In vitro antibacterial studies of some transition metal complexes of Schiff base derived from 2-aminophenol and furan-2-carbaldehyde

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ABSTRACT

A novel Schiff-base ligand and its Ni(II) & Cu(II) complexes were synthesized and well characterized by elemental analyses and spectral studies. Physical and analytical data suggest that the Schiff base acts as tridentate ligand towards metal ions via azomethine-N, deprotonated-O of 2-aminophenol and O-atom of furan moiety. The synthesized ligand, along with its metal complexes were screened for their in-vitro antibacterial activity against four bacterial pathogens (E. coli, Bacillus subtilis, Staphylococcus aureus and P. vulgareous). The results of these studies revealed that the free ligand and its metal complexes showed significant antibacterial potency.

Keywords: Schiff base, Antibacterial activities, Carbaldehyde, Cis-platin

INTRODUCTION

The bio-functional activity of metal based complexes in medicine and chemotherapy has spurring the growth of interest in the scientific world in the past few decades, after the successful clinical use of Cis-platin as an anticancer drug [1-4]. Most of the metals being unnatural to human body, because of having no effective mechanism for its rejection and toxic behavior, there has been rapid expansion in research and development of novel metal based drugs with improved pharmacological properties [5,6]. Transition metal chemistry of Schiff base has gained momentum in the late 19th century and since then metal based drugs of Schiff bases drew significant interest of researchers in the medical science for their immense biological activities [7,8]. Drug resistance against various pathogens is the major cause of morbidity and mortality throughout the world, so novel antimicrobial drugs are still in demand for biological monitoring of diseases [9-11]. Besides their biological importance, they are also considered to perform various catalytic functions due to chelating behavior [12] and capacity of stabilizing different metals in various oxidation states with unusual structural features. Various studies reveal Azomethine linkage (C=N) in Schiff base, a responsible part for the biological activities. Many reports have shown that some drugs have greater activity when administered as metal complex as that as free organic compounds [13,14].

In the present investigation, the synthesis and characterization of novel Schiff base ligand and its transition metal complexes have been reported. The novel ligand has been prepared by using 2-aminophenol and furan-2-carbaldehyde, NiCl₂.6H₂O and CuCl₂.2H₂O salts have been selected for the synthesis of metal complexes, due to having high degree of affinity for coordination with synthesized Schiff base at potential donor N and O atoms [15-19]. In vitro antibacterial evaluation of the novel ligand and metal complexes was carried against E. coli, B. Subtilis, S. aureus and P. vulgareous to know the potentiality of such compounds.
MATERIALS AND METHODS

All the chemicals and solvents used for the synthesis were of analytical grade and purchased from local dealers of BDH and Merck chemical co. They were used without further purification. The infrared spectra of the ligand and metal complexes were run as KBr discs in the range 4000-400 cm$^{-1}$ on a Shimadzu Infrared Spectrophotometer. Elemental analysis (C H N) was performed by using a Flash EA 1112 Series elemental analyzer. $^1$H NMR spectra were determined in DMSO (internal standard TMS) on Bruker spectrometer. NiCl$_2$.6H$_2$O and CuCl$_2$.2H$_2$O salts were used for the synthesis of metal complexes of Schiff base. Mass spectra were carried out, TOF-MS on water KC-455 model in DMSO. The molecular structures of the complexes were optimized by CsChem 3D Ultra-11 programme.

Synthesis of novel Schiff base ligand

The novel Schiff base ligand was prepared by condensation of 2-aminophenol (1.0905 gm; 10 mmol) with furan-2-carbaldehyde (0.9602 gm; 10 mmol) in 25 ml distilled ethanol and the mixture was refluxed for 5 hrs at 30°C. The resulting solution was allowed to evaporate by slow diffusion process in air for a week [20]. The crystals of novel ligand were collected, washed several times with distilled ethanol and recrystallized from hot ethanol and dried in vacuum desiccators.

\[
\text{2-aminophenol} + \text{furan-2-carbaldehyde} \rightarrow (E)-2-(\text{furan-2-ylmethyleneamino})\text{phenol}
\]

Figure 1. Scheme for the synthesis of Novel Ligand [LH]. (Red mark groups indicating donor sites)

Synthesis of Metal complexes

The synthesized novel ligand in hot ethanol was added to hot ethanolic solution of 5 mmol of metal chlorides drop wise and refluxed for 5 hours and dried at room temperature. A good crystalline form of the complexes was obtained. All the complexes were characterized by spectral techniques accordingly.

