Synthesis, Characterization and in vitro Biological Evaluation of a New Schiff Base Derived from Drug and its Complexes with Transition Metal Ions

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New series of copper (II), cobalt (II), zinc (II), nickel (II), manganese (II), iron (II) complexes of a novel Schiff base were prepared by the condensation of sulphadizine and pyridoxal hydrochloride. The ligand and metal complexes were characterized by utilizing different instrumental procedures like microanalysis, thermogravimetric examination and spectroscopy. The integrated ligand and transition metal complexes were screened against various bacteria and fungus. The studies demonstrated the enhanced activity of metal complexes against reported microbes when compared with free ligand.

Keywords: Schiff base, metal complex, sulphadizine, Pyridoxal hydrochloride, Antibacterial

In biochemistry Interaction between transition metals and proteins is universal [1]. These interactions can be used to study the interaction between metal complexes and amino acid Schiff bases [2]. Schiff bases are broadly utilized as bi- or tri- dentate ligands in coordination chemistry as they have capability of forming exceptionally stable transition metal complexes [3-4]. Schiff base and their metal complexes are significantly important in biological systems such as anti-inflammatory drugs[5-8], antimicrobial[9-14], antispasmodic[15], Tuberculosis [16], anti-cancer [17-18], antioxidants [19], anthelmintic [20].

Schiff bases are also act as catalysts, intermediates in organic synthesis, pigments, colorants, polymeric stabilizers [21] and corrosion inhibitors [22]. A literature survey affirmed that metal complex shows higher biological activity as the free organic compounds [23].

Increase of biological activity reported by the implementation of the transition metals of Schiff bases [24]. Schiff bases have assumed a huge part in the improvement of coordination science and have been embroiled as a vital point in the advancement of inorganic biochemistry and optical materials [25].

The metal complexes of Schiff base got from various drugs are great antibacterial operators. In the present work an attempt has been made to expand the degree of derivatization by giving greater versatility through Schiff base ligand. Here we report preparation, characterization and biological studies of New Schiff base transition metal complexes obtained from sulphadizine and pyridoxal hydrochloride.

Experimental part

Analytical grade chemicals and solvent used in these studies. Sulphadizine and pyridoxal hydrochloride were taken from BDH. Other pure chemicals and solvent were purchased from Alfa Aesar and used without purification.

Microanalysis was performed utilizing normal strategies. Metals were assessed by atomic absorption spectroscopy. Basic investigations were resolved on a CE-440 Elemental Analyzer, FT-IR spectra were recorded with a Perkin Elmer Spectrum-100 spectrometer utilizing KBr plates. NMR spectra were measured on a J eol ECS 400 spectrometer.

Mass spectra were measured with the assistance of Thermo Scientific Exactive TM Plus Orbitrap spectrometer. Thermogravimetric examinations for the Schiff base and its complexes were completed on a SDT-Q600 instrument. Magnetic moments were estimated using Evans balance with anhydrous calcium chloride. Electronic absorption spectra of all the complexes were recorded on a Shimadzu-1800 spectrophotometer. DMSO (10^{-3} mol L^{-1}) used as solvent for conductance measurements using conductivity meter (4510-J enway).

Preparation of Schiff base

Sulphadizine (2.0 m mole) was dissolved in 2 mL (1M) sodium hydroxide. To this, ethanolic solution of pyridoxal hydrochloride (2.0 m mole) was added and refluxed for one hour. A clear orange colored solution was collected for isolation of Schiff base ligand by crystallization. The crystalline product was dried under vacuum and kept in a desiccator for further use.

Schiff base Ligand

[N-4-(3-hydroxy-5-carboxymethyl)-2-methylpyridine-4-methylamino)-pyrimidin-2-yl-benzene sulfonamide].

Yield 85% (Orange). m.p. 195-197°C. IR (KBr, cm^{-1}) 3243 (OH), 1610 (HC=N azomethine), 1580 (C=N-pyrimidine), 1187(O=S=O), 1095(C-N). Anal. Calcd. For C_{15}H_{12}N_{4}O_{2}S_{2} (367); Calcd: C, 49.55; H 3.89; N, 16.05; Found: C, 49.43; H 3.89; N, 16.13 %.

1H NMR (DMSO-D_{6}, δ ppm) 8.05 (-CH=N), 6.27- 8.01 (phenyl); 1C NMR (DMSO-D_{6}, δ ppm) 165.4 (-CH=N), 157.7-158.5 (pyrimidine), 125.4-147.2 (phenyl). MS (EI); m/z (%) = 400.1069 [M+].

