Synthesis and physico-chemical characterization of a new Mannich base, N-(morpholinobenzyl)benzamide and its Cu(II), Co(II), Ni(II) and Zn(II) complexes

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A new Mannich base, N-(morpholinobenzyl)benzamide (MBB), formed by the condensation of morpholine, benzamide and benzaldehyde and its Cu(II), Co(II), Ni(II) and Zn(II) complexes have been synthesized. Their probable structures have been determined on the basis of their microanalytical, IR, UV-vis, 1H NMR, 13C NMR and FAB-mass spectral data. All the complexes exhibit square planar geometry. The monomeric and non-electrolytic nature of the complexes are evidenced by their magnetic susceptibility and low conductance data. The electrochemical behaviour of the complexes in acetonitrile solution has been studied by cyclic voltammetric method. The biological activities of the ligand and its metal chelates against the bacteria E.coli, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa and Shigella flexuri are also reported. The complexes have higher activity than that of the free Mannich base and the control.

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It is well known from the literature that the compounds containing amide moiety have a strong ability to form metal complexes and exhibit a wide range of biological activities. The coordination chemistry of amide group has received much attention due to its diverse coordinating behaviour and the role it plays in biological process. An amide group offers two potential binding sites, i.e., through oxygen and nitrogen for complexation with protons and metal ions. It is now generally accepted that for neutral amide groups, both protonation and metal ion binding will be at the amide oxygen. Upon deprotonation the binding shifts to the amide nitrogen. But, for certain reasons, like bite size and steric hindrance, the coordination may also take place at amide nitrogen. In continuation of our earlier studies, we have synthesized a new Mannich base, N-(morpholinobenzyl)benzamide (MBB) which also contains amide moiety. The synthesis of MBB and its complexation characteristics with Cu(II), Co(II), Ni(II) and Zn(II) salts and the antibacterial activity of the complexes are described in this note.

Experimental
All the reagents used were Merck products. Spectroscopic grade solvents were used for spectral and cyclic voltammetric measurements. The carbon, hydrogen, and nitrogen contents in each complex were performed at RSIC, CDRI, Lucknow. 1H NMR and 13C NMR spectra of the samples were measured in DMSO-d6 at Madurai Kamaraj University, Madurai. The IR spectra were recorded in KBr pellets using a Perkin-Elmer 783 spectrophotometer. The UV-Vis spectra of the complexes were recorded on a Shimadzu UV-1601 spectrophotometer using DMSO as solvent. Magnetic susceptibility measurements of the complexes were carried out using Gouy balance. Copper sulphate was used as the calibrant. Cyclic voltammogram of the copper complex was recorded on a Shimadzu UV-1601 spectrophotometer using DMSO as solvent. Magnetic conductivity measurements of the complexes were carried out using a Systronic conductivity bridge with a dip type cell, using 10−3 M solution of complexes in ethanol. The FAB mass spectra were recorded on a JEOL SX 102/Da−6000 mass spectrophotometer/data system using argon/xenon (6 KV, 10 MA) as the FAB gas. The accelerating voltage was 10 KV and the spectra were recorded at room temperature. m-Nitrobenzene alcohol (NBA) was used as the matrix.

Antimicrobial activity
The in vitro biological screening effects of the investigated compounds were tested against the bacteria, S. aureus, S. typhi, K. pneumoniae, S. flexneri, P. aeruginosa, E. coli by the Well diffusion method using agar nutrient as the medium. The test solutions were prepared by dissolving the compounds in DMSO. In a typical procedure, a well was made on the agar medium inoculated with microorganisms. The well was filled with the test solution using micropipette and the plate was incubated at 35°C for 24 h. During this period, the test solution was diffused
and the growth of the inoculated microorganisms was affected. The inhibition zone developed on the plate was measured. Here ampicillin is used as the control.

