

Pharmacological Activity of Newly Synthesized and Characterized N, O-donor Tetraaza Macrocyclic Metal Complexes

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Abstract

Aim: To synthesize a new series of metal based therapeutic agents with good antimicrobial and antioxidant properties and their structural characterization. **Methodology:** Synthesis of a series of transition metal complexes of type $[M(C_{16}H_{11}N_4O_2)X_2]$, where $M=Co(II)$, $Ni(II)$, and $Cu(II)$, $X = Cl^-$, NO_3^- and CH_3COO^- derived from template condensation of 1,3-dicarbonyl-phenyl-dihydrazide and 1*H*-indole-2,3-dione(isatin) and their characterization by ultraviolet-visible, Fourier-transformed infrared spectroscopy, electron paramagnetic resonance, mass spectral studies. Antimicrobial activity was determined using agar well diffusion method against four bacterial strains and two fungal strains. Antioxidant potential of compounds was screened by 2, 2-diphenyl-1-picrylhydrazyl scavenging activity method. **Result:** All the synthesized complexes were found to have six coordinated octahedral geometry. All test compounds possess varied but significant antimicrobial activity against four bacterial and two yeast strains. Minimum inhibitory concentrations lie between 32-128 $\mu g/ml$ for the bacterial strains. Some of the test compounds have shown significant % radical scavenging activity. **Conclusion:** Tetradentate ligand derived from template condensation coordinates readily with all divalent metal salts and affords the synthesis of octahedral complexes. 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.

Key words: Antibacterial, antifungal, antioxidant, electron paramagnetic resonance spectra, macrocyclic complex

INTRODUCTION

The design and study of metal containing macrocyclic molecules have attracted the attention of researchers for a number of reasons: Their pharmacological, physiological activities, their role in molecular processes, drug development, and usefulness in many catalytic applications.^[1-5] Macrocyclic compounds and their derivatives have been proven to be excellent host system for a guest metal ion.^[6,7] These compounds have shown remarkable bacterial inhibiting potential either by penetrating through bacterial cell walls to inactivate their enzymes or by generating H_2O_2 to kill the bacteria. The presence of metal ions accelerates the drug action and efficiency of organic therapeutic agents.^[8-11] The structure and geometry of coordination compounds also influence their antibacterial action with other factors such as thermodynamic stability, hydrolytic stability, kinetics of ligation, and molecular weight to release the metal ion to desired active site.^[12-14]

Hydrazones which are characterized by the presence of (C=N-N=C) unit have shown their remarkable physiological and biological activities. The presence of two interlinked nitrogen offer an additional donor site to the guest ion and make them more versatile and flexible host system. The coordination abilities of these ligands have attracted our attention and aroused our interest in elucidating new structures and evaluating their antimicrobial potential, as there is an increasing demand to search new therapeutics to deal with the problem of bacterial resistance of classical drugs.^[15-18]

Prompted by above facts the synthesis and characterization of the chemical structure derived from the template

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condensation of 1,3-dicarbonyl-phenyl-dihydrazide and isatin (*1H*-indole-2,3-dione) with divalent transition metal salts have been discussed. Besides characterization of synthesized species by modern instrumentation techniques, i.e., ultraviolet-visible (UV-vis), IR, nuclear magnetic resonance (NMR), mass, electron paramagnetic resonance (EPR), thermogravimetric analysis (TGA), compounds were also evaluated for their antibacterial and antifungal potential against various pathogenic strains of bacteria and fungi. The complexes were also evaluated for their antioxidant ability as it becomes great if the compounds are good antimicrobial as well as a good antioxidant.

EXPERIMENTAL

Materials

All the reagents and solvents used in this study were of analytical reagent 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 - such as methanol, ethanol ether, dimethyl sulfoxide (DMSO), and N, N-dimethylformamide (DMF) - were used as such without any distillation.

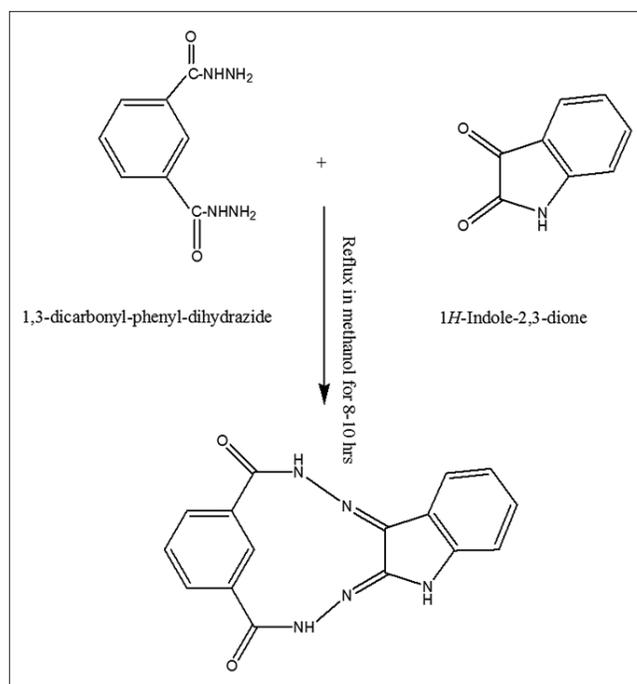
Synthesis of precursor

1,3-dicarbonyl-phenyl-dihydrazide

To a hot stirring ethanolic solution of diethylbenzene-1,3-dicarboxylate (0.01 mol, 2.22 g) Hydrazine hydrate (98% 2 cc) was added and refluxed for 4-5 h. The reaction mixture was then allowed to cool at room temperature; a white color precipitate was formed. The cooled mixture was filtered through the crucible and washed with ethanol and ether. The precipitate was allowed to dry in air and then put into desiccator for further use.

