Antibacterial Activity and DNA Binding Properties of Bivalent Metal Complexes of Cuminaldehyde Acetoylhydrazone

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ABSTRACT

Metallo-hydrazones having the formula [M(IBAH)₂] (where, M = Ni(II), Cu(II) and Zn(II); IBAH is p-Isopropylbenzaldehyde acetoylhydrazone) are prepared and confirmed on the basis of physico-chemical and spectral analyses. Conductivity data revealed that the complexes are non-electrolytes. Metal-DNA interactions are investigated using absorption spectrophotometry. Binding constant (Kₘ) data revealed that the copper complex interact DNA more strongly than other complexes. Antibacterial activity studies indicated higher activity for complexes than the metal free hydrazone ligand. The copper compound displays higher activity. DNA binding constants are correlated with the activity of metal compounds in this article.

Keywords: New metallo-hydrazones, Spectral characterization, DNA binding constants, Antibacterial activity.

INTRODUCTION

Hydrazones exhibit a broad spectrum of biological activities such as antibacterial⁴, antitubercular⁵, antioxidant⁶, antiviral⁷ and anticancer⁸ activities. Hydrazones constitute an important class of compounds for new drug development⁹. Hydrazones derived from acetichydrazide are named as acetohydrazone. Compared to simple hydrazone Schiff bases, acyl and aryl hydrazones have an extra donor site in >C=O. This presents a wide range of properties in them. The discovery that acetohydrazone show higher activity than benzylohydrazone evoked considerable interest⁶,⁷ to investigate metal complexes of former type of ligands.

Survey of literature revealed that a very few acetohydrazones are used as chromogenic reagents⁸ and in the study of transition⁹-¹¹ and lanthanide¹²,¹³ metal complexes. Hydrazones derived from aldehydes contain hydrogen atom on azomethine carbon atom. It is reported¹⁴ that hydrazones having azomethine hydrogen atom shows higher activity. Hence it is of interest to investigate hydrazones derived from aldehydes rather than ketones.
Our customary foods contain carbonyl compounds showing beneficial effects to human health. The chemical name of cuminaldehyde is p-Isopropylbenzaldehyde (IB). It is known to present in cumin, essential oils of eucalyptus, myrrh, cassia etc. The aromatic aldehyde, viz. p-Isopropylbenzaldehyde (IB) is an important ingredient of cumin. It has medicinal properties. For example, it is known to inhibits the fibrillation of alpha-synuclein present in cumin. Hence it is of interest to use p-Isopropylbenzaldehyde in the synthesis of new hydrazone ligand and its metal complexes.

Studies on Metal-DNA interactions are expected to predict biological activity of compounds. The compound which bind DNA strongly may find application in the drug development. Survey of literatures revealed that metal complexes with organic ligands showed higher biological activity than metal free organic compounds. Hence it is considered worthwhile to investigate novel hydrazone ligand originated from p-Isopropylbenzaldehyde and its metal complexes. We have studied nucleic acid binding of various metal complexes in the past to develop antimicrobial agents. To renew our interests, herein we communicate our results on spectral analysis and biological applications of bivalent metal complexes with p-Isopropylbenzaldehyde acetoxyhydrazone (IBAH).

**EXPERIMENTAL**

Acetylhydrazide and p-Isopropylbenzaldehyde were bought from Sigma Aldrich and utilized without further purification. Metal salts (ZnCl$_2$, NiCl$_2$6H$_2$O and CuCl$_2$2H$_2$O) were of Merck AR quality. Solvents were distilled before use.

**Synthesis of IBAH ligand**

Round bottom flask (100 mL capacity) was charged with 20 mL of methanolic solution of acetic hydrazide (0.91g; 0.012 mol) and 20 mL of methanolic solution of p-Isopropylbenzaldehyde (5 mL, 0.03 mol). Glacial acetic acid (few drops) was added as catalyst to the contents of flask. The reactants were heated on water bath for 2.5 h and cooled. The product was collected and washed with few drops 50% methanol and desolvated in vacuum. The compound was ammassed and treated with methanol followed by filtration. The compound was recrystallized from methanol. Yield: 70.58%; m.p., 130-132 oC. Molecular formula: C$_{12}$H$_{16}$N$_2$O. Formula weight, 204.1. Synthesis of IBAH is depicted in Scheme 1.

