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 \([\text{M(C}_{16}\text{H}_{11}\text{N}_{4}\text{O}_{2})X_{2}]\), where \(\text{M} = \text{Co(II)}, \text{Ni(II)}, \text{and Cu(II)}, \text{X} = \text{Cl}^{-}, \text{NO}_{3}^{-}\) and \(\text{CH}_{3}\text{COO}^{-}\) derived from template condensation of 1,3-dicarbonyl-phenyl-dihydrazide and 1\(\text{H}\)-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 \(\mu\text{g/ml}\) 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 \(\text{H}_{2}\text{O}_{2}\) 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 \((\equiv\text{N}-\equiv\text{N}=\equiv)\) 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-dicarboxyl-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-dicarboxyl-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 desicator for further use.

**Synthesis of macrocyclic ligand**

A calculated amount of 1,3-dicarboxyl-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. The resulting solution was refluxed at 135-40°C for ½ h. Subsequently, methanolic solution (20 ml) of isatin (0.01 mol, 1.65 g) 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-dicarboxyl-phenyl-dihydrazide and 1H-indole-2,3-dione (isatin) in 1:1 molar ratio](image)

**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.\(^\text{[19]}\)

A solution of divalent Co(II), Ni(II), or Cu(II) salt of Cl\(^-\), NO\(_2\)\(^-\), or CH\(_3\)COO\(^-\) (0.01 mol) salt dissolved in the minimum quantity of methanol (≈ 20 ml) was added to a hot stirring solution of 1,3-dicarboxyl-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 ½ 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].
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 \(1 \times 10^7\) 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 µl 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 µl 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 µg/ml of the medium. Different concentrations (10 µg, 50 µg, 100 µg/ml) for test samples were prepared in DMSO and 200 µl 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:\(^{[21]}\)

\[
\%\text{ Inhibition} = \left( \frac{C - T}{C} \right) \times 100
\]

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 µg/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 \(1.5 \times 10^8\) cells/ml (0.5 McFarland) was prepared. To each tube, 100 µl 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 µg/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.

**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: $[M \{C_{16}H_{11}N_{2}O_{2}\}^\times X]_\times$, where $M = \text{Co(II)}, \text{Ni(II)}, \text{or Cu(II)} X = \text{Cl}, \text{NO}_{3}^{-}$ or \text{CH}_{3}\text{COO}^{-}$, and $[C_{16}H_{11}N_{4}O_{2}]$ corresponds to the synthesized macrocyclic ligand. 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/Ω⋅cm$^2$/mol. It was concluded from the results that complexes are non-electrolytic in nature.$^{[24]}$ Therefore, these complexes may be formulated as (MLX$_{x}$) 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.

**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 $\nu_{\text{N-H}}$, $\nu_{\text{C=N}}$, $\nu_{\text{C=O}}$ respectively.$^{[23]}$ Disappearance of a pair of bands corresponding to $\nu_{\text{N-H}}$

| Table 1: Physical properties i.e., molar conductance, molecular weight and elemental analysis of all synthesized species |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Molecular formula | Melting point (°C) | Color | Percentage yield | Mol. weight based on formula |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| C$_{16}$H$_{11}$N$_{2}$O$_{2}$L | 245 | Light yellow | 30 | 290.14 |
| Ni(C$_{16}$H$_{11}$N$_{2}$O$_{2}$) (OAc)$_{2}$ | 250 | Yellow green | 64 | 467.56 |
| Ni(C$_{16}$H$_{11}$N$_{4}$O$_{2}$) (NO$_{3}$)$_{2}$ | 247 | Green | 65 | 472.50 |
| Ni(C$_{16}$H$_{11}$N$_{4}$O$_{2}$)Cl$_{2}$ | 254 | Yellow green | 64 | 419.46 |
| Co(C$_{16}$H$_{11}$N$_{2}$O$_{2}$) (OAc)$_{2}$ | 255 | Yellow brown | 64 | 467.42 |
| Co(C$_{16}$H$_{11}$N$_{2}$O$_{2}$) (NO$_{3}$)$_{2}$ | 260 | Yellow brown | 64 | 473.34 |
| Cu(C$_{16}$H$_{11}$N$_{2}$O$_{2}$) (NO$_{3}$)$_{2}$ | 267 | Green | 63 | 477.42 |
| Cu(C$_{16}$H$_{11}$N$_{2}$O$_{2}$) (OAc)$_{2}$ | 242 | Bluish green | 65 | 471.50 |
| Cu(C$_{16}$H$_{11}$N$_{2}$O$_{2}$) (Cl)$_{2}$ | 240 | Brown | 64 | 424.35 |