Synthesis of macrocyclic ligand

A calculated amount of 1,3-dicarbonyl-phenyl-dihydrazide (0.01 mol, 2.52 g) was dissolved in methanol (50 ml) with continuous stirring and 4-5 drops of Conc. HCl was added to it. Subsequently, a solution of *1H*-Indole-2,3-dione (isatin) (0.01 mol, 1.65 g) in 20 ml methanol was added to it and continuously refluxed for 8-10 h at 35-40°C. The resulting solution after cooling at room temperature was kept for 28 h in refrigerator. The dark colored precipitate was obtained which was filtered, washed with methanol and kept in desiccator. The yield of product was ~30% [Scheme 1].

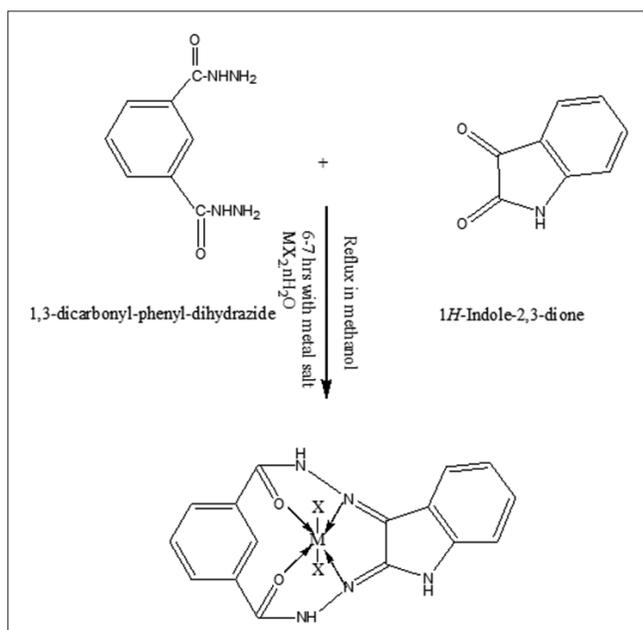


Scheme 1: Synthesis of tetraazamacrocyclic ligand derived from 1,3-dicarbonyl-phenyl-dihydrazide and *1H*-Indole-2,3-dione (isatin) in 1:1 molar ratio

Synthesis of Co (II), Ni (II) and Cu (II) complexes

All the complexes were synthesized by template method because of the low yield of macrocyclic ligand. The template method is effective for the synthesis of macrocyclic complexes which involves the condensation reaction between suitable dicarbonyl compounds and dihydrazides carried out in the presence of appropriate metal ions which serve as templates in directing the steric course of the reaction. In this metal template effect, the metal ion-through coordination organizes the linear substrates to facilitate the condensation process which may lead toward macrocyclic products and a higher yield of the product may be obtained.^[19]

A solution of divalent Co(II), Ni(II), or Cu(II) salt of Cl^- , NO_3^- , or CH_3COO^- (0.01 mol) salt dissolved in the minimum quantity of methanol (≈ 20 ml) was added to a hot stirring solution of 1,3-dicarbonyl-phenyl-dihydrazide (0.01 mol, 2.52 g) in methanol (50 ml) and 4-5 drops of dil. HCl was added. The resulting solution was refluxed at 35-40°C for $\frac{1}{2}$ h. Subsequently, methanolic solution (20 ml) of isatin (0.01 mol, 1.65 g) was added to it and refluxing was continued for 6-7 h. The mixture was then concentrated to half of its volume and allowed to cool at room temperature through evaporation. Dark-colored precipitates were filtered, washed with methanol, acetone, and diethyl ether and dried in vacuum. The obtained yields were ≈ 60 -65%. The complexes were only soluble in DMF and DMSO [Scheme 2].



Scheme 2: Synthesis of metal macrocyclic complex MLX_2 where $M=Co(II)$, $Ni(II)$, or $Cu(II)$ $X = Cl^-$, NO_3^- or CH_3COO^- template condensation of 1,3-dicarbonyl-phenyl-dihydrazide and 1H-indole-2,3-dione with metal salt

In vitro antimicrobial activity

All the newly synthesized complexes were evaluated for their antibacterial activities toward bacterial and fungal strains using agar well-diffusion method.^[20]

Antibacterial activity

Test organisms were chosen on the basis of their clinical importance on causing diseases in human, e.g. *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive), *Escherichia coli*, and *Salmonella typhi* (Gram-negative) for antibacterial screening. Mueller-Hinton Agar (MHA) was used as the culture medium for antibacterial screening.

