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Synthesis of Biologically Active Divalent Tetraaza Macrocyclic Metal Complexes

*** Preeti Jain** Department of Applied Chemistry, Gautam Buddha University, Gautam Budh Nagar, India, * Corresponding author

Vandna Singh Department of Applied Chemistry, Gautam Buddha University, Gautam Budh Nagar, India

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(C22H14N4O2)X2], where M=Zn(II), X = Cl-, NO3- and CH3COO- derived from template condensation of 1,3-dicarbonyl-phenyl-dihydrazide and 9,10-phenanthrenequinone 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 gram+ve bacterial strains.

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

I.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 9,10-phenanthrenequinone 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

2.1Materials

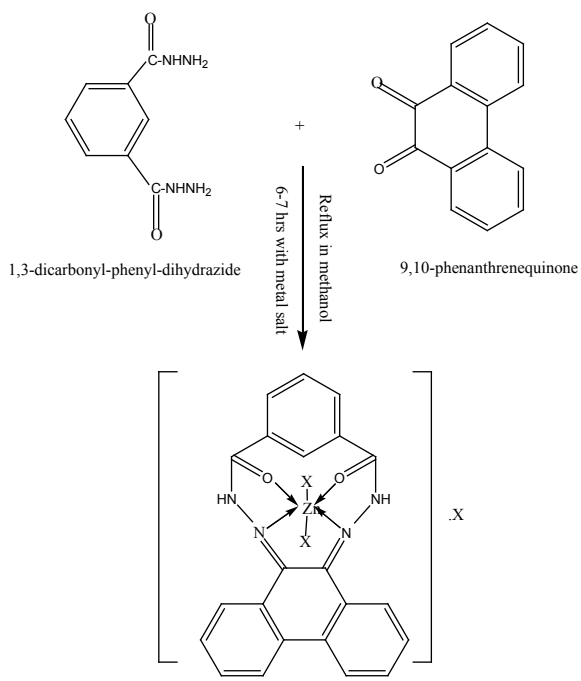
All the reagents and solvents used in this study were of AR grade. Diethylbenzene-1, 3-dicarboxylate, 9,10-phenanthrenequinone and 98% hydrazine hydrate were purchased from Sigma(USA) and Aldrich(Germany).

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 (≈ 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^{0}C for half an hour. Subsequently, 9,10-phenanthrenequinone (0.01mol, 2.08gm) 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 ≈ 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 9,10-phenanthrenequinone

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 i.e Staphylococcus aureus and bacillus subtilis (gram +ve) bacterial strains. Muellor 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

3.1Analytical and physical measurement

The analytical data showed a 1:1 stoichiometry for all synthesized species and suggested formula for macrocyclic complexes as: $[Zn\mathbf{[}C_{22}H_{14}N_4O_2\mathbf{]}X_2]$, 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 12-15 Ω^{-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.

Table 1: Physical properties i.e. Molar conductance, molecular weight and elemental analysis of all synthesized species

Mol. Formula	Molar cond. (Ω cm^{-2} mol^{-1})	Melting point(^{0}C)	Colour	%Yield	Mol. Weight based on formula	Elemental analysis, Calculated(found)					
						C	H	N	O	M	Cl
$Zn(C_{22}H_{14}N_4O_2)(OCOCH_3)_2$	12	265	Dark Yellow	65%	549	56.7	3.6	10.1	17.4	11.8	
$Zn(C_{22}H_{14}N_4O_2)(NO_3)_2$	14	295	Light brown	64%	555	47.5	2.54	15.1	23.0	11.77	
$Zn(C_{22}H_{14}N_4O_2)Cl_2$	15	290	Light brown	62%	502	52.5	2.8	11.1	14.1	6.3	13.01

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 3270cm-1, 1620cm^{-1} , 1687cm^{-1} and may be assigned due to vibrational absorption for $\nu_{N\text{-}H,}$ $\nu_{C=N,}$ $(\nu_{-C=O})_{hydrazide}$ respectively. Disappearance of a pair of bands corresponding to (ν_{NH2}) stretch at 3310 & 3370 cm-1and 1710cm^{-1} $(\nu_{C=O})_{isatin}$ while appearance of a strong band at 1630 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 320-350cm^{-1} may be arising due to $\nu_{(M\text{-}Cl)}$ vibrations.

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 200nm-900nm. [16]

The Zn (II) complex exhibit a single band at 34550 cm-1 assignable to charge transfer from ligand to metal. These complexes are diamagnetic due to completely filled d-orbital and geometry may be assigned octahedral [16]

3.4 ^{1}H NMR spectroscopy

The ^{1}H 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 $–CH_2$ proton. The multiplets at 7.20 – 7.88 ppm may be assigned to hydrogens of aromatic rings [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 $(\mathbf{C_{22}H_{14}N_4O_2})(OCOCH_3)_2$ at 548 m/z which is corresponding to the M^{-1} values as the calculated mass of the complex is 549 a.m.u. This peak may be assigned to molecular ion peak for complex. Subsequent peaks are observed at 488 and 468 in the spectrum which may be assigned to removal of first and remaining two acetate groups respectively from the molecule.

3.7 Biological studies

3.7.1Antibacterial assay

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

$Zn(C_{22}H_{14}N_4O_2)(OCOCH_3)_2$: 14.2mm for S.aureus and 13.5 mm for B. subtilis

$Zn(C_{22}H_{14}N_4O_2)(NO_3)_2$: 16.8mm for S.aureus and 15.8mm for B. subtilis

$Zn(C_{22}H_{14}N_4O_2)Cl_2$: 13.5mm for S.aureus and 11.8mm for B. subtilis

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

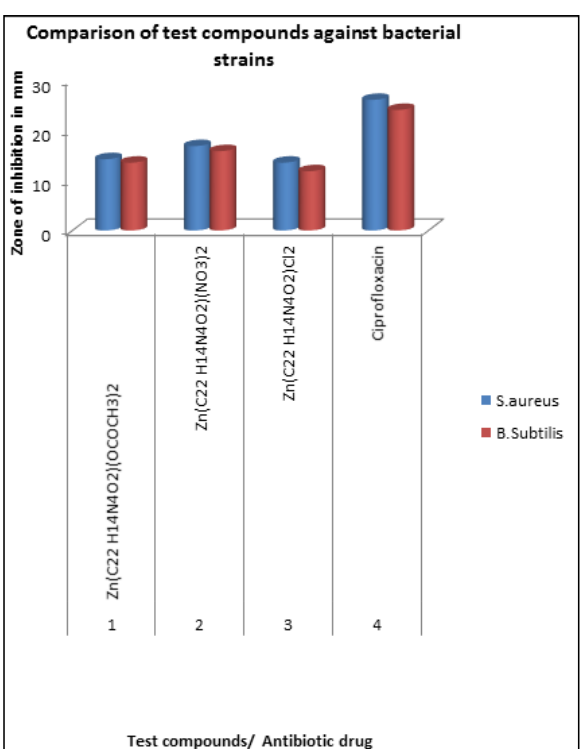


Fig.1: Comparison of zone of inhibition for all synthesized compounds against both bacterial strains

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 may be due to strong electron withdrawing effect. 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 9,10-phenanthrenequinone 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 ^{1}H 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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