**Preparation of metallo-hydrazones**

The IBAH ligand (1.5 g; 0.007 mol) was transferred to 100-mL beaker and dissolved in 20 mL ethanol. In another 100-mL beaker, CuCl$_2$.2 H$_2$O (0.007 mol) was dissolved in 15 mL of ethanol. These two solutions were mixed in a clean R.B flask and refluxed for 2 hours. On cooling, a green stained product was formed. The compound was ammassed and washed with few drops 50% methanol and desolvated in vacuum. Nickel(II) and zinc(II) complexes of IBAH were prepared similarly. Melting points and yields of compounds are delineated in Table 1. Particulars of equipment employed in the present study, deoxyribonucleic acid (DNA) binding and antibacterial activity experiments are given in our previous articles.

**RESULTS AND DISCUSSION**

The ligand (IBAH) is characterized on the basis of spectroscopic studies. FT-IR spectroscopy: 3264, 3082, 2945, 1648 and 1542 are designated to secondary amine (N-H), aromatic C- H, aliphatic C-H, >C=O and azomethine (>C=N) elongating vibrations correspondingly. 1H-NMR spectroscopy (in deuterated chloroform): δ 10.64 (s, 1H) 8.95 (s, 1H ) (7.29 - 7.75) (m, 4H), 2.32 (s, 3H) 1.271 (d, 6H) 2.97 (septet 1H) are respectively assigned to azomethine (CH=N-), >NH , Ar-H, CH$_3$ of acetoyl, -CH$_3$ protons of isopropyl and -CH methine protons of isopropyl group sequentially. Mass spectrometry: Mass plot of IBAH (Fig. 1) shows a peak at m/z = 204.1 which coincides with formula (C$_{12}$H$_{16}$N$_2$O) weight of IBAH.

Based on spectral analysis the structure (Fig. 2) of IBAH ligand is proposed. Mass spectral fragmentation pattern of IBAH (Fig. 3) is shown below.
Structural characterization of metal complexes

Metal chlorides reacted with \( p \)-isopropylbenzaldehyde acetoxyhydrazone (IBAH) in basic medium under reflux conditions to produce corresponding metal complexes. The complexes are stable, non-hygroscopic, moderately soluble in CH\(_3\)OH and C\(_2\)H\(_5\)OH but easily soluble in dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). Color, yield, ESI-MS & conductivity data of Cu(II), Ni(II) and Zn(II) compounds are stated in Table 1. Molar conductivity outcome show that the coordination compounds are non-electrolytes\(^{21}\).

Electronic spectroscopy

UV-Visible data of metal complexes and designation of peaks are summarized in Table 2. The electronic spectrum of Cu(II) complex shows (Fig. 4) peak at 14,792 cm\(^{-1}\) (676 nm) having shoulder peak at 16,447 cm\(^{-1}\) in the visible region. This band is assigned to \( ^2E_g \rightarrow ^2T_g \) electron transfer in favour of octahedral geometry. The presence of shoulder peak at 16,447 cm\(^{-1}\) (608 nm) may be due to Jahn-Teller effect which is due to geometric distortion generally noticed for copper(II) octahedral compounds where the two axial bonds can be shorter or longer than those of the equatorial bonds.

Peaks are observed at 16,366 and 10, 111 in the electronic spectrum of nickel complex. These peaks are related to \( ^3A_2g \rightarrow ^3T_2g(F) \) and \( ^3A_2g \rightarrow ^3T_1g(P) \) electron transfers respectively in support of octahedral geometry. A peak in higher energy region due to \( ^3A_2g \rightarrow ^3T_2g \) transition is not observed due to the domination of \( \pi \rightarrow \pi^* \) transition.

IR spectroscopy

The coordination sites of IBAH ligand are uncovered by examining IR spectra of metal-free ligand and complexes. Spectral data are stated in Table 3. In the spectrum of the IBAH bands are observed due to \( \nu_{\text{NH}} \), \( \nu_{\text{C=O}} \) and \( \nu_{\text{C-N}} \) stretching modes. Both \( \nu_{\text{NH}} \) (3264 cm\(^{-1}\)) and \( \nu_{\text{C=O}} \) (1648 cm\(^{-1}\)) bands of ligand are absent in the spectra of complexes due to enolization and subsequent deprotonation of ligand during complex formation (Scheme 2).
Table 3: Infrared Spectral data of IBAH ligand and its complexes