**Synthesis of Mannich base**

Benzamide (1.21 g, 10 mmol) in 20 mL of ethanol was mixed with morpholine (0.9 mL, 10 mmol) with stirring to get a clear solution under ice cold condition. To the contents, benzaldehyde (1 mL, 10 mmol) was added dropwise using dropper with stirring for ca. 15-20 min. The reaction mixture was then kept at room temperature for ca. 5 days. The colourless solid obtained was filtered and recrystallized from ethanol (Yield: 70%; m.p. 85°C).

**Synthesis of metal complexes**

A solution of 5 mmol of MC\_2 [M=Cu(II), Co(II), Ni(II) and Zn(II)] and the Mannich base (10 mmol, 2.92 g) in 40 mL ethanol and chloroform mixture (1:6, v:v) was boiled under reflux at 58°C (b.p. of azeotropic mixture) for ca. 3 h. The resulting solution was concentrated and then cooled to 0°C for 12 h and the precipitated complexes were filtered, washed with ethanol and dried in vacuo. The proposed structure of the Mannich base ligand and the complexes is given in Scheme 1.

![Scheme 1 — Proposed structure of: (a) the Mannich base ligand, and (b) its complexes](image)

**Results and discussion**

The physical appearance and analytical data of MBB and its complexes are summarized in Table 1. Elemental analyses show that the metal ions form 1:2 complexes with the ligand, MBB. All the complexes are coloured except the Zn(II) complex and stable at room temperature. They are soluble in CHCl\_3, DMF and DMSO but insoluble in water. Molar conductances of the complexes reveal their non-electrolytic nature. The magnetic susceptibility values of the complexes at room temperature indicate that they are mononuclear.

The mass spectra of the ligand and its copper complex were recorded. The base peak observed (both for the ligand and the complex) at m/z 122 indicates the presence of benzamide moiety. The molecular ion peak for the ligand is observed at m/z = 296 (C\_{18}H\_{20}O\_2N\_2). Other important peaks at m/z 105 and 120 correspond to the presence of PhCO and PhCONH respectively. The molecular ion peak for copper complex is observed at m/z = 654 (C\_{36}H\_{38}O\_4N\_4Cu) and the other peaks at m/z 577, 521 and 444 correspond to the release of Ph, PhCONHCH and PhCONHCHPh, respectively. The data confirm the stoichiometry of the metal complexes being ML\_2 type.

In MBB, the IR bands observed at 3330, 1600, and 1100 cm\(^{-1}\) have been assigned to \(\nu(\text{NH})\), amide \(\nu(\text{C}=\text{O})\) and \(\nu(\text{C}-\text{N}-\text{C})\) of morpholine group respectively. In all the complexes, the \(\nu(\text{N}-\text{H})\) band appeared in the region 3330-3425 cm\(^{-1}\). The amide \(\nu(\text{C}=\text{O})\) and \(\nu(\text{C}-\text{N}-\text{C})\) of morpholine bands displayed substantial negative shifts with fairly low intensity indicating coordination through the oxygen of amide moiety and nitrogen of morpholine entity present in the ligand. Some new bands were found around 420-400 cm\(^{-1}\) and 540-520 cm\(^{-1}\) which may be due to M-N and M-O bonds.

\(^1\)H NMR spectra of the ligand and its zinc complex were recorded in DMSO-\(d_6\) solution. The spectrum of the ligand shows the signals as follows: a broad absorption around 6.2 \(\delta\) due to the NH proton; morpholine N-CH\_2 at 2.2 \(\delta\) and morpholine O-CH\_2 at 3.7 \(\delta\); the methylene proton appeared in 6.7 \(\delta\); the multiplet observed around 7.2 to 8.2 \(\delta\) is assignable to the phenyl group. In the ligand spectrum, one absorption peak at 10.1 \(\delta\) was observed which can be assigned to N=C-OH proton. The disappearance of the peaks at 10.1 \(\delta\) and 6.2 \(\delta\) in the spectrum of zinc complex indicates that the coordination is taking place.
Table 1—Analytical data of the complexes [Found (Calc'd), %]