For quantitative analysis, a microbial suspension having approximately 1×10^7 cfu/ml (0.5 McFarland) was taken from a 24 h old culture. 20 ml of MHA was poured into each sterile petri plates, 100 μ l inoculum of the test microbe was swabbed onto each petri plate already loaded with nutrient agar media and kept for 30 min for incubation (adsorption). Wells were bored using sterile borer (6 mm), into the nutrient agar loaded and seeded petri plates. These bores were loaded with 100 μ l of the test compound at concentration 2.0 mg/ml prepared in DMSO. The measurement of diameter of bacterial growth inhibition zones in mm with zone reader for each test compound was used for quantitative analysis.

Antifungal activity

Fungal strains *Candida albicans* and *Saccharomyces cerevisiae* (yeast) strains were chosen for antifungal screening

of test compounds. Malt yeast extract agar (MYEA) was used as the culture medium for antifungal screening. To check the growth of bacterial culture in the medium, requisite quantity of the standard antibiotic (amphotericin-B) was added, so as to get their desirable final concentration of 100 μ g/ml of the medium. Different concentrations (10 μ g, 50 μ g, 100 μ g/ml) for test samples were prepared in DMSO and 200 μ l of each sample was spread on MYEA media containing sterilized petri plates. Mycelial discs taken from the standard cultures of fungi were grown on MYEA medium for 5-7 days. Standard cultures inoculated at 28°C were also used as the control. The efficacy of each sample was determined by measuring radial mycelial growth.

The radial growth of the fungus colony was measured in two directions at the right angle to each other, and the average of two replicates was recorded in each case. Data were expressed as percent inhibition over control from the size of colonies. The percent inhibition was calculated using the formula:^[21]

$$\% \text{ Inhibition} = (C - T) \times 100 / C.$$

Where,

C = Diameter of fungus colony in the control plate after 96 h incubation;

T = Diameter of the fungus colony in tested plate after the same incubation period.

Media containing DMSO was used as negative control parallel, whereas media loaded with ciprofloxacin (standard antibiotic) and amphotericin-B (standard antifungal drug) were used as positive control. All the experiments were performed thrice against each strain.

Minimum inhibitory concentrations (MIC) are important in diagnostic laboratories to confirm resistance of microorganisms to antimicrobial agents and also to monitor the activity of new antimicrobial agents. The macro dilution method was used to determine MIC of selected test compounds.^[22] In this method, the test concentrations of chemically synthesized compounds were made from 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.50, and 0.25 μ g/ml in the sterile tubes. Mueller-Hinton Broth (MHB) medium was prepared, and 100 ml sterile MHB was poured in each sterile tube. Two-fold serial dilutions were carried out. Microbial suspension of approximately 1.5×10^8 cells/ml (0.5 McFarland) was prepared. To each tube, 100 μ l of standard inoculum was added. Ciprofloxacin (antibiotic) was used as positive control. All the tubes were incubated for 24 h at 37°C.

Antiradical activity

Free radical induced oxidative damage of DNA in humans is well-known problem, so the antiradical potential of some of the synthesized compound was investigated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method.^[23]

Stock solution of 10^{-3} M of DPPH was prepared in methanol, and the solutions of all test compounds of concentration 0, 100, 250, and 500 $\mu\text{g/ml}$ were prepared in DMF. To 1 ml of sample solution, 3 ml of methanolic solution of DPPH (10^{-3} M) was added and kept for 30 min for incubation at room temperature.

Free radical scavenging potential of synthesized complexes was observed by measuring their absorbance at 517 nm at room temperature.

of 20 ml of 10^{-3} M of their solutions were measured at room temperature. All the complexes showed molar conductance 10-15/ $\Omega/\text{cm}^2/\text{mol}$. It was concluded from the results that complexes are non-electrolytic in nature.^[24] Therefore, these complexes may be formulated as (MLX_2) however various analytical, spectroscopic, and magnetic studies were performed and enabled the possible structure of the synthesized complexes to be predicted.

All complexes gave satisfactory micro elemental analyses results as shown in Table 1.

RESULTS AND DISCUSSION

Analytical and physical measurement

The analytical data showed a 1:1 stoichiometry for all synthesized species and suggested formula for macrocyclic complexes as: $[\text{M} [\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2]_2 \text{X}_2]$, where M = Co(II), Ni(II), or Cu(II) X = Cl, NO_3^- or CH_3COO^- , and $[\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2]$ corresponds to the synthesized macrocyclic ligand. The metal complexes were dissolved in DMF and molar conductivities

Infrared (IR) 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 [Table 2]. Ligand gave three specific IR absorption bands in the IR region at 3270/ cm , 1620/ cm , 1687/ cm and may be assigned due to vibrational absorption for $\nu_{\text{N-H}}$, $\nu_{\text{C=N}}$, $(\nu_{\text{C=O}})_{\text{hydrazide}}$, respectively.^[25] Disappearance of a pair of bands corresponding to (ν_{NH_2})