**RESULTS AND DISCUSSION**

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The bands present between 320 and 350/cm may be arising due to $\nu_{(M-O)}$ vibrations.

The appearance of various absorption peaks in the region 1450-1590/cm may be assigned due to $\nu_{C=O}$ aromatic stretching vibrations of the aromatic ring of 1,3-dicarboxyl-phenyl-dihydrzide 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 intraligand 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].\[28\]

### 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

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Vibrational absorption frequencies (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$(\nu_{\text{NH}})$</td>
</tr>
<tr>
<td>C$<em>{16}$H$</em>{11}$N$<em>{2}$O$</em>{2}$-L compound 1</td>
<td>3270</td>
</tr>
<tr>
<td>Ni (C$<em>{16}$H$</em>{11}$N$<em>{2}$O$</em>{2}$)(OAc)$_{2}$ compound 2</td>
<td>3269</td>
</tr>
<tr>
<td>Ni (C$<em>{16}$H$</em>{11}$N$<em>{2}$O$</em>{2}$)(NO$<em>{2}$)$</em>{2}$ compound 3</td>
<td>3269</td>
</tr>
<tr>
<td>Ni (C$<em>{16}$H$</em>{11}$N$<em>{2}$O$</em>{2}$)Cl$_{2}$ compound 4</td>
<td>3257</td>
</tr>
<tr>
<td>Co (C$<em>{16}$H$</em>{11}$N$<em>{2}$O$</em>{2}$)(OAc)$_{2}$ compound 5</td>
<td>3251</td>
</tr>
<tr>
<td>Co (C$<em>{16}$H$</em>{11}$N$<em>{2}$O$</em>{2}$)(NO$<em>{2}$)$</em>{2}$ Compound 6</td>
<td>3240</td>
</tr>
<tr>
<td>Cu (C$<em>{16}$H$</em>{11}$N$<em>{2}$O$</em>{2}$)(NO$<em>{2}$)$</em>{2}$ compound 7</td>
<td>3262</td>
</tr>
<tr>
<td>Cu (C$<em>{16}$H$</em>{11}$N$<em>{2}$O$</em>{2}$)(OAc)$_{2}$ Compound 8</td>
<td>3269</td>
</tr>
<tr>
<td>Cu (C$<em>{16}$H$</em>{11}$N$<em>{2}$O$</em>{2}$)(Cl)$_{2}$ compound 9</td>
<td>3180</td>
</tr>
</tbody>
</table>

IR: Infrared
spectral bands at 11212-11169/cm ($\nu_1$), 14972-14267/cm ($\nu_2$) and 20885-20663/cm ($\nu_3$) [Table 2] in UV-vis region. As the ground state in Co(II) is 4$T_{1g}$ (F), these bands may be corresponding to the transitions 4$T_{1g}$ (F)→$T_{2g}$ (F), ($\nu_1$) 4$T_{1g}$ (F)→$A_{2g}$ (F) ($\nu_2$), and 4$T_{1g}$ (F)→$T_{1g}$ (P), ($\nu_3$), respectively. The position of absorption bands indicates an overall octahedral geometry for Co (II) complexes.

