SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES
OF ONS DONOR N-4-DISUBSTITUTED THIOSEMICARBAZONE
LIGAND AND ITS TRANSITION METAL COMPLEXES

Vrushali S. Gavhane¹, Vijayanand D. Ingale² Vishal G. Shinde³, Anjali S. Rajbhoj⁴,
Suresh T. Gaikwad⁵

Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad
(M.S.) India-431 004.

ABSTRACT

Four novel transition metal complexes Cr(III), Mn(II), Co(II) and Ni(II) of 1-(2-hydroxybenzylidene)-4-ethyl-4-phenylthiosemicarbazide (HEPT) have been synthesized and characterized by elemental, spectroscopic (FTIR,¹H-NMR), molar conductivity and X-ray diffraction studies. The FTIR spectral data suggested the attachment of central metal ion to ligand moiety through the azomethine nitrogen, thioketalon sulphur and phenolic oxygen. The molar conductance measurements of the complexes in DMSO determines electrolytic nature of Cr(III), Mn(II) and Co(II) complex, while Ni(II) complex is nonelectrolyte in nature. On the basis of spectral studies an octahedral geometry may be assigned for all four complexes. The HEPT ligand and its transition metal complexes have been tested for their antibacterial activity against Staphylococcus aureus, Bacillus subtilis species and antifungal activity against Aspergillus niger and Fusarium oxysporum species at 250 ppm and 500 ppm concentration. All complexes showed considerable activity almost equal to the activity of ciprofloxacin.

KEYWORDS: N-4-Disubstituted-3-thiosemicarbazone, XRD, Biological activity.

INTRODUCTION

The condensation product of primary amines with carbonyl compounds are often referred as Schiff bases.¹⁴ Schiff bases are compounds containing azomethine linkage(C=N) also referred to as imines. Their synthesis and properties are widely reviewed.⁵⁻¹⁰ The synthesis
and study of well arranged metal complex containing Schiff base ligand with ONS donor atom is an very interesting area of coordination chemistry.\textsuperscript{[11-12]} Thiosemicarbazones are considered as versatile ligands due to their capability to form complexes with variety of transition metal salts results in quite stable complexes.\textsuperscript{[13-14]} N-4-disubstituted thiosemicarbazones obtained by condensation of N-4-disubstituted thiosemicarbazide with salicylaldehyde form NS / NSO donor ligands.

West and his co-workers have been reported numerous new thiosemicarbazone ligands and their transition metal complexes. The newly synthesized ligands were 2-pyridine formide-N-(4)-methylthiosemicarbazone\textsuperscript{[15]}, 2- pyridine formamide-3-piperidyl thiosemicarbazone\textsuperscript{[16]}, pyrazineformide –N-(4)-methylthiosemicarbazone\textsuperscript{[17]} The ligands and complexes were characterized by molar conductivities, magnetic susceptibilities and X-ray techniques. The synthesis of three bis(thiosemicarbazone) compounds formed by the reaction of benzyl with either thiosemicarbazide, 4-Methyl thiosemicarbazide were reported by Alsop\textsuperscript{[18]}, Gerbeleu.\textsuperscript{[19]}

have shown that in thiosemicarbazone metal complexes bonding may also occurs through the hydrazinic nitrogen and the amide nitrogen if sulphur centre is substituted. Thiosemicarbazones which comprise a well known group of NS donors have been extensively used for complex formation in the recent past and are widely employed in medical sciences.\textsuperscript{[20]} This class of thiosemicarbazone complexes is important because of their antifungal\textsuperscript{[21]}, antimalarial, antiviral\textsuperscript{[22]}, antitumor\textsuperscript{[23]} activities. In extension of this sense the present paper describes the synthesis, characterization and biological analysis of four novel complexes of 1-(2-hydroxybenzylidene)-4-ethyl-4-phenylthiosemicarbazide (HEPT).

**MATERIALS AND METHODS**

1-(2-hydroxybenzylidene)-4-ethyl-4-phenylthiosemicarbazide (HEPT) was prepared by adopting and modifying a reported procedure of Scovil.\textsuperscript{[24]} Salicylaldehyde were of reagent grade purchased from Sigma-Aldrich. All other chemicals were used of AR grade and used as supplied. The solvents were distilled before use.