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

Molecular formula	Melting point ($^{\circ}\text{C}$)	Color	Percentage yield	Mol. weight based on formula	Elemental analysis, calculated (found)					Cl
					C	H	N	O	M	
$\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2=\text{L}$ compound 1	245	Light yellow	30	290.14	66 (64.4)	3.47 (3.45)	19.3 (19)	11.02 (11.2)		
$\text{Ni}(\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2)(\text{OAc})_2$ compound 2	250	Yellow green	64	467.56	51.43 (50.9)	3.45 (3.42)	12 (12.2)	20.55 (20.4)	12.57 (12.4)	
$\text{Ni}(\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2)(\text{NO}_3)_2$ Compound 3	247	Green	65	472.50	40.63 (41)	2.13 (2.11)	17.77 (17.5)	27.06 (27)	12.41 (12.3)	
$\text{Ni}(\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2)\text{Cl}_2$ Compound 4	254	Yellow green	64	419.46	45.77 (46.2)	2.4 (2.3)	13.34 (13.2)	16.84 (17)	7.62 (8)	13.9 (14)
$\text{Co}(\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2)(\text{OAc})_2$ compound 5	255	Yellow	65	467.42	51.41 (51.2)	3.45 (3.3)	11.97 (11.7)	20.54 (20.1)	12.61 (11.9)	
$\text{Co}(\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2)(\text{NO}_3)_2$ Compound 6	260	Yellow brown	64	473.34	40.61 (40.3)	2.13 (2.11)	17.76 (17.5)	27.05 (27)	12.45 (12.4)	
$\text{Cu}(\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2)(\text{NO}_3)_2$ compound 7	267	Green	63	477.42	40.22 (40.1)	2.11 (2.1)	17.59 (17.6)	26.79 (26.6)	13.3 (13.1)	
$\text{Cu}(\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2)(\text{OAc})_2$ Compound 8	242	Bluish green	65	471.50	50.9 (50.1)	3.42 (3.4)	11.87 (11.7)	20.34 (20.1)	13.47 (13.5)	
$\text{Cu}(\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2)\text{Cl}_2$ compound 9	240	Brown	64	424.35	45.25 (45.2)	2.37 (2.3)	13.17 (13.01)	16.69 (17)	7.53 (7.7)	14.9 (15)

Table 2: Comparison of IR spectral peaks showing complexation of ligand with metal (4000-400/cm)

Compound	Vibrational absorption frequencies (cm ⁻¹)							
	($\nu_{\text{-NH}}$)	($\nu_{\text{-CONH}}$)	($\nu_{\text{C=N}}$)	($\nu_{\text{C-N}}$)	($\nu_{\text{-M-O}}$)	($\nu_{\text{C=O}}$) _{acetate}	($\nu_{\text{C-O}}$) _{acetate}	($\nu_{\text{-M-N}}$)
C ₁₆ H ₁₁ N ₄ O ₂ =L compound 1	3270	1687	1640	1290	-	-	-	-
Ni (C ₁₆ H ₁₁ N ₄ O ₂)(OAc) ₂ compound 2	3269	1622	1620	1300	522	1669	1258	420
Ni (C ₁₆ H ₁₁ N ₄ O ₂)(NO ₃) ₂ compound 3	3260	1640	1622	1280	525			422
Ni (C ₁₆ H ₁₁ N ₄ O ₂) Cl ₂ compound 4	3257	1632	1610	1285	530			425
Co (C ₁₆ H ₁₁ N ₄ O ₂)(OAc) ₂ compound 5	3251	1652	1605	1302	524	1679	1260	436
Co (C ₁₆ H ₁₁ N ₄ O ₂)(NO ₃) ₂ Compound 6	3240	1639	1595	1287	521			450
Cu (C ₁₆ H ₁₁ N ₄ O ₂)(NO ₃) ₂ compound 7	3262	1640	1626	1195	528			435
Cu (C ₁₆ H ₁₁ N ₄ O ₂)(OAc) ₂ Compound 8	3269	1635	1598	1095	530	1672	1261	446
Cu (C ₁₆ H ₁₁ N ₄ O ₂)(Cl) ₂ compound 9	3180	1630	1622	1120	520			432

IR: Infrared

stretch at 3300 and 3350/cm and 1710/cm ($\nu_{\text{C=O}}$)_{isatin} while the appearance of a strong band at 1620/cm indicates the condensation between both of the molecules.^[26,27]

The band corresponding to $\nu_{\text{C=N}}$ appears at lower frequency by 10-30/cm in the complexes and indicates the coordination of nitrogen atoms of azomethine groups to metal atom^[28] as a result of lower electron density around nitrogen atom of C=N bond [Table 2].

The values show in Table 2 indicates that the peak corresponding to $\nu_{\text{C=O}}$ group of the -CONH moiety has been shifted to lower side and appears around 1620–1640/cm 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.

Other bands present in the range 1300-1000/cm in all the complexes may be assigned to (C-N) stretch.^[29]

In nitrate complexes, absorption bands are observed at 1430-1410, 1315-1280, and 1050-1017/cm frequency suggests unidentate coordination of both nitrate groups to central metal ion.

Two specific bands corresponding to ($\nu_{\text{C=O}}$)_{acetate} and ($\nu_{\text{C-O}}$)_{acetate} are appeared in the region 1670-1650/cm and 1290-1250/cm, respectively, in all acetate complexes. A difference between these two peaks indicates the unidentate coordination of the acetate ion with the central metal ion.

