (0.01mol, 2.52gm) in methanol(50 ml) and 4-5 drops of dil. HCl were added. The resulting solution was refluxed at 35-40°C for half an hour. Subsequently, isatin (0.01mol, 1.65gm) was added to it and refluxing was continued for 6-7 hours. The mixture was then allowed to cool at room temperature through evaporation. Dark-colored precipitates, were filtered, washed with methanol and dried in vacuum. The obtained yields were \approx 60-65 %. The complexes were only soluble in DMF and DMSO. Scheme



Scheme: Synthesis of metal macrocyclic complex $ZnLX_2$ where, $X = Cl^-$, NO_3^- and CH_3COO^- template condensation of 1,3-dicarbonyl-phenyl-dihydrazide and 1H-indole-2,3-dione

2.4 In vitro antimicrobial activity

All the newly synthesized complexes were evaluated for their antibacterial activities towards bacterial and fungal strains using agar well diffusion method [11]. Bacterial cultures were procured from microbial type culture collection IMTECH Chandigarh and sub cultured on nutrient agar medium.

Test microorganism and medium

Test organisms were chosen on the basis of their clinical importance on causing diseases in human eg. *Staphylococcus aureus* and *Bacillus subtilis* (gram +ve) bacterial strains. Mueller Hinton Agar (MHA) was used as the culture medium for antibacterial screening.

The qualitative and quantitative analysis of antibacterial potential of the newly synthesized ligand and its metal complexes against all microbial strains has been studied by Agar well diffusion method [11-12]. Zone of inhibition was measure by using a standard zone reader in mm. Ciprofloxacin (antibiotic) was used as positive control and DMSO was used ad negative control. All the experiments for each strain were performed in triplicates.

3. RESULTS AND DISCUSSION

3.1 Analytical and physical measurement

The analytical data showed a 1:1 stoichiometry for all synthesized

species and suggested formula for macrocyclic complexes as: $[Zn(C_{16}H_{10}N_4O_2)_2X_2]$, where $X = Cl^-$, NO_3^- or CH_3COO^- . The metal complexes were dissolved in DMF and molar conductivities of 20 ml of 10^{-3} M of their solutions were measured at room temperature. All the complexes showed molar conductance $10-15 \Omega^{-1} cm^2 mol^{-1}$ which indicates the non electrolytic nature of all complexes. All complexes gave satisfactory micro elemental analyses results, as shown below.

Physical properties i.e. Formula, color, Molecular weight, molar conductivity(Ωcm^{-1}) % of C, H, N, O, M, Cl Found(calculated).

Complex 1: $Zn(C_{16}H_{10}N_4O_2)(OCOCH_3)_2$, Light brown, 473, $10.5 \Omega cm^{-1}$, C=50.7(50.2)%, H=3.4(3.2)%, N=11.8(11.7)%, O=20.2(20)%, M=13.8(13.7)%.

Complex 2 $Zn(C_{16}H_{10}N_4O_2)(NO_3)_2$, light brown, 479, $14.2 \Omega cm^{-1}$, C=40.06(40)%, H=2.1(1.9)%, N=17.52(17.4)%, O=26.68(26)%, M=13.63(13.5)%

Complex 3; $Zn(C_{16}H_{10}N_4O_2)Cl_2$, light brown, 426, $15 \Omega cm^{-1}$, C=45(44)%, H=2.3(2.1)%, N=13.1(13)%, O=16.6(16.2)%, M=7.5(7.3)%, Cl=15.33(15.3)%

3.2 Infra- red spectral Analysis

To understand the binding mode of ligand to metal in complexes, IR spectra of free ligand and its metal complexes were compared successfully. Ligand gave three specific IR absorption bands in the IR region at $3270 cm^{-1}$, $1620 cm^{-1}$, $1687 cm^{-1}$ and may be assigned due to vibrational absorption for ν_{N-H} , $\nu_{C=N}$ ($\nu_{C=O}$ hydrazide) respectively. Disappearance of a pair of bands corresponding to (ν_{NH_2}) stretch at 3300 & $3350 cm^{-1}$ and $1710 cm^{-1}$ ($\nu_{C=O}$ isatin) while appearance of a strong band at $1620 cm^{-1}$ indicates the condensation between both of the molecules [13].

The band corresponding to $\nu_{C=N}$ appears at lower frequency by $10-30 cm^{-1}$ in the complexes and indicates the coordination of nitrogen atoms of azomethine groups to metal atom [14] as a result of lower electron density around nitrogen atom of C=N bond.

The values given in the table 2 indicates that the peak corresponding to $\nu_{C=O}$ group of the -CONH moiety has been shifted to lower side and appears around $1620-1640 cm^{-1}$ in the spectra of all the complexes and suggesting the involvement of oxygen of the carbonyl group in coordination with metal ion. Shifting of C=N and C=O band of dihydrazide confirms the tetradentate coordination of ligand to metal.

The bands appeared in the region $450-420 cm^{-1}$ are attributed to (M-N) vibrations and indicate the coordination of azomethine nitrogen to metal [15]. The bands present between $320-350 cm^{-1}$ may be arising due to $\nu_{(M-Cl)}$ vibrations.

