Research Article

Synthesis, Characterisation, and Biological Evaluation of Zn(II) Complex with Tridentate (NNO Donor) Schiff Base Ligand

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The present paper deals with the synthesis and characterization of metal complex of tridentate Schiff base ligand derived from the inserted condensation of 2-aminobenzimidazole (1H-benzimidazol-2-amine) with salicylaldehyde (2-hydroxybenzaldehyde) in a 1:1 molar ratio. Using this tridentate ligand, complex of Zn(II) with general formula ML has been synthesized. The synthesized complex was characterized by several techniques using molar conductance, elemental analysis, FT-IR, and mass and 1HNMR spectroscopy. The elemental analysis data suggest the stoichiometry to be 1:1 [M:L]. The complex is nonelectrolytic in nature as suggested by molar conductance measurements. Infrared spectral data indicate the coordination between the ligand and the central metal ion through deprotonated phenolic oxygen, imidazole nitrogen of benzimidazole ring, and azomethine nitrogen atom. Spectral studies suggest tetrahedral geometry for the complex. The pure compound, synthesized ligand, and metal complex were screened for their antimicrobial activity.

1. Introduction

Metal complexes of Schiff base have played a central role in the development of coordination chemistry [1]. Various Schiff base complexes have been widely studied because they have antimicrobial, anticancer, analgesic, anti-inflammatory, antifertility, and herbicidal applications [2, 3]. Chelating ligands containing N, N, and O donor atoms show broad biological activity and are of special interest because of the ways in which they are bonded to the metal ions [4]. It is known that existence of metal ions bonded to biologically active compounds may enhance their activities [5–7]. Even though many Schiff bases using salicylaldehyde and substituted salicylaldehydes and amines had been studied [8–11] as ligands, no work had been done with salicylaldehyde and 2-aminobenzimidazole as the basic nucleus of Schiff bases. Schiff base metal complexes had been a widely studied subject to their industrial and biological applications [12]. The synthesis, physicochemical characterization, and biological activity of 2-aminobenzimidazole complexes with different metal ions have been reported [13]. An efficient synthesis of Schiff bases containing benzimidazole moiety catalysed by transition metal nitrates had been done [14]. The synthesis and antimicrobial activity of Cu(II), Co(II), and Ni(II) complexes with 2-aminobenzimidazole were available [15].

2. Experimental

2.1. Chemicals. All the chemicals used were of AR/GR grade. Pure sample of 2-aminobenzimidazole (Ab), molecular formula C7H7N3, molecular weight 133.15 g/mol, melting point 229–231°C was obtained from Himedia Pharmaceuticals Ltd. (Figure 1). Metal salt of ZnCl2 was of Merck chemicals. Solvents used were ethanol, acetone, and DMF.

2.2. Synthesis of Schiff Base. The Schiff base was prepared by mixing equimolar amounts of 2-aminobenzimidazole and the aromatic aldehyde in ethanol. The mixture was refluxed for about 4 hours. Concentration of the solution was done to
reduce it to one-half of its original volume and kept for 4-5 days when deep yellow crystals were formed in the reaction mixture, which were filtered, washed thoroughly with the same solvent, and recrystallized with acetone. The yellow Schiff base product obtained (m.p. 142°C) produced in 54% yield was subjected to analysis (Figure 2).

2.2.1. Synthesis of the Complex. For the synthesis of complex, Ab-S-Zn, ligand-metal ratio was determined by conductometric titration using monovariation method on Systronics conductivity meter using dip type electrode. 20 mL of the ligand (0.01 M) was diluted to 200 mL using pure ethanol and titrated against ZnCl₂ (0.02 M) solution prepared in the same solvent. Conductance was recorded after each addition of metal salt solution. Graph is plotted between corrected conductance and volume of metal salt added.

From the equivalence point in the graph, it has been concluded that the complex formation of the ligand with the metal takes place in the ratio 1:1 (L: M). Conductometric titration supported 1:1 (L: M) ratio in the complex, which was further supported by Job's method of continuous variation as modified by Turner and Anderson.