The bands appeared in the region 450-420/cm are attributed to (M-N) vibrations and indicate the coordination of

azomethine nitrogen to metal.^[29] The bands present between 320 and 350/cm may be arising due to $\nu_{\text{(M-Cl)}}$ vibrations.

The appearance of various absorption peaks in the region 1450-1590/cm may be assigned due to $\nu_{\text{(C=C)}}$ aromatic stretching vibrations of the aromatic ring of 1,3-dicarbonyl-phenyl-dihydrazide and isatin moiety.^[29]

UV-vis spectral analysis and magnetic measurement

Electronic spectral technique is very helpful to evaluate the results obtained from other techniques of structural investigation. The position of peaks and the number of d-d transitions are greatly helpful to evaluate the geometry proposed by other techniques of structural investigation which is achieved by the metal ion in the coordination sphere. The electronic absorption spectra of ligand and all complexes were recorded in DMSO at room temperature ranging 200 nm-900 nm. Broad peaks were observed near 35000-36000/cm region in ligand which is assigned to intra-ligand transitions of >C=O bond and >C=N bond. These bands were observed slightly lower side in the complexes due to coordination of oxygen atom of >C=O and nitrogen atom of >C=N bond with the metal ion and known as charge transfer band [Table 3].^[30]

Cobalt complexes

Magnetic moment of cobalt complexes lie between 4.30 and 4.70 Bohr magneton (B.M.) which indicates three unpaired electron and an octahedral arrangement.^[31] The Co(II) complexes gave three electronic

Table 3: Ligand field parameters of nickel complexes

Complexes	μ_{eff} (B.M.)	d-d transition bands λ_{max} (cm ⁻¹)	Dq (cm ⁻¹)	B' (cm ⁻¹)	β	ν_2/ν_1	LFSE (Kcal mol ⁻¹)
Ni (L) (OCOCH ₃) ₂	2.97	11140, 16860, 25853	1114	619.2	0.595	1.513	38.19
Ni (L)(NO ₃) ₂	2.96	11242, 17304, 25784	1124	624.1	0.599	1.53	38.53
Ni (L) Cl ₂	3.10	11090, 16976, 25,869	1109	638.33	0.613	1.53	38.02

B.M.: Bohr magneton

spectral bands at 11212-11169/cm (ν_1), 14972-14267/cm (ν_2) and 20885-20663/cm (ν_3) [Table 2] in UV-vis region. As the ground state in Co(II) is $4T_1g$ (F), these bands may be corresponding to the transitions $4T_1g(F) \rightarrow T_2g(F)$, (ν_1) $4T_1g(F) \rightarrow A_2g(F)$ (ν_2), and $4T_1g(F) \rightarrow T_1g(P)$, (ν_3), respectively. The position of absorption bands indicates an overall octahedral geometry for Co (II) complexes.^[32]

Nickel complexes

μ_{eff} of Ni (II) complexes at room temperature lies in the range 2.96-3.10 B.M., [Table 3] which reveals the presence of two unpaired electrons and an octahedral arrangement of molecule. Electronic spectra of complexes show bands in the region of 11242-11090 (ν_1), 17860-16304 (ν_2) and 25869-25784/cm (ν_3). An examination of above spectral bands indicates that the complexes have an overall octahedral geometry. The ground state of Ni (II) in an octahedral coordination is $3A_2g$, so these bands may be assigned to the Ni Complexes $3A_2g(F) \rightarrow 3T_2g(F)$, $3A_2g(F) \rightarrow T_1g(F)$ $3A_2g(F) \rightarrow T_1g(P)$ transitions, respectively.^[32]

Cu complexes

Cu complexes have shown one broad absorption band for d-d transitions at 15918-13,861/cm. It may be assigned to $^2Eg \rightarrow ^2T_2g$ transitions. Although three close transitions were expected for Cu (II) octahedral complexes, they are very close in energy and only one broadband envelop is observed.^[48] The magnetic moment (1.43-1.88 B.M.) lies well in the region reported for d^9 system with an unpaired electron.^[32]

Various ligand field parameters [Table 2] - such as the ligand field splitting energy (10 Dq), Racah inter-electronic repulsion parameter (B'), covalency factor (β), and ligand field stabilization energy (LFSE) - have been calculated and reported for nickel complexes using Underhill AE and Billing DE equations (equation 1-4)^[33] which further confirms the interaction of metal with ligand in octahedral manner. The value of B' for complexes is lower than the value of B for free ion in gaseous state. The value of $\beta < 1$ which confirms considerable amount of orbital overlap and hence covalent characteristics of metal-ligand bond Table 3.^[33]

For Ni (II) octahedral complexes,

$$B' = (\nu_2 + \nu_3 - 3\nu_1) / 15 \quad (1)$$

B for free Ni (II) = 1041;

$$\beta = B' / B \quad (2)$$

$$Dq = \nu_1 / 10 \quad (3)$$

$$LFSE = 12Dq / 350 \quad (4)$$

All the complexes have also shown some common peaks in the range 35000-36000/cm which are attributed to charge transfer absorption peaks.