**1.1-Synthesis of Schiff base ligand- (1-(2-hydroxybenzylidene)-4-ethyl-4- phenylthiosemicarbazide (HEPT).**

Schiff base ligand HEPT was prepared by mixing together ethanolic solution of N-4- Ethyl,
Phenyl-3-Thiosemicarbazide (0.01 M, 1.95 gm) and Salicylaldehyde (0.01M, 1.22 gm) in equimolar ratio. The resulting mixture refluxed for 30 minutes. This reaction mixture was chilled (overnight). Yellow colored shiny crystals were separate out which was filtered, washed repeatedly with alcohol and finally with ether. It was recrystallized from DCM-Alcohol mixture and subsequently dried over CaCl2 in vacuum desicator. The purity of ligand was checked by TLC, microanalysis, melting point. The data are given in (Table 1)
The ligand is insoluble in alcohol slightly soluble in acetone, dichloromethane but completely soluble in DMF and DMSO. Yield of the ligand: 80%, M.P-170oC. Proposed molecular formula: C16H17N3OS (M.Wt. 229.39). The reaction of ligand formation can be represented as below (Scheme-1).

Scheme-1: Synthesis of ligand HEPT.

1.2-Synthesis of Metal Complexes
Hot methanolic solution of metal salt (1mmol) eg. Cobaltous chloride hexahydrate(0.23gm), Chromium chloride hexahydrate (0.26gm), Nickel chloride hexahydrate (0.23gm) were mixed with hot methanolic solution of ligand (2mmol). The reaction mixture was then refluxed for 1-2 hour on heating mantle. The solid precipitate formed was allowed to keep overnight then it was filtered, washed and dried in vacuum over anhydrous calcium chloride in desiccators (Yield-75-78%). The relevant data included in the (Table 1)

1.3-Physical measurement
Microanalytical data of the complexes were obtained from sophisticated test and Instrumentation Centre Cochin. Molar conductance was measured on the Elico (CM82T) conducting bridge. Molar conductance of Cr(III), Mn(II), and Co(II) complexes shows that they are electrolytic in nature while Ni(II) complex is nonelectrolyte. IR spectra (KBr) were recorded on FTIR spectrum BX-II spectrophotometer. The melting points of all the complexes were determined by open capillary method. ¹H NMR were recorded in DMSO solution using Bruker 400 MHz spectrometer.
Table-1. Physical and analytical data of ligand (HEPT) and their metal complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mol. Wt.</th>
<th>Molar Cond. ($\Omega$-1 cm$^{-2}$ mol$^{-1}$)</th>
<th>Color</th>
<th>Yield</th>
<th>Elemental Analysis (%)</th>
<th>Found (Calculated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td>HEPT</td>
<td>299.39</td>
<td>—</td>
<td>Brown</td>
<td>80%</td>
<td>64.12</td>
<td>06.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(64.1)</td>
<td>(05.7)</td>
</tr>
<tr>
<td>[Cr(C$_2$H$_3$N$_6$O$_2$S$_2$)]</td>
<td>650.77</td>
<td>135</td>
<td>Brown</td>
<td>76%</td>
<td>58.95</td>
<td>05.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(59.0)</td>
<td>(05.2)</td>
</tr>
<tr>
<td>[Mn(C$_2$H$_3$N$_6$O$_2$S$_2$)]</td>
<td>653.71</td>
<td>146</td>
<td>Brown</td>
<td>78%</td>
<td>58.72</td>
<td>05.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(58.7)</td>
<td>(05.2)</td>
</tr>
<tr>
<td>[Co(C$_2$H$_3$N$_6$O$_2$S$_2$)]</td>
<td>657.71</td>
<td>116</td>
<td>Dark Brown</td>
<td>75%</td>
<td>58.10</td>
<td>05.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(58.3)</td>
<td>(05.1)</td>
</tr>
<tr>
<td>[Ni(C$_2$H$_3$N$_6$O$_2$S$_2$)]</td>
<td>657.47</td>
<td>027</td>
<td>Yellow</td>
<td>75%</td>
<td>58.01</td>
<td>05.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(58.4)</td>
<td>(05.1)</td>
</tr>
</tbody>
</table>

RESULT AND DISCUSSION

$^1$HNMR spectra of ligand

The $^1$HNMR spectrum of the ligand (HEPT) was recorded in DMSO. The $^1$HNMR spectrum of ligand (HEPT) shows a signal at 11.52, 10.74$\delta$ and 8.34 $\delta$ corresponding to OH, NH and CH proton respectively. Aromatic protons show a multiplet at 6.81-7.52 $\delta$. The assignments are in agreement with the values already reported.\cite{25}

FTIR Studies

The tentative assignments of the IR spectral bands to establish the structural identity of the ligands and its metal complexes given in (Table no.2). To study the binding mode of ligand to metal in complexes, IR spectrum of ligand was compared with IR spectrum of metal complexes.