0.02 M solution of Ab-S and 0.02 M metal salt solution were prepared separately in pure ethanol and mixed. Colour of the resulting solution was yellow green. The pH of the solution was raised to 7.5 by adding N/10 NaOH solution. This solution was refluxed for 4 hours and kept for four days when pale green coloured product was obtained. This product was then filtered, washed with the same solvent, dried over fused CaCl₂, weighed, and subjected to analysis.

2.3. Physical Measurements. Elemental analysis was carried out on Vario MICRO V2.20 Elemental Analyser System GmbH, from IIIM, Jammu. Metal contents were determined gravimetrically [16]. The infrared spectra were recorded on FT-InfraRed Spectrophotometer Model RZX (Perkin Elmer) using KBr pellets, from SAIF, Punjab University, Chandigarh. Molar conductance measurement was made in 10⁻³ M DMF solution on a Systronics direct reading conductivity meter (Model 303).

The melting points of the ligand and the complex were recorded in open capillaries on a capillary melting point apparatus. The NMR spectra were recorded at SAIF/CIL, PU, Chandigarh, by NMR spectrophotometer. The mass spectra was recorded at SAIF/CIL, PU, Chandigarh, by LC-MS spectrometer model Q-ToF Micro Waters.

2.4. In Vitro Antimicrobial Activity. Qualitative analysis for screening of antibacterial activity of Schiff base and its complex was carried out by agar well diffusion method (Table 3) [17, 18] with modifications. The compounds were tested against two gram positive and two gram negative bacteria at concentration 10⁻³ M. 20 mL of sterilized nutrient agar was inoculated with 100 mL of bacterial suspension (10⁸ CFU/mL) and then poured on to sterilized petri plate. The agar plate was left to solidify at room temperature.

A well of 6 mm was aseptically bored into the agar plate. Then, 20 mL of the complexes (diluted with DMSO, 1:1) was added in each well. Chloramphenicol (10 μg) was used as a positive reference to determine the sensitivity of bacteria. The plates were kept at 4°C for 2 hours to allow the dispersal and then incubated.

Definite volumes of peptone (5 gm), beef extract (3 gm), and agar (15 gm) were dissolved in distilled water to prepare the nutrient agar and pH was adjusted to 7.2. This solution was sterilized by autoclaving at 15 psi for 20 minutes.

The in vitro antifungal activity by agar well diffusion method [19] was standardized using Griseofulvin. Sabouraud’s dextrose agar medium was used as the nutrient medium (HiMedia fungi used). The medium was prepared by dissolving in water and autoclaving at 121°C for 15 minutes. Standard cultures of Candida albicans and Aspergillus niger were employed for the present study.

Griseofulvin standard was prepared at a final concentration of 10 μg/mL in sterile distilled water. The surface of Sabouraud’s agar plate was dried at 35°C. Later bores in plate were made using sterile cork borer. A control having only DMSO was maintained in each plate. The plates were incubated at 35°C for 48 hrs. Later the values of zones of inhibition were recorded. The plates were left for diffusion for one hour in the refrigerator and then incubated at 25°C and the inhibition zone for C. albicans was recorded after 24 hours of incubation. The zone of A. niger was recorded after 36 hours of incubation.

3. Results and Discussion

The reaction in solution between the ligand and transition metal cation may proceed by the following general equation:

\[ MX_n + nHL \rightarrow ML_n + nHX \]  

where \( n = 1 \) or 3; \( M = \text{Zn(II)} \); and \( X = \) corresponding anion of metal salt.

The metal complex is formed in 1:1 stoichiometric ratio. The ligand and the metal (II) complex were isolated pure from EtOH in good yields and they are of various colours. The ligand is yellow whereas the complex is pale green in colour. The complex gets decomposed up to 245°C. They are very air stable solids at room temperature without decomposition for a long time. The complex is nonhygroscopic and insoluble.
in water and other common organic solvents but soluble in DMF and DMSO. The molar conductance value of the complex (measured in 10⁻³ M DMF) is 18.0 Scm²mol⁻¹, indicating the nonelectrolytic nature [20] of the complex. The analytical data and molar conductance values are given in Table 1.