Nickel complexes

$\mu_{\text{eff}}$ 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 ($\nu_1$), 17860-16304 ($\nu_2$) and 25869-25784/cm ($\nu_3$). 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 3$A_{2g}$, so these bands may be assigned to the Ni Complexes 3$A_{2g}$ (F)→3$T_{2g}$ (F), 3$A_{2g}$ (F)→3$T_{1g}$ (F) 3$A_{2g}$ (F)→$T_{1g}$ (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 $^2E_{g}$$\rightarrow$$^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 ($\beta$), and ligand field stabilization energy (LFSE) - have been calculated and reported for nickel complexes using Underhill AE and Billing DE equations (equation1-4)[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]

<table>
<thead>
<tr>
<th>Complexes</th>
<th>$\mu_{\text{eff}}$ (B.M.)</th>
<th>d-d transition bands $\lambda_{\text{max}}$ (cm$^{-1}$)</th>
<th>Dq (cm$^{-1}$)</th>
<th>B' (cm$^{-1}$)</th>
<th>$\beta$</th>
<th>$\nu_2/\nu_1$</th>
<th>LFSE (Kcal mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni (L)(OCOCH$_3$)$_2$</td>
<td>2.97</td>
<td>11140, 16860, 25853</td>
<td>1114</td>
<td>619.2</td>
<td>0.595</td>
<td>1.513</td>
<td>38.19</td>
</tr>
<tr>
<td>Ni (L)(NO$_3$)$_2$</td>
<td>2.96</td>
<td>11242, 17304, 25784</td>
<td>1124</td>
<td>624.1</td>
<td>0.599</td>
<td>1.53</td>
<td>38.53</td>
</tr>
<tr>
<td>Ni (L) Cl$_2$</td>
<td>3.10</td>
<td>11090, 16976, 25,869</td>
<td>1109</td>
<td>638.33</td>
<td>0.613</td>
<td>1.53</td>
<td>38.02</td>
</tr>
</tbody>
</table>

B.M.: Bohr magneton

For Ni (II) octahedral complexes,

\[
B'=(\nu_2+\nu_3-3\nu_1)/15
\]  

$B$ for free Ni (II) =1041;

\[
\beta=B'/B
\]

\[
Dq=\nu_1/10
\]

\[
\text{LFSE}=12\text{Dq}/350
\]

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.

$g_{||}$&$g_{\perp}$ values of complexes 7 were calculated and found to be 2.28 and 2.09, respectively, which follows the pattern $g_{||}>g_{\perp}>2.002$ 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.

$^1$H NMR spectrum

$^1$H 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 $-\text{NH}$ protons of $-\text{CONH}$ moiety. Another broad singlet for two protons at $\delta=10.2$ ppm may be assigned to $-\text{NH}$ 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°C to 750°C at a heating rate of 10°C/min. No degradation of molecule below 200°C indicates the presence of coordinated or uncoordinated water in the molecule. It was observed that decomposition starts at 200°C and goes up to 720°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. 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.
Complexes were showing greater susceptibility to bacteria than the respective ligand as earlier reported in literature\textsuperscript{[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.\textsuperscript{[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