Preparation of Schiff base metal complexes

The ligand (L) and chloride salts of copper (II), cobalt (II), zinc (II), nickel (II), manganese (II), iron (II) were dissolved in ethanol separately. Both solutions of Schiff base and metal salt were mixed in the ratio 2:1. The reaction mixture was then refluxed for 2 h. After preparation, the colored precipitates of Schiff base-metal complexes were filtered off, washed with water, ethyl alcohol and dried under reduced pressure at room temperature.
Iron (II) complex
Yield 71% (Pink), m.p. (decomp.) 219-222 °C. IR (KBr, cm⁻¹) 3225 (OH), 1612 (HC=N azomethane), 1571 (C=N-pyrimidine), 1177 (O=S=O), 441 (M-N), 362 (M-O).
UV (DMSO) λ_max (cm⁻¹) 26800; 23950, 32679; B.M (5.43 µS cm⁻¹). Anal. Calcd. For C_{22}H_{20}N_8S_2Fe, M=539.34; Calcd: C, 44.68; H 3.51; N, 14.48; Fe, 6.09 Found: C, 44.44; H 3.37; N, 14.51; Mn, 6.0%. The metal ligand bond was verified by comparing the FTIR spectra of the Schiff base ligand with metal complexes. The FTIR spectra predicted all the absorption bands of the Schiff base ligands and some new bands at specific frequencies confirmed the modes of absorption and the completion of the ligands with the metal ions through nitrogen and oxygen. The azomethine group of ligand 1610 cm⁻¹ was shifted to higher value (1622 cm⁻¹) in all the complexes thus suggested the coordination of metal to the Schiff base ligand. Absorption spectra of increased conjugation and coordination to metal ions. The electronic spectrum of Mn (II) and Fe(II) complexes shows 6A₁g → 3T₁g(P), 3A₂g(F) → 4A₁g 4Eg transitions respectively. The magnetic moment values of both complexes supports octahedral geometry i.e. 5.92 B.M and 5.43 B.M.

Results and discussions
The synthesis of ligand was accomplished by refluxing the drug and aldehyde in a molar ratio 1:1 in ethanol. The metal complexes of ligand were prepared using metal chloride and ligand in a 2:1 molar ratio. The characterization is done with Elemental analyzer FT-IR, NMR, Mass spectroscopy, TG and micro-analytical data. The metal complexes of ligand were prepared using metal chloride and ligand in a 2:1 molar ratio. All the metal complexes are amorphous solids and have decomposition point. They are insoluble in water, organic solvents, partially soluble in acetone and completely soluble in DMF and DMSO. The structures of synthesized Schiff base ligand along with metal complexes were investigated by different techniques.

NMR Spectra
\(^{1}H\) NMR and \(^{13}C\) NMR Spectra were taken in \(d_2\)-DMSO. The peaks of all the proton and carbon atoms were fixed in their expected region. The NMR spectra of Schiff base ligand was confirmed the absence of aldehyde peak at \(9-10\) and presence of azomethine at \(8.05\). \(^{1}C\) NMR spectra also verify azomethine peak at \(165.4\). The dimagnetic copper complexes showed a slight change in spectra because of increased conjugation and coordination to metal ions.

IR Spectra
The metal ligand bond was verified by comparing the FTIR spectra of the Schiff base ligand with metal complexes. The FTIR spectra predicted all the absorption bands of the Schiff base ligands and some new bands at specific frequencies confirmed the modes of absorption and the completion of the ligands with the metal ions through nitrogen and oxygen. The azomethine group of ligand 1610 cm⁻¹ was shifted to higher value (1622 cm⁻¹) in all the complexes thus suggested the coordination of metal to ligand bond through azomethine (HC=N). Absorption bands of the sulfonamides moiety in the synthesized ligands and in metal complexes have same frequency. Further definitive proof of the coordination of the Schiff-bases with the metal ions was confirmed by the appearance new bands at 436-482 and 359-381 cm⁻¹ designate to the metal nitrogen (M-N) and metal-oxygen (M-O) extending vibrations, individually [27]. These bands were not present in the spectra of the free ligands, therefore affining the presence of O and N in the coordination.

Electronic spectra and magnetic susceptibility
The electronic absorption spectra of transition metal complexes were recorded in \(10^{-3}\) M solutions of each complex in DMSO in the range 2000-10000 cm⁻¹ at room temperature.

The electronic spectrum of Mn (II) and Fe(II) complexes shows \(\Lambda_A \to \Lambda_A (G), \Lambda_A \to \Lambda_A (E), \Lambda_A (F) \to \Lambda_T (P)\) and \(\Lambda_A \to \Lambda_A (G)\) transitions respectively. The magnetic moment values of both complexes supports octahedral geometry i.e. 5.92 B.M and 5.43 B.M.

The electronic absorption spectrum of the Cu(II) complex showed two bands at 15336 cm⁻¹ and 27027 cm⁻¹ corresponding to the transition \(\Lambda_A \to \Lambda_A\). No spectral
bands were found below 10000 cm\(^{-1}\) which supports octahedral geometry. Also, the magnetic moment value (1.93 B.M) for the Cu (II) complexes suggests the octahedral geometry with \(d^2\) \(\pi^2\) ground state.

The spectrum of the Ni (II) complex showed d-d bands in the region 16760 and 24435 cm\(^{-1}\) showed the spin-allowed transitions \(^3A_2g(F) \rightarrow ^3T_1g(F)\) and \(^3A_2g(F) \rightarrow ^3T_1g(P)\), respectively, with the octahedral configuration. The magnetic moment (3.14 B.M) value recommended two unpaired electrons Ni (II) ion also consistent with an octahedral geometry for the Ni(II) complex.