<table>
<thead>
<tr>
<th>Compound/Complex</th>
<th>Colour</th>
<th>M</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>Mol.Wt.</th>
<th>1M mho cm² mol⁻¹</th>
<th>μeff (B.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{C}_1\text{H}_2\text{O}_3\text{N}_2])</td>
<td>Colourless</td>
<td>—</td>
<td>72.80</td>
<td>6.27</td>
<td>9.05</td>
<td>296.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([\text{C}_6\text{H}_5\text{O}_4\text{N}_2\text{Cu})]</td>
<td>Green</td>
<td>9.76</td>
<td>65.82</td>
<td>5.71</td>
<td>8.03</td>
<td>654.25</td>
<td>17.6</td>
<td>1.79</td>
</tr>
<tr>
<td>([\text{C}_6\text{H}_5\text{O}_4\text{N}_2\text{Co})]</td>
<td>Pink</td>
<td>9.10</td>
<td>65.91</td>
<td>5.41</td>
<td>8.12</td>
<td>649.43</td>
<td>13.8</td>
<td>3.29</td>
</tr>
<tr>
<td>([\text{C}_6\text{H}_5\text{O}_4\text{N}_2\text{Ni})]</td>
<td>Green</td>
<td>9.12</td>
<td>66.00</td>
<td>5.10</td>
<td>8.01</td>
<td>649.41</td>
<td>14.8</td>
<td>—</td>
</tr>
<tr>
<td>([\text{C}_6\text{H}_5\text{O}_4\text{N}_2\text{Zn})]</td>
<td>Colourless</td>
<td>9.17</td>
<td>65.12</td>
<td>5.22</td>
<td>7.94</td>
<td>656.07</td>
<td>8.9</td>
<td>—</td>
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Table 2—IR spectral data of ligands and their complexes

<table>
<thead>
<tr>
<th>Compound/Complex</th>
<th>(\nu) (N-H) cm⁻¹</th>
<th>(\nu) (C=O) cm⁻¹</th>
<th>(\nu) (O-C) cm⁻¹</th>
<th>(\nu) (N-O) cm⁻¹</th>
<th>(\nu) (M-O) cm⁻¹</th>
<th>(\nu) (M-N) cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{C}_1\text{H}_2\text{O}_3\text{N}_2])</td>
<td>3330</td>
<td>1600</td>
<td>1100</td>
<td>—</td>
<td>—</td>
<td>420</td>
</tr>
<tr>
<td>([\text{C}_6\text{H}_5\text{O}_4\text{N}_2\text{Cu})]</td>
<td>3400</td>
<td>1575</td>
<td>1080</td>
<td>540</td>
<td>420</td>
<td>420</td>
</tr>
<tr>
<td>([\text{C}_6\text{H}_5\text{O}_4\text{N}_2\text{Co})]</td>
<td>3350</td>
<td>1580</td>
<td>1095</td>
<td>520</td>
<td>420</td>
<td>420</td>
</tr>
<tr>
<td>([\text{C}_6\text{H}_5\text{O}_4\text{N}_2\text{Ni})]</td>
<td>3425</td>
<td>1595</td>
<td>1075</td>
<td>540</td>
<td>400</td>
<td>420</td>
</tr>
<tr>
<td>([\text{C}_6\text{H}_5\text{O}_4\text{N}_2\text{Zn})]</td>
<td>3380</td>
<td>1600</td>
<td>1085</td>
<td>540</td>
<td>420</td>
<td>420</td>
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</table>

via the dissociation of the –OH proton of the ligand. The signal due to morpholine N-CH₂ proton also shifted downfield and appeared at 2.6 δ in the complex. This is an indication of the coordination of morpholine nitrogen.

\(^{13}\text{C}\) NMR spectral data show the presence of the expected number of signals corresponding to the number of chemically different types of carbon atoms present in the compounds. The spectrum of the ligand MBB shows 8 signals of which four at 78.48, 77.04, 170.32 and 169.42 ppm, in which the former two are due to morpholine carbons and the latter two are due to C=O and –CH carbons respectively. The chemical shifts for the ring carbons are C(1) 131.45, C(2) 130.02, C(3) 128.22 and C(4) 133.81 ppm respectively. Zn(MBB)₂ shows four signals at 78.88, 78.08, 171.24 and 170.05 ppm which are due to morpholine, C=O, –CH carbons respectively. The chemical shifts of the ring carbons are C(1) 130.81, C(2) 130.41, C(3) 128.02 and C(4) 133.64 ppm.