<table>
<thead>
<tr>
<th>IBAH</th>
<th>Cu(IBAH)₂</th>
<th>Ni(IBAH)₂</th>
<th>Zn(IBAH)₂</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3264</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>νN-H</td>
</tr>
<tr>
<td>3082</td>
<td>3014</td>
<td>3027</td>
<td>3024</td>
<td>νC=O aromatic</td>
</tr>
<tr>
<td>3045</td>
<td>2920</td>
<td>2915</td>
<td>2913</td>
<td>νC=H aliphatic</td>
</tr>
<tr>
<td>1648</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>νC=N</td>
</tr>
<tr>
<td>1648</td>
<td>1615</td>
<td>1620</td>
<td>1612</td>
<td>νC=O aromatic</td>
</tr>
<tr>
<td>1542</td>
<td>1528</td>
<td>1522</td>
<td>1532</td>
<td>νC=N</td>
</tr>
<tr>
<td>1465</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>νC=O aromatic</td>
</tr>
<tr>
<td>1378</td>
<td>1367</td>
<td>1318</td>
<td>1312</td>
<td>νC=O aromatic</td>
</tr>
<tr>
<td>1264</td>
<td>1260</td>
<td>1242</td>
<td>1248</td>
<td>νC=O aromatic</td>
</tr>
<tr>
<td>1185</td>
<td>1173</td>
<td>1178</td>
<td>1165</td>
<td>νC=O aromatic</td>
</tr>
<tr>
<td>604</td>
<td>616</td>
<td>632</td>
<td>-</td>
<td>νM-O</td>
</tr>
<tr>
<td>574</td>
<td>538</td>
<td>542</td>
<td>-</td>
<td>νM-N</td>
</tr>
<tr>
<td>513</td>
<td>520</td>
<td>524</td>
<td>-</td>
<td>νM-N</td>
</tr>
</tbody>
</table>

The bands of νC=O and νC≡N are shifted to lower wave numbers indicating involvement of acetyl >C=O and azomethine (>C≡N-) nitrogen groups in chelation. Spectral data suggest that the IBAH behaves as uninegative bidentate ligand in complexes. Bands in low energy regions, 604-632 and 513-524 cm⁻¹ are related to νM-O and νM-N vibrations respectively.

ESR spectroscopy

ESR spectrum of Cu complex in DMF at liquid nitrogen temperature (LNT) is depicted in Fig. 5. Table 4 gives ESR data of copper(II) complex in solid state & in DMF solvent. The g, g∥, g⊥ and α² and λ values of Cu complex suggest covalent nature of Metal- ligand (M-L) bond and the location of unpaired electron in d<sup>2</sup>- orbital. This finding indicates covalent nature of metal-ligand (M-L) bond. The orbital reduction parameters (K∥, K⊥) reveal the occurrence of in-plane π-bonding in the complex. The axial symmetry parameter values of complex in solution state at R.T is below 4.0. The values indicate absence of quid pro quo interaction.

Table 4: Spectral data of [Cu(IBAH)₂] complex in DMF at LNT

<table>
<thead>
<tr>
<th>g∥</th>
<th>g⊥</th>
<th>g avg</th>
<th>G</th>
<th>A∥ x 10⁻⁵</th>
<th>A⊥ x 10⁻⁵</th>
<th>K∥</th>
<th>K⊥</th>
<th>λ</th>
<th>α²</th>
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<tr>
<td>2.38</td>
<td>2.07</td>
<td>2.17</td>
<td>5.64</td>
<td>0.00262</td>
<td>0.00014</td>
<td>0.997</td>
<td>1.091</td>
<td>520</td>
<td>0.3816</td>
</tr>
</tbody>
</table>

ESR data of complex at RT are given in brackets.

Deoxyribonucleic acid Interactions

Interactivity of complexes with CT-DNA was examined by UV-Visible spectroscopy. Spectra of copper complex are shown in Figure 7.

Metal derivatives of IBAH showed strong peak due to M→L charge transfer transitions. The binding constant (Kₘ) is calculated using standard equation. DNA-binding constants of complexes are given in the Table 5. Metal complexes (except Cu(II) complex) showed considerable hypsochromic shift (Blue shift) (Δλ<sub>max</sub> = 0.8-1.5 nm) with raising amounts of DNA. High binding constants (4.3-17.2 x 10⁶ M⁻¹) of present complexes are suggestive of intercalation mode of binding. The binding constant (Kₘ) order is Cu(IBAH)₂ > Ni(IBAH)₂ > Zn(IBAH)₂. The observed trend indicates that Cu(IBAH)₂ interacts DNA more firmly.

Deoxyribonucleic acid Interactions

On the basis of analytical, molar conductivity, UV-Visible, FT-IR ESR spectroscopy studies a general structure (Fig. 6) is tentatively suggested.