\[
\text{NiCl}_2.6\text{H}_2\text{O} + \text{LH} \rightarrow \text{ML}_2.6\text{H}_2\text{O}
\]

\[
\text{CuCl}_2.2\text{H}_2\text{O} + \text{LH} \rightarrow \text{ML}_2.2\text{H}_2\text{O}
\]

Figure 2. Structure of metal complexes [ML$_2$]

In Vitro Antibacterial Assay

Antibacterial sensitivity of different compounds conjugated with different metals was studied by Kirby Bauer paper disc diffusion method, using Muller Hinton's agar nutrient media for bacterial growth. Fresh culture of standard bacteria viz. E. coli, B. subtilis, S. aureus and P. vulgarus were collected from Nepal public health laboratory Teku, Kathmandu (Nepal) and the inoculum was spread over nutrient agar media, prepared as usual in Petri dish using stick swab and inoculated at 37°C for 24 hrs. Paper discs of 6 mm diameter (Whatman no. 1) impregnated with different compounds at the concentration of 0.001 g/ml were placed on the previously seeded bacterial culture. Two paper discs, one impregnated with Gentamicin (0.001 g/ml) and other with DMSO were used as positive and negative control respectively [21,22]. Afterwards, the Petri plates inoculated with bacteria were incubated at 37°C for 24 hrs and the diameter of inhibition zone around each disc was measured in mm.
RESULTS AND DISCUSSION

The synthesized compounds were crystalline, colored, nonhygroscopic, and soluble in water. Composition and identity of the complexes were carried out by elemental analysis. The structure of the synthesized ligand and its metal complexes were established with the help of spectral techniques. The spectroscopic data for the newly synthesized metal complexes are in good agreement with proposed formulation.

Elemental composition analysis

The elemental analysis (CHN) data for the novel ligand, and its metal complexes are summarized in the table below.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Empirical Formula</th>
<th>Mol. Wt.</th>
<th>Colour</th>
<th>% Yield</th>
<th>C Found (Calc.)</th>
<th>H Found (Calc.)</th>
<th>N Found (Calc.)</th>
<th>O Found (Calc.)</th>
<th>M Found (Calc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novel Ligand</td>
<td>C₁₁H₉NO₂</td>
<td>187.19</td>
<td>Dirty green</td>
<td>81.5%</td>
<td>70.48</td>
<td>4.91</td>
<td>7.47</td>
<td>17.14</td>
<td></td>
</tr>
<tr>
<td>Ni(II) Complex</td>
<td>C₂₂H₁₆N₂NiO₄</td>
<td>431.07</td>
<td>Creamson</td>
<td>75%</td>
<td>61.29</td>
<td>3.81</td>
<td>6.53</td>
<td>14.83</td>
<td>13.60</td>
</tr>
<tr>
<td>Cu(II) Complex</td>
<td>C₂₂H₁₆CuN₂O₄</td>
<td>435.92</td>
<td>Yellowish brown</td>
<td>78%</td>
<td>60.60</td>
<td>3.76</td>
<td>6.47</td>
<td>14.00</td>
<td>14.55</td>
</tr>
</tbody>
</table>

Infrared spectral analysis

The IR spectra of novel ligand has been compared with the spectra of metal complexes that revealed the binding mode of ligands to metal ions which is precisely confirmed by change in the positions of absorption peaks. The loss of absorption peaks at 3317-3385 cm⁻¹ for ν(N-H) stretch of 2-aminophenol and at 1706 cm⁻¹ for ν(C=O) of furan-2-carbaldehyde and appearance of peak at 1643 cm⁻¹ for ν(C≡N) in novel ligand are good indicative for the formation of Schiff base. One more medium intensity band at the region of 3600-3640 cm⁻¹ is assignble to phenolic OH group whose negative shift in metal complexes indicated metal coordination with phenolic oxygen [23]. Medium intensity bands in the range of 1643 cm⁻¹ due to ν(C≡N) in the novel ligand undergone negative shift about 25-40 cm⁻¹ which was in the range of 1613 - 1595 cm⁻¹ in metal complexes, indicating the imine nitrogen atom involvement in coordination to metal ions [24]. This fact is further supported by the appearance of medium intensity band in the region below 500 cm⁻¹ assignable to νM=N vibration. The appearance of one more medium intensity band in the region of 420 - 405 cm⁻¹ assignable due to νM=O vibration, also indicated the metal oxygen binding mode in the complexes [25]. Thus from the IR- spectral assignments it is clear that the compounds may be bonded to metal ions through the imine nitrogen, deprotonated O and O-atom of furan ring.