### Table 1: Analytical and physicochemical data of Schiff base and its metal complex.

<table>
<thead>
<tr>
<th>Ligand/complex</th>
<th>M : L</th>
<th>Mol. Wt.</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>M</th>
<th>Colour (yield%)</th>
<th>M.Pt. (°C)</th>
<th>Scm² mol⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>[HL] C₆H₁₂N₅O</td>
<td>—</td>
<td>237.25</td>
<td>70.87</td>
<td>70.80</td>
<td>4.67</td>
<td>(4.58</td>
<td>17.71</td>
<td>(17.59)</td>
<td>—</td>
</tr>
<tr>
<td>C₁₀H₁₄N₅OCl Zn</td>
<td>1 : 1</td>
<td>(337.09)</td>
<td>50.80</td>
<td>(49.76)</td>
<td>2.37</td>
<td>(2.46</td>
<td>14.41</td>
<td>(13.47</td>
<td>19.65</td>
</tr>
</tbody>
</table>

#### 3.1. IR Spectral Study.

The comparative analysis of the IR spectra of the complex and of the free ligand (Table 2) revealed that the absorption band characteristic of the stretching vibrations of –C=N– (azomethine group) is shifted towards higher wave number from 1609 cm⁻¹ in the spectrum of the ligand to 1642 cm⁻¹ in the complex [8, 21]. This indicates the coordination of the nitrogen of this group with the metal ion [22]. A broadband appearing at ~3566 cm⁻¹ assigned to ν₁O-H in the Schiff base was no longer found in the spectra of the investigated metal complex [23] and, instead, appearance of a new band at 1272 cm⁻¹ due to ν₁(C=N) stretching vibrations indicated deprotonation and coordination of the hydroxyl oxygen to the metal ion [24]. In addition, the appearance of new band at 335 cm⁻¹ showing the involvement of M–Cl bond in complex formation, supporting the IR spectral study. Further conclusive evidence of coordination of the Schiff base with the metal ion was shown by the appearance of low frequency new band at 648 cm⁻¹ due to metal-oxygen ν₁(M–O) vibrations [23, 26] and was observed in the spectra of the thus confirming participation of the oxygen atom in the coordination. Further coordination of metal to azomethine nitrogen is confirmed by a new band at 508 cm⁻¹ with the involvement of the imidazole ring nitrogen in the coordination with metal ion [27] due to ν₁(M–N).

#### 3.2. ¹H NMR Spectra.

¹H NMR spectrum of the ligand (Ab-S) shows a signal at 12.39 ppm as singlet due to phenolic –OH [28]. In the complex Ab-S-Zn, the absence of any signal due to phenolic proton (–OH) in this region indicates the involvement of phenolic oxygen in coordination via deprotonation [29], thus further supporting the IR spectral observations. In the ligand, the signal at 9.63 ppm as singlet due to proton of azomethine group [30] shows an upfield shifting of 1.18 ppm and appears at 10.81 ppm in the complex indicating the coordination of azomethine nitrogen with metal ion [31]. The signals for the ligand were obtained at 8.16–6.84 ppm [32] due to protons of aromatic rings (8H, Ar–H) which gets shifted to higher field in the complexes due to shielding and appears as singlet and multiplet.


The mass spectrum of [Zn(C₁₄H₁₀N₃OCl)] shows a molecular ion peak at m/z 339.09 (I) due to [Zn(L)]⁺⁺ which suggests the monomeric nature of the complex. The intense peak at m/z 238.1 (IV) corresponds to loss of ligand molecule L⁺⁺, that is, (C₁₄H₁₀N₃O)⁺⁺ from (II), indicating molecular mass of the ligand. Other peaks of appreciable intensity observed at m/z values of 306.54 (II) due to loss of (Cl)⁻ from (I), at m/z 249.64 (III) due to loss of (C₁₀H₇)⁺ from (II), and at m/z 183.1 (V) due to loss of (C₇H₄)⁺ from (IV), and base peak at m/z 134.1 (VI) is due to loss of (C₆H₄N₂)⁺ from (IV). An intense peak formed at (VI) indicates loss of the 2-aminobenzimidazole ring. The relative intensities of these peaks give an assessment of the stabilities of the various fragments. Such type of fragmentation patterns has been reported by many workers [33–35].