EPR analysis

EPR analysis is very informative to understand the metal ion environment in complexes. X-band spectra of all three copper complexes 7, 8, and 9 were recorded in DMSO at room temperature (300 K) and LNT (77 K).

The absorption spectrum of copper complexes at room temperature shows an isotropic unresolved intense band is observed in a high field region [Figure 1]. Peak was not split even at the liquid nitrogen temperature [Figure 1]. It shows magnetic equivalence of Cu nuclei in the powder sample and possibly the exchange interactions are not enough for hyperfine splitting of lines.

g_{\parallel} & g_{\perp} values of complexes 7 were calculated and found to be 2.28 and 2.09, respectively, which follows the pattern $g_{\parallel} > g_{\perp} > 2.002$ and indicates the presence of unpaired electron of copper predominantly in the dx^2-y^2 orbital.^[34] The relation of g values shows considerable amount of covalent characters of M-N bond of complexes in the ligand environment. The G values were also calculated and found to be around 3.2 which suggests the covalent characters of complexes and indicates negligible exchange interaction of Cu-Cu in polycrystalline copper complexes.

¹H NMR spectrum

¹H NMR spectrum of the synthesized ligand was recorded in DMSO-*d*₆ at room temperature. A singlet for two protons

was observed at $\delta=9.4$ ppm which may be assigned to $-NH$ protons of $-CONH$ moiety. Another broad singlet for two protons at $\delta=10.2$ ppm may be assigned to $-NH$ proton of isatin moiety.

Two multiples were observed at $\delta=7.0-8.0$ ppm corresponding to four protons each and may be assigned to aromatic protons of both aromatic rings.

TGA

The TGA spectrum of complex $Cu(L)(OAc)_2$ was recorded at temperature from $50^\circ C$ to $750^\circ C$ at a heating rate of $10^\circ C/min$. No degradation of molecule below $200^\circ C$ indicates the presence of coordinated or uncoordinated water in the molecule. It was observed that decomposition starts

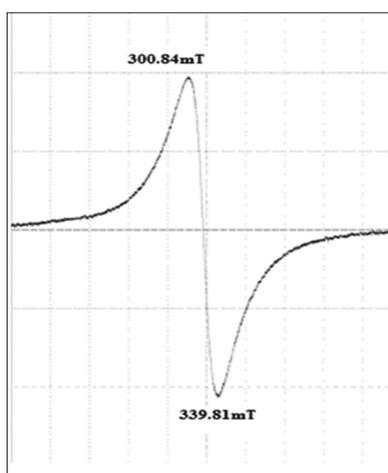


Figure 1: X-band electron paramagnetic resonance spectrum of $Cu(L)(NO_3)_2$ at room temperature

at $200^\circ C$ and goes up to $720^\circ C$ (78.88%) in approximately one step which shows the degradation of entire unit as it resembles to the calculated value. Further horizontal constant curve is showing the presence of metal oxides residue as the remaining part as the observed value resembles with the calculated value of corresponding metal oxide (CuO).

Mass spectral analysis

ESI mass spectrums of all complexes were recorded to confirm the proposed structures. A less intense peak was observed for complex 3 $Ni(L)Cl_2$ at m/z 420.0 which is corresponding to the M^+ values as the calculated mass of the complex is 419.82 a.m.u. This peak may be assigned to molecular ion peak for complex. Subsequent peaks are observed at 385 and 350.0 in the spectrum which may be assigned to removal of first and second chloride groups, respectively, from the molecule. Table 4 is showing the prominent peaks observed in mass spectra of all the complexes.

Antimicrobial activity result and discussion

All the synthesized macrocyclic metal complexes were tested for their *in vitro* antibacterial activity by agar well-diffusion method (Table 5) and detection of MIC against selected microbial strains by macrodilution method (Table 6). It was observed that all the complexes of $Co(II)$, $Ni(II)$, and $Cu(II)$ are a more effective against Gram-positive bacteria than Gram-negative bacteria as reported earlier in literature.^[35] Compound 6 is the most effective against all the bacterial strains. Compound 3 and 4 are least effective against all the strains. Compounds 4 and 9 were most effective against both of the fungal strains