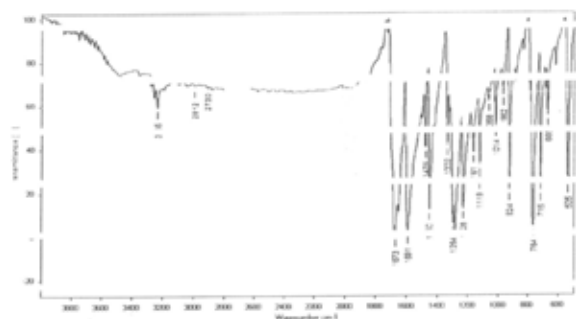


Fig.1 FT-IR spectrum of complex $Zn(C_{16}H_{10}N_4O_2)(OCOCH_3)_2$ at room temperature in the range of $600-4000 cm^{-1}$

3.3 UV-VIS Spectral analysis and magnetic measurement

The electronic absorption spectra of ligand and all complexes were recorded in DMSO at room temperature ranging $200 nm-900 nm$. [16]

The Zn (II) complex exhibit a single band at $32550 cm^{-1}$ assignable to charge transfer from ligand to metal. These complexes are dia-

magnetic due to completely filled d-orbital and geometry may be assigned octahedral [16]

3.4 1H NMR spectroscopy

The 1H NMR spectrum of Zinc(II) complex showed a singlet at 8.45 ppm due to protons of the -CONH moiety [34]. A singlet at 2.4 ppm may be due to $-CH_3$ proton [35]. The multiplets at 7.20 – 7.88 ppm may be assigned to hydrogens of aromatic rings [36]. A broad singlet at 10.80 ppm may be assigned to proton of the -NH group of isatin [16]

3.5 Mass spectral analysis

ESI Mass spectrums of all complexes were recorded to confirm the proposed structures. A less intense peak was observed for complex $Zn(C_{16}H_{10}N_4O_2)(OCOCH_3)_2$ at m/z 471.5 which is corresponding to the M^{+1} values as the calculated mass of the complex is 473 a.m.u. This peak may be assigned to molecular ion peak for complex. Subsequent peaks are observed at 412 and 292.0 in the spectrum which may be assigned to removal of first and remaining two acetate groups respectively from the molecule.

3.7 Biological studies

3.7.1 Antibacterial assay

The Zone of inhibition for both of the bacterial strains was observed and following results were obtained:

$Zn(C_{16}H_{10}N_4O_2)(OCOCH_3)_2$: 12mm for S.aureus and 11.7mm for B. subtilis

$Zn(C_{16}H_{10}N_4O_2)(NO_3)_2$: 16.5mm for S.aureus and 18.8mm for B. subtilis

$Zn(C_{16}H_{10}N_4O_2)Cl_2$: 15.3mm for S.aureus and 13.5mm for B. subtilis

Ciprofloxacin (standard antibiotic): 26mm for S.aureus and 24mm for B. subtilis

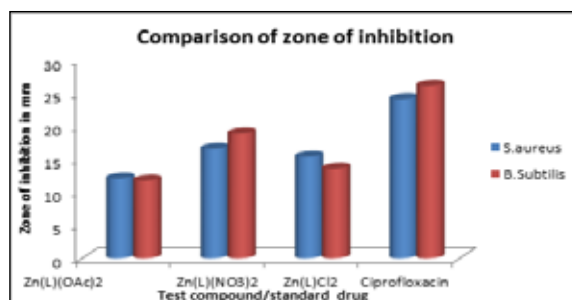


Fig.2: Comparison of zone of inhibition for all synthesized compounds against both bacterial strains

3.7.2 Determination of bacterial susceptibility

It was observed that all the compounds are more effective against gram +ve bacterial strains. Compound 2 is most effective against all the bacterial strains. Compound 1 and 3 are less effective against both of the strains. It indicates that the effect of individual metal, its electron density, coordination potential, dipole moment, conductance also affect its overall biological behavior [17].

The antimicrobial results of the ligand exhibited a considerable enhancement on coordination with the transition metal ions against all bacterial strains. [18-19]

Conclusion

Present investigation shows that the tetradentate ligand derived from template condensation of 1, 3-dicarbonyl-phenyl-dihydrazide and isatin coordinates readily with all divalent zinc salts and afford the synthesis of octahedral complexes.

However detailed spectroscopic study including UV, IR, NMR and Mass spectrum was needed to investigate the influence of structure and coordination on the reactivity of the corresponding ligand. Mass spectral study confirms the formation of a monocentric complex.

An investigation of biological behavior of all synthesized species has shown significant antimicrobial and antiradical results.

A detailed structural and biological investigation of this series of complexes would throw more light on the influence of metal coordination on the reactivity of macrocyclic molecules which may be further explored and used as alternative therapeutic agents.

Experimental protocol

The microanalysis was realized using an elemental analyzer (Perkin Elmer 2400) at IIT Delhi. The magnetic susceptibility measurements of the compounds were carried out by Gouy balance at room temperature; the IR spectra were recorded on Thermo Scientific Nicolet S 50 FT-IR Spectrometer in the range 4000-400 cm^{-1} using ATR. UV-Visible spectra in DMSO were recorded on PerkinElmer Lambda 25 spectrophotometer ranging 200-900nm. The molar conductance was measured on digital conductivity meter. ESI Mass spectra were obtained from JEOL-ACCU TOF JMS-T100LC mass spectrometer ranging 50.0-1000amu. The ^1H NMR spectra was recorded at room temperature in DMSO on a Bruker AVANCE II 400 NMR spectrometer (400 MHz) from IIT Delhi

The metal contents in the complexes were determined by AAS. Melting points were determined by using capillaries in electrical melting point apparatus.

Abbreviations:

B.M.: Bohr magneton;
DMF: N, N-dimethylformamide;
DMSO: Dimethylsulphoxide;
CFU: Colony forming unit;
MHA: Mueller Hinton agar;
IR: Infrared;
AAS: Atomic Absorption Spectroscopy

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