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Antibacterial activity studies

The compounds under investigation are tested for antibacterial activity as outlined before\(^8\) by taking Gram-positive (*Micrococcus luteus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria using agar well diffusion method against ciprofloxacin as positive control. About 24 h old bacterial cultures are used to prepare bacterial lawns. Agar wells were prepared and injected with sample solutions prepared using DMF. The agar plates are incubated at 35°C for 24 h and inhibition zones are measured and expressed Table 6 in millimeter units.

Results in Table 6 indicate that the present coordination compounds show higher activity than metal-free IBAH ligand. Data are depicted in the form of bar graph (Fig. 8) which indicates that Cu(IBAH)\(_2\) complex shows more activity possibly due to its strong binding to DNA.

Metal complexes displayed higher antibacterial activity\(^29,30\) than the metal free ligand. Increased activity of coordination compounds may be explained by using Tweedy’s chelation theory\(^31\) and Overtone’s concept\(^32\). The ligand forms organic coat around the metal ion in the formation of complex. As a result the complex becomes lipophilic. According to latter concept, the cell membrane allows the passage of complexes as they are lipid-soluble. Hence lipid solubility of compound is principal requirement for showing this action. On complex formation, the polarity of metal ion is significantly reduced due to delocalization of π-electrons. On entering into the cell the complex undergo dissociation and releases metal ion which inhibits enzymatic activity of microorganisms more effectively\(^33\).

### Table 6: Zone inhibition (in mm) data of IBAH ligand and its metal complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount Taken (μg/μL)</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>M. luteus</th>
<th>B. subtilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>5</td>
<td>11.68±0.05</td>
<td>10.24±0.01</td>
<td>9.78±0.02</td>
<td>10.36±0.04</td>
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<tr>
<td>IBAH</td>
<td>100</td>
<td>1.57±0.19</td>
<td>1.56±0.11</td>
<td>1.49±0.37</td>
<td>1.03±0.43</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1.82±0.35</td>
<td>2.48±0.01</td>
<td>2.73±0.25</td>
<td>2.87±0.04</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>2.64±0.44</td>
<td>2.65±0.28</td>
<td>2.68±0.08</td>
<td>2.29±0.22</td>
</tr>
<tr>
<td>Cu( IBAH)(_2)</td>
<td>100</td>
<td>6.45±0.25</td>
<td>3.42±0.39</td>
<td>4.09±0.32</td>
<td>3.93±0.16</td>
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<tr>
<td></td>
<td>200</td>
<td>7.05±0.36</td>
<td>5.46±0.05</td>
<td>5.92±0.42</td>
<td>5.84±0.38</td>
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<td>300</td>
<td>6.31±0.08</td>
<td>6.29±0.03</td>
<td>6.38±0.14</td>
<td>5.68±0.24</td>
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<tr>
<td>Ni( IBAH)(_2)</td>
<td>100</td>
<td>2.14±0.06</td>
<td>1.75±0.06</td>
<td>1.92±0.42</td>
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<tr>
<td></td>
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<td>3.95±0.18</td>
<td>2.22±0.03</td>
<td>2.33±0.50</td>
<td>2.70±0.13</td>
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<tr>
<td></td>
<td>300</td>
<td>4.09±0.22</td>
<td>4.86±0.36</td>
<td>3.57±0.27</td>
<td>4.58±0.11</td>
</tr>
<tr>
<td>Zn( IBAH)(_2)</td>
<td>100</td>
<td>2.02±0.17</td>
<td>3.08±0.23</td>
<td>2.73±0.04</td>
<td>1.89±0.19</td>
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<tr>
<td></td>
<td>200</td>
<td>4.94±0.01</td>
<td>4.33±0.18</td>
<td>4.52±0.41</td>
<td>2.51±0.28</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>5.73±0.23</td>
<td>6.14±0.31</td>
<td>3.43±0.29</td>
<td>4.73±0.35</td>
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</table>
A new organic ligand viz. p-Isopropylbenzaldehyde acetoxythidrazone (IBAH) and its metal complexes are synthesized and characterized for the first time. A general structures for the complexes is proposed based on analytical and electronic spectral data. High DNA binding constants suggest that the complexes bind DNA via intercalation. Complexes showed higher antibacterial activity. Among all compounds, the copper complex binds DNA more firmly and inhibits bacteria effectively. The Cu(II)(IBAH)_2 complex not only binds DNA strongly but also inhibits bacteria more effectively. The observation revealed a direct relationship between DNA binding constant and antibacterial activity. Thus antibacterial activities of metal complexes are concomitant with their DNA binding constants.

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Conflicts of interest

Regarding the publication of this article, the authors declare that there is no conflict of interests.

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