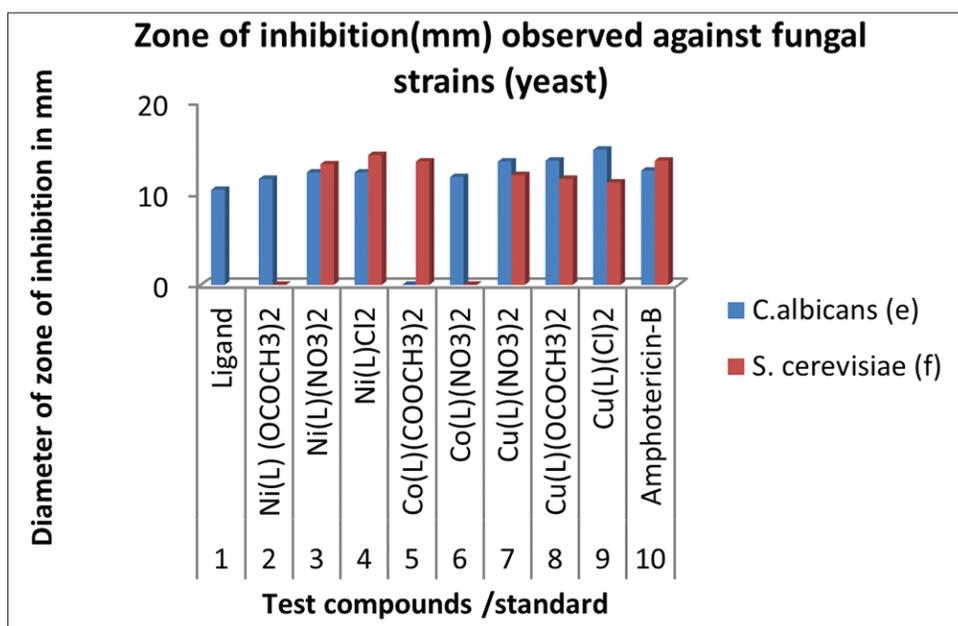


Figure 2: Comparison of zone of inhibition of all synthesized compounds against all fungal strains

Table 4: Mass spectral values of some complexes

Complexes	Mol. weight	Molecular ion peak[M+] at m/z	Other significant peaks
Ni(C ₁₆ H ₁₁ N ₄ O ₂)(OAc) ₂	467.81	466.4	289.2, 345.8, 405.9
Ni(C ₁₆ H ₁₁ N ₄ O ₂)(NO ₃) ₂	472.80	471.2	290.2, 346.7, 408.1
Ni(C ₁₆ H ₁₁ N ₄ O ₂)Cl ₂	419.82	420.0	291.2, 350, 385.0
Co(C ₁₆ H ₁₁ N ₄ O ₂)(OAc) ₂	467.45	466	290.2, 346.6, 406.2
Co(C ₁₆ H ₁₁ N ₄ O ₂)(NO ₃) ₂	473.44	472.5	288.9, 346.9, 409.8
Cu(C ₁₆ H ₁₁ N ₄ O ₂)(NO ₃) ₂	477.55	476.7	291.8, 352.3, 414.4
Cu(C ₁₆ H ₁₁ N ₄ O ₂)(OAc) ₂	471.23	471.2	291.8, 351.3, 411
Cu(C ₁₆ H ₁₁ N ₄ O ₂)(Cl) ₂	424.24	423.8	291.8, 353.4, 388.1

Table 5: Determination of zone of inhibition in mm for all bacterial and fungal strains

Compound	Diameter of zone of inhibition in mm					
	a	b	c	d	e	f
Ligand	12	11.7	-	-	10.4	-
Ni(L)(OCOCH ₃) ₂	14.5	-	10.6	-	11.6	-
Ni(L)(NO ₃) ₂	15.3	-	-	10.6	12.3	13.2
Ni(L)Cl ₂	-	-	12.5	11.2	12.3	14.2
Co(L)(COOCH ₃) ₂	17.6	17.8	10.7	11.7	-	13.5
Co(L)(NO ₃) ₂	18.2	19.2	12.3	10.5	11.8	-
Cu(L)(NO ₃) ₂	14.5	15.4	11.6	11.5	13.5	12
Cu(L)(OCOCH ₃) ₂	14.7	14.6	11	-	13.6	11.6
Cu(L)(Cl) ₂	13.9	13.8	11.6	11.8	14.8	11.2
Ciprofloxacin	28	26	24	24	-	-
Amphotericin-B	-	-	-	-	12.5	13.6

-: Means no significant activity; here bacterial strains are, a: *Staphylococcus aureus*, b: *Bacillus subtilis*, c: *Escherichia coli*, d: *Salmonella typhi*, fungal strains are, e: *Candida albicans*, and f: *Saccharomyces cerevisiae*

Table 6: Minimum inhibitory concentration (µg/ml) for all test compounds against bacterial strains

Compound	MIC (µg/ml)	
	a	b
Ni(L)(OCOCH ₃) ₂	128	-
Ni(L)(NO ₃) ₂	128	-
Co(L)(OCOCH ₃) ₂	64	128
Co(L)(NO ₃) ₂	64	64
Cu(L)(NO ₃) ₂	128	64
Cu(L)(OCOCH ₃) ₂	128	128
Cu(L)(Cl) ₂	128	128
Ciprofloxacin	6	6

a: *Staphylococcus aureus*, b: *Bacillus Subtilis*, -: Means no activity, MIC: Minimum inhibitory concentrations

(Figure 2). Complexes were showing greater susceptibility to bacteria than the respective ligand as earlier reported in literature^[14,15] and indicates the enhancement of lipophilic nature of ligand on coordination and better

penetration of bacterial cell wall (bacterial susceptibility). Compounds of Co(II) are the most effective against all the strains. It indicates that the effect of individual metal, its electron density, coordination potential, dipole moment, conductance also affect its overall biological behavior. MIC was detected only for Gram-positive bacterial strains as the synthesized compounds are less effective against Gram-negative strains.

The antimicrobial results of the ligand exhibited a considerable enhancement on coordination with the transition metal ions against all bacterial strains.^[15-20]

Antioxidant activity results

The antioxidant potential of selected compounds was checked by DPPH scavenging method.