The electronic absorption spectra of the ligand and its Cu(II), Co(II) and Ni(II) complexes were recorded in DMSO solution. The green coloured Cu(II) complex showed a broad band at 17090 cm⁻¹ which is assigned to \(2^2\text{B}_1g\rightarrow2^2\text{A}_1g\) transition indicating square planar structure of the complex. \(^{14,15}\) Co(II) complex exhibits a band at 19294 cm⁻¹ which is assigned to \(1^2\text{A}_1g\rightarrow1^2\text{B}_1g\) for square planar geometry. Ni(II) complex showed a band at 16233 cm⁻¹ which is assigned to \(1^2\text{A}_1g\rightarrow1^2\text{B}_1g\) transition conforming a square planar geometry for the complex.

These facts are also supported by their magnetic measurements. The magnetic moments of Cu(II) (1.79 BM) and Co(II) (3.29 BM) complexes confirm the square planar assignment for them. Further, the Ni(II) complex is diamagnetic in nature indicating that it has square planar configuration.

Cyclic voltammogram of copper complex was recorded in dry acetonitrile solution at room temperature. The copper complex shows a single peak in the cathodic side which corresponds to the reduction of Cu(II) to Cu(I) at \(E_{p,c}=-0.76\) V. After an initial scan if the potential is reversed towards the anodic direction the peak corresponding to the formation of the couple Cu(0) to Cu(II) at \(E_{p,a}=0.14\) V and is attributable to the oxidation of deposited metal to Cu(II). \(^{16}\) The two-electron nature of the process is established by the comparison of \(i_p\) and \(i_a\) values.

**Antimicrobial activity**

The *in vitro* biological screening effects of the investigated compounds were tested against six bacteria: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Shigella flexneri* by the Well diffusion method. The zone of inhibition values of the compounds against the growth of microorganisms are summarized in Table 3. A comparative study of the ligand and its complexes indicates that the metal chelates exhibit higher activity than the free ligand and the control (ampicillin). The order of activity towards *Shigella flexneri* and
Table 3—Antibacterial activity of the ligands and their complexes (zone of inhibition in mm)

<table>
<thead>
<tr>
<th>Compound</th>
<th>S. flexneri</th>
<th>S. typhi</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
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<tr>
<td>Ampicillin</td>
<td>18</td>
<td>18</td>
<td>16</td>
<td>11</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>MBB</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>9</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Cu(II) complex</td>
<td>22</td>
<td>20</td>
<td>25</td>
<td>18</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Co(II) complex</td>
<td>19</td>
<td>18</td>
<td>15</td>
<td>15</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Ni(II) complex</td>
<td>14</td>
<td>10</td>
<td>13</td>
<td>10</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Zn(II) complex</td>
<td>20</td>
<td>23</td>
<td>12</td>
<td>17</td>
<td>17</td>
<td>20</td>
</tr>
</tbody>
</table>

Staphylococcus aureus is Cu > Zn > Co > Ni; Pseudomonous aeruginosa is Cu > Co > Zn > Ni; Klebsiella pneumoniae and Salmonella typhi is Zn > Cu > Co > Ni; Escherichia coli is Cu > Co > Zn > Ni. The increase in antimicrobial activity is due to faster diffusion of metal complexes as a whole through the cell membrane or due to the combined activity effect of the metal and ligand. Such increased activity of the metal chelates can be explained on the basis of Overtone’s concept and the Tweedy’s chelation theory. The lipid membrane that surrounds the cell favours the passage of only lipid soluble materials due to which lipophilicity is an important factor which controls the antimicrobial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of $\pi$-electron over the whole chelate ring and enhances the liposolubility of the complexes. This increased liposolubility enhances the penetration of the complexes into the lipid membrane and blocks the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restrict further growth of the organisms.

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References