#### 3.4. Antimicrobial Study.

The pure compound does not show any biological activity but the synthesized Schiff base and the complex showed moderate to good biological activity against all microbes. In case of bacterial strains E. coli showed good activity in both Schiff base and the complex whereas others showed moderate to zero activity [36].

But in case of fungal strains A. niger has zone of inhibition ranging between 1.15 mm and 3.41 mm with the metal complex having maximum zone of inhibition more than the derived Schiff base. Further, in case of C. albicans, the effective antifungal activity was shown again by the complex with 3.20 mm zone of inhibition (Table 4), whereas the Schiff base showed moderate activity having 1.35 mm zone of inhibition compared to the complex [37].

### Table 2: Important IR spectral bands (cm⁻¹) of the ligand and its complex.

<table>
<thead>
<tr>
<th>Ligand/complex</th>
<th>ν₁(C=N)</th>
<th>ν₁(νₓH,C=N)</th>
<th>ν₁(νₓH,C=N)</th>
<th>ν₁(C=O)</th>
<th>ν₁(M–Cl)</th>
<th>ν₁(M–N)</th>
<th>ν₁(M–O)</th>
<th>ν₁(chelate ring)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab-S</td>
<td>1609 s</td>
<td>1569 s</td>
<td>1439 s</td>
<td>1273 s</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>[Zn(Ab-S)Cl]</td>
<td>1642 b</td>
<td>1519 w</td>
<td>1465 s</td>
<td>1272 s</td>
<td>335 s</td>
<td>508 m</td>
<td>648 s</td>
<td>1319 s</td>
</tr>
</tbody>
</table>
Table 3: Antibacterial activity of pure compound, its ligand, and metal complex.

<table>
<thead>
<tr>
<th>Compounds/complexes (100 µg/mL)</th>
<th>B. subtilis</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ab-S</td>
<td>0.9</td>
<td>—</td>
<td>1.15</td>
<td>1.15</td>
</tr>
<tr>
<td>[Zn(Ab-S)Cl]</td>
<td>1.3</td>
<td>—</td>
<td>1.15</td>
<td>—</td>
</tr>
<tr>
<td>Chloramphenicol 10 µg (4 mm)</td>
<td>2.0</td>
<td>2.15</td>
<td>2.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Table 4: Antifungal activity of pure compound, its ligand, and metal complex.

<table>
<thead>
<tr>
<th>Compounds/complexes (100 µg/mL)</th>
<th>Candida albicans Diameter of growth of inhibition zone (mm)</th>
<th>Aspergillus niger Diameter of growth of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ab-S</td>
<td>1.35</td>
<td>1.15</td>
</tr>
<tr>
<td>[Zn(Ab-S)Cl]</td>
<td>3.20</td>
<td>3.41</td>
</tr>
<tr>
<td>Griseofulvin (10 µg/mL)</td>
<td>3.45</td>
<td>3.95</td>
</tr>
</tbody>
</table>

Figure 3: Structure of the metal complex.

Thus, it can be said that complexation or chelation increases the antimicrobial activity.

4. Conclusion

The study of the reaction between the transition metal and the derived Schiff base indicates its high stability. This encourages the synthesis and careful investigation of the nature of bonding between the Schiff base and the transition metal cation of important biological role, using physicochemical method of analyses. It is clear from above discussion that the fragmentation pattern and spectral studies of the complex confirm and illustrate the proposed geometry obtained by elemental analysis, IR, H NMR, and mass spectra.

Hence, the proposed tentative structure of the metal complex is given as in Figure 3.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References


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