¹H NMR spectral analysis

¹H NMR spectral comparison of novel ligand and metal complexes was made to confirm the binding nature of ligand with metal ions viz. Ni (II) & Cu(II). The integral intensities of each signal in the ¹H NMR spectra of novel ligand and its metal complexes were found to agree with the number of different types of protons present.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>δ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁₁H₉NO₂ (Ligand)</td>
<td>5.0 (s) [OH-Ar]; 6.5-7.7 [CH-Ar]; 8.45(s) [CH imine]; 6.1-7.5(s) [CH furan ring]</td>
</tr>
<tr>
<td>Ni(II) complex</td>
<td>8.9(s) [¹H-imine]; 6.5-7.7 [8¹H Ar-CH]; 6.1-7.5 [CH furan ring]</td>
</tr>
<tr>
<td>Cu(II) complex</td>
<td>9.10( s) [¹H-imine]; 6.5-7.7 [8¹H Ar-CH]; 6.1-7.5 [CH furan ring]</td>
</tr>
</tbody>
</table>

TOF-Mass spectral analysis

The proposed molecular formula of metal complexes was confirmed by the TOF mass spectral analysis by comparing their formula weight with m/z values. The mass spectra contain molecular ion peak (M⁺) at m/z 430.05 for Ni(II) complex and 435.04 for Cu (II) complex. These data are in good agreement with the respective molecular formula.

Molecular Modeling

3D molecular modeling of the proposed structure of the complexes was performed using CsChem3D Ultra -11 program package. The correct stereochemistry was assured through the manipulation and modification of the molecular coordinates to obtain reasonable low energy molecular geometries. The optimized structures of the complexes were performed by MM2 programme contained CS chem. Office programme. They showed octahedral geometry as suggested by molecular modeling [15,21,26].
Antibacterial Studies:
The results of the antimicrobial activities are summarized in Table 3. In vitro antibacterial screening of the ligand and metal complexes was assayed against various bacterial strains and the potentiality of the complexes was evaluated by measuring the diameter of zone of inhibition in mm. Antimicrobial results showed that all the synthesized compounds possess biological activity. Comparative study of ligand and its metal complexes showed moderate to better antibacterial activity [27]. Such increased activity of metal complexes may be considered due to chelation of metal ions with Schiff base with enhanced lipophilicity due to delocalization of pi-electrons over the whole chelate ring [28]. This increased lipophilicity enhances the penetration of complexes into the lipid membranes and blocks the metal binding sites in enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism. The data revealed that the activity of the ligand enhanced on complexation but less than the standard used. Overall comparison of observed data gives information that metal complexes are more active than free ligand against all bacteria.

### Table 3. Diameter of zone of inhibition in mm

<table>
<thead>
<tr>
<th>Compounds</th>
<th>E. coli</th>
<th>Bacillus subtilis</th>
<th>S. aureus</th>
<th>P. vulgaris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Gentamicin)</td>
<td>20</td>
<td>24</td>
<td>19</td>
<td>24</td>
</tr>
<tr>
<td>Free Ligand</td>
<td>14</td>
<td>18</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Ni-complex</td>
<td>17</td>
<td>22</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Cu-complex</td>
<td>15</td>
<td>13</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Concentration of sample = 0.001 g/ml of DMSO
Concentration of control drug = 0.001 g/ml.
(-) = No activity
Zone of inhibition (mm): >19 highly active, >15 & <19 moderately active, <15 less active.

Figure 3. ChemOffice simulated space filling molecular model
[ Ni-complex (left) & Cu-complex (right)]

Figure 4. Comparative antibacterial activities of complexes with standard drug
CONCLUSION

Bio-active azomethine linkage (C=N) of tridentate novel ligand has been studied extensively and is an entity which has interesting biological features. The ligand coordinates to metal ions using the azomethine N and deprotonated O atom of 2-aminophenol moiety. In spite of this, there is also involvement of O atom of furan for coordination. The free ligand coordinates to metal ions in the ratio of 2:1. The synthesized complexes were characterized by elemental analysis, MS, IR, 1H NMR and the structure was optimized by Chem Office Ultra-11 programme package. All the synthesized compounds (free ligand and metal complexes) were evaluated in vitro against four bacterial pathogens and showed better antibacterial activity.

Acknowledgments

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REFERENCES