\begin{table}
\centering
\caption{Mass spectral values of some complexes}
\small
\begin{tabular}{|c|c|c|c|}
\hline
Complexes & Mol. weight & Molecular ion peak $[M^+]$ at m/z & Other significant peaks \\
\hline
Ni(C\textsubscript{16}H\textsubscript{11}N\textsubscript{4}O\textsubscript{2})(OAc)\textsubscript{2} & 467.81 & 466.4 & 289.2, 345.8, 405.9 \\
Ni(C\textsubscript{16}H\textsubscript{11}N\textsubscript{4}O\textsubscript{2})(NO\textsubscript{3})\textsubscript{2} & 472.80 & 471.2 & 290.2, 346.7, 408.1 \\
Ni(C\textsubscript{16}H\textsubscript{11}N\textsubscript{4}O\textsubscript{2})Cl\textsubscript{2} & 419.82 & 420.0 & 291.2, 350, 385.0 \\
Co(C\textsubscript{16}H\textsubscript{11}N\textsubscript{4}O\textsubscript{2})(OAc)\textsubscript{2} & 467.45 & 466 & 290.2, 346.6, 406.2 \\
Co(C\textsubscript{16}H\textsubscript{11}N\textsubscript{4}O\textsubscript{2})(NO\textsubscript{3})\textsubscript{2} & 473.44 & 472.5 & 288.9, 346.9, 409.8 \\
Cu(C\textsubscript{16}H\textsubscript{11}N\textsubscript{4}O\textsubscript{2})(NO\textsubscript{3})\textsubscript{2} & 477.55 & 476.7 & 291.8, 352.3, 414.4 \\
Cu(C\textsubscript{16}H\textsubscript{11}N\textsubscript{4}O\textsubscript{2})(OAc)\textsubscript{2} & 471.23 & 471.2 & 291.8, 351.3, 411 \\
Cu(C\textsubscript{16}H\textsubscript{11}N\textsubscript{4}O\textsubscript{2})(Cl)\textsubscript{2} & 424.24 & 423.8 & 291.8, 353.4, 388.1 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Determination of zone of inhibition in mm for all bacterial and fungal strains}
\small
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Compound & Diameter of zone of inhibition in mm \\
\hline
Ligand & 12 & 11.7 & - & - & 10.4 & - \\
Ni(L)(OCOCH\textsubscript{3})\textsubscript{2} & 14.5 & - & 10.6 & - & 11.6 & - \\
Ni(L)(NO\textsubscript{3})\textsubscript{2} & 15.3 & - & - & 10.6 & 12.3 & 13.2 \\
Ni(L)Cl\textsubscript{2} & - & - & 12.5 & 11.2 & 12.3 & 14.2 \\
Co(L)(OCOCH\textsubscript{3})\textsubscript{2} & 17.6 & 17.8 & 10.7 & 11.7 & - & 13.5 \\
Co(L)(NO\textsubscript{3})\textsubscript{2} & 18.2 & 19.2 & 12.3 & 10.5 & 11.8 & - \\
Cu(L)(NO\textsubscript{3})\textsubscript{2} & 14.5 & 15.4 & 11.6 & 11.5 & 13.5 & 12 \\
Cu(L)(OCOCH\textsubscript{3})\textsubscript{2} & 14.7 & 14.6 & 11 & - & 13.6 & 11.6 \\
Cu(L)(Cl)\textsubscript{2} & 13.9 & 13.8 & 11.6 & 11.8 & 14.8 & 12.1 \\
Ciprofloxacin & 28 & 26 & 24 & 24 & - & - \\
Amphotericin-B & - & - & - & - & 12.5 & 13.6 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Minimum inhibitory concentration (μg/ml) for all test compounds against bacterial strains}
\small
\begin{tabular}{|c|c|}
\hline
Compound & MIC (μg/ml) \\
\hline
Ni(L)(OCOCH\textsubscript{3})\textsubscript{2} & 128 & - \\
Ni(L)(NO\textsubscript{3})\textsubscript{2} & 128 & - \\
Co(L)(OCOCH\textsubscript{3})\textsubscript{2} & 64 & 128 \\
Co(L)(NO\textsubscript{3})\textsubscript{2} & 64 & 64 \\
Cu(L)(OA\textsubscript{c})\textsubscript{2} & 128 & 64 \\
Cu(L)(NO\textsubscript{3})\textsubscript{2} & 128 & 64 \\
Cu(L)(OCOCH\textsubscript{3})\textsubscript{2} & 128 & 128 \\
Cu(L)(Cl)\textsubscript{2} & 128 & 128 \\
Ciprofloxacin & 6 & 6 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a}: Staphylococcus aureus, \textsuperscript{b}: Bacillus Subtilis, \textsuperscript{-}: Means no activity, MIC: Minimum inhibitory concentrations.
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: \[^{[23]}\]
\[
\%\text{Radical scavenging activity} = \left( \frac{O.D_{\text{control}} - O.D_{\text{sample}}}{O.D_{\text{control}}} \right) \times 100
\]

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 the 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 factors 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 analysis of complexes was carried out by TGA Perkin Elmer thermo analyzer. The 'H NMR spectra were recorded at room temperature in DMSO-d6 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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