The electronic spectra of Co (II) complexes in DMSO exhibited bands around 17665 cm\(^{-1}\) and a high-energy band at 21755 cm\(^{-1}\) designed \(^4T_1g(F) \rightarrow ^4T_2g(F)\), \(^4T_1g(F) \rightarrow ^4T_1g(P)\) transitions respectively, for a high-spin octahedral geometry. The magnetic susceptibility measurements (4.21 B.M) for the solid Co(II) complexes are also indicative of three unpaired electrons per Co(II) ion consistent with their octahedral environment\[28-29\]. The spectrum of Zn(II) complex exhibited only one band at 28640 cm\(^{-1}\) which was assigned to a ligand→metal charge transfer. The zinc (II) complexes of ligand were observed to be diamagnetic obviously and in this manner, their magnetic properties could not be calculated.

**Thermal studies**

Thermogravimetric analyses (TGA) for the transition metal complexes were done from room temperature to 1000°C. Calculated and found mass losses are shown in the table below.

**Biological activity**

Antimicrobial and antifungal activity of all the synthesized transition metal complexes and Schiff base ligand were tested against *Escherichia coli*, Enterobacter aerogenes, Staphylococcus aureus, Bacillus pumilus, Klebsiella oxytoca, Clostridium butyrium, Aspergillus niger and Mucor. The results showed enhanced activity when coordinated with transition metals (table 2). This enhancement in activity of the metal complexes as compared to free ligand can be explained by chelation therapy. The metal complexes were found to be active against aspergillus niger and Mucor whereas parent drug and ligand showed no antifungal activity.

### Table 1

**THERMAL ANALYSIS DATA OF THE METAL COMPLEXES**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Metal Chelates</th>
<th>Temperature Range (°C)</th>
<th>Mass Loss % Found (Calculated)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>[Co(L-H)(_2)(H(_2)O)(_2)]</td>
<td>145-240 240-410</td>
<td>4.05(3.72) 30.12(29.80)</td>
<td>Loss of 2H(_2)O Loss of 2-imino-pyrimidine + Loss of 2SO(_2)</td>
</tr>
<tr>
<td>3.</td>
<td>[Zn(L-H)(_2)(H(_2)O)(_2)]</td>
<td>152-241 241-402</td>
<td>3.78(3.69) 29.44(29.11)</td>
<td>Loss of 2H(_2)O Loss of 2-imino-pyrimidine + Loss of 2SO(_2)</td>
</tr>
<tr>
<td>5.</td>
<td>[Mn(L-H)(_2)(H(_2)O)(_2)]</td>
<td>138-238 238-402</td>
<td>3.80(3.73) 30.22(29.93)</td>
<td>Loss of 2H(_2)O Loss of 2-imino-pyrimidine + Loss of 2SO(_2)</td>
</tr>
</tbody>
</table>

**Table 2**

**ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SCHIFF BASE LIGAND AND THEIR METAL COMPLEXES (ZONE OF INHIBITION; 400 µg mL\(^{-1}\))**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>L. coli (mm)</th>
<th>E. Aerogenes (mm)</th>
<th>S. aureus (mm)</th>
<th>B. pumilus (mm)</th>
<th>K. oxytoca (mm)</th>
<th>C. Butyrium (mm)</th>
<th>A. Niger (mm)</th>
<th>Mucor (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Cu(L-H)(_2)(H(_2)O)(_2)]</td>
<td>22</td>
<td>19</td>
<td>29</td>
<td>13</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>[Co(L-H)(_2)(H(_2)O)(_2)]</td>
<td>15</td>
<td>14</td>
<td>17</td>
<td>13</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>[Zn(L-H)(_2)(H(_2)O)(_2)]</td>
<td>23</td>
<td>20</td>
<td>26</td>
<td>13</td>
<td>17</td>
<td>17</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>[Ni(L-H)(_2)(H(_2)O)(_2)]</td>
<td>17</td>
<td>13</td>
<td>17</td>
<td>14</td>
<td>13</td>
<td>10</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>[Mn(L-H)(_2)(H(_2)O)(_2)]</td>
<td>20</td>
<td>12</td>
<td>19</td>
<td>13</td>
<td>11</td>
<td>11</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>[Fe(L-H)(_2)(H(_2)O)(_2)]</td>
<td>14</td>
<td>12</td>
<td>15</td>
<td>12</td>
<td>10</td>
<td>12</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Ligand</td>
<td>12</td>
<td>10</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Drug</td>
<td>11</td>
<td>7</td>
<td>12</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
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</table>
Conclusions
The results obtained after analysis, proved that after derivatization, the antibacterial activities is improved against selected microbes. The metal complexes show activity against Aspergillus niger and Mucor whereas the parent drug and ligand showed no antifungal activity. These observations, in accordance with different studies, recommend that metal based drugs have potential as therapeutics.

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