It was observed that the absorbance for solution containing test compounds has been significantly reduced in comparison to pure DPPH solution (no test compound) [Table 7], which

reveals the antioxidant nature of the complexes. All tests were performed thrice and an average value is reported. DMF was used as a negative control and DPPH was used as positive control [Figure 4].

Radical scavenging activity was calculated using the following formula:^[23]

$$\% \text{Radical scavenging activity} = \left[\frac{\text{O.D.}_{\text{control}} - \text{O.D.}_{\text{sample}}}{\text{optical density}_{\text{control}}} \right] \times 100$$

Here OD=Optical density

It was observed that all the test compounds have shown varied antioxidant potential at different concentrations. Co(II) complex is comparatively more active than other Ni(II) and Cu(II) complex.

CONCLUSION

The present study evaluated the antioxidant and antimicrobial behavior of macrocyclic complexes. The analytical, spectral and magnetic moment studies suggest an octahedral geometry for all of these complexes. Mass spectral study confirms a monocentric complex, while thermal studies have shown the pattern of degradation of compounds with temperature which further confirms the geometry of the compounds. All the complexes were found to be potent against microbial strains and have antioxidant activity up to different levels. The compound no. 6 was found to be best antibacterial agent whereas complexes of Cu(II) have shown the highest antifungal activity. Compound no. 5 have shown maximum % of radical scavenging activity. Due to complexity of biological system, it is rather difficult to stipulate the exact mechanism for such activities however; the chelation/coordination may be suggested as one of the factor responsible for these activities.

Experimental protocol

The microanalysis for C, H, and N, O, and M was realized using an elemental analyzer (Perkin Elmer 2400) at Indian Institute of technology 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 Fourier Transform-IR spectrometer in the range 4000-400/cm using ATR. UV-visible spectra in DMSO were recorded on PerkinElmer Lambda 25 spectrophotometer ranging 200-900 nm. The molar conductance was measured on digital conductivity meter (HPG-3001). The metal contents in the complexes were determined by Atomic absorption spectroscopy. EPR spectra of Cu (II) complexes were recorded at room temperature and at liquid nitrogen temperature on ESR-JEOL-Japan from IIT Bombay. ESI Mass spectra were obtained from JEOL-ACCU TOF JMS-T100LC mass spectrometer ranging 50.0-1000.0. Thermal

Table 7: Percentage radical scavenging activity at variable concentration

Compound	Concentration in $\mu\text{g/ml}$ (%)			
Percentage radical scavenging activity	50.0	100.0	250.0	500.0
BHA	32.3	47.2	74.6	88.3
$\text{Co}(\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2)(\text{OAc})_2$	28.55	35.54	49.07	59.43
$\text{Ni}(\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2)(\text{OAc})_2$	20	35.94	45.94	52.28
$\text{Cu}(\text{C}_{16}\text{H}_{11}\text{N}_4\text{O}_2)(\text{OAc})_2$	22.48	33.29	46.10	56.10

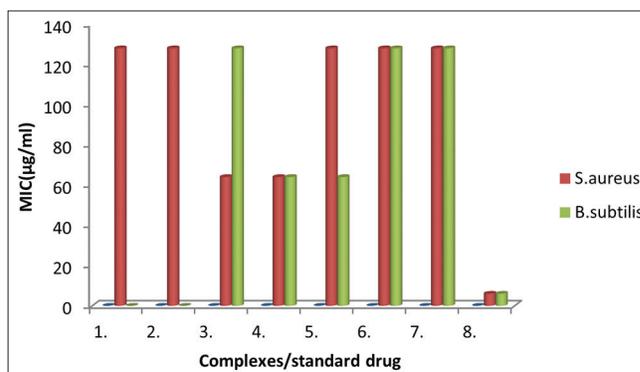


Figure 3: Comparison of minimum inhibitory concentration ($\mu\text{g/ml}$) of new compounds with standard drug against Gram-positive bacterial strains

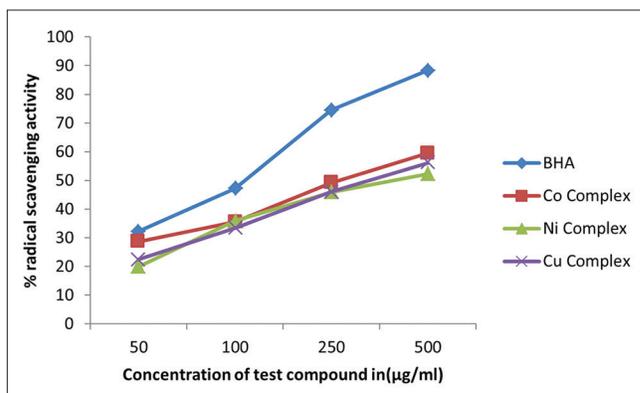


Figure 4: Comparative study of antioxidant activity

analysis of complexes was carried out by TGA Perkin Elmer thermo analyzer. The ^1H NMR spectra were recorded at room temperature in DMSO- d_6 on a Bruker AVANCE II 400 NMR spectrometer (400 MHz) from IIT Delhi.

Melting points were determined using capillaries in electrical melting point apparatus.

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