In the solid state the ligand (HEPT) remains in the thione form as $\nu$(S-H) band expected to be at 2570 cm$^{-1}$ is absent. A sharp $\nu$ (N-H) band is observed at 3027, 2980 cm$^{-1}$ for O-N-S donor ligand. A sharp $\nu$(C=S) band and a low intensity $\delta$(C=S) band are seen in ligand at 1304, 746 cm$^{-1}$ while in case of metal complexes it is in the range of 1270-1370 cm$^{-1}$ and 700-770cm$^{-1}$. Ligand (HEPT) shows $\nu$(OH) band at 3220 cm$^{-1}$ due to intramolecular hydrogen bonding and free (OH) band in complexes at 3400-3450 cm$^{-1}$ and at 1485cm$^{-1}$, indicating presence of coordinated or lattice water in these complexes.\cite{26}

Other characteristics absorption bands at 1518, 1611, 1186 cm$^{-1}$ due to C=N, N-N and C-O vibration respectively, while in metal complexes of ligand (HEPT) shows bands in the range of 1540-1600cm$^{-1}$ it predicts that azomethine nitrogen takes part in coordination.\cite{27} Shifting of $\nu$(C-O) band at 1120 to 1200 cm$^{-1}$ confirms the presence of
v(M-O) band, also the bands in the range of 520-570 and 440-510 shows v(M-N) and v(M-S) bond.

Table- 2: FTIR spectral data of the ligand (HEPT) and its metal complexes (in cm\(^{-1}\)).

<table>
<thead>
<tr>
<th>Ligand / Metal Complexes</th>
<th>(N) (C-O)</th>
<th>(N) (O-H)</th>
<th>(\delta) (O-H)</th>
<th>(\nu) (N-H)</th>
<th>(\nu) (C=N)</th>
<th>(N) (N-N)</th>
<th>(N\delta) (C=S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPT</td>
<td>1120</td>
<td>3277</td>
<td>1485</td>
<td>2980</td>
<td>1518</td>
<td>1611</td>
<td>1304, 746</td>
</tr>
<tr>
<td>[Cr(C(_3)H(_4)N(_6)O(_2)S(_2)])</td>
<td>1201</td>
<td>—</td>
<td>1438</td>
<td>2972</td>
<td>1535</td>
<td>1598</td>
<td>1288, 700</td>
</tr>
<tr>
<td>[Mn(C(_3)H(_4)N(_6)O(_2)S(_2)])</td>
<td>1157</td>
<td>—</td>
<td>1420</td>
<td>2929</td>
<td>1539</td>
<td>1599</td>
<td>1290, 762</td>
</tr>
<tr>
<td>[Co(C(_3)H(_4)N(_6)O(_2)S(_2)])</td>
<td>1122</td>
<td>—</td>
<td>1439</td>
<td>2971</td>
<td>1546</td>
<td>1596</td>
<td>1273, 761</td>
</tr>
<tr>
<td>[Ni(C(_3)H(_4)N(_6)O(_2)S(_2)])</td>
<td>1134</td>
<td>3445</td>
<td>1443</td>
<td>2978</td>
<td>1495</td>
<td>1579</td>
<td>1276, 754</td>
</tr>
</tbody>
</table>

**X-Ray Diffraction Study**

The X-ray diffractogram of complexes were recorded in the range 0 to 70° 20 value and wavelength of 1.540598 A. The major reflexes were measured and corresponding d' values were calculated using Bragg's equation. The independent indexing of major reflexes was carried out using least square method. All the reflections have been indexed for h, k, l values using reported procedures in the literature.[28] The 20 value of each peak, relative intensity and diffractogram associated data depict the inter planar spacing and lattice constants a, b and c for each unit cell parameter as shown in [Table no.3] To determine the density values of the complexes were used specific gravity method.[29] To calculate the unit cell volume of Cr (III) complexes for triclinic crystal system by the following equation was used.

To calculate unit cell volume of Mn (II) and Fe(III) complex for monoclinic system the equation used are,

\[
V = abc \sin \beta
\]

For Ni(II) complex, volume for orthorhombic system is determined by equation,

\[
V = abc
\]

The systematic arrangement of XRD studies on metal complexes are Lattice constant, unit cell volume, crystal system, d-value, 20 value.[30]
Table-3 Lattice constant, Unit cell volume. Crystal system of metal complexes.

<table>
<thead>
<tr>
<th>Unit cell of compound</th>
<th>Lattice constant</th>
<th>Crystal System</th>
<th>HKL</th>
<th>Unit cell Volume</th>
<th>Inter axial angle</th>
<th>2θ</th>
<th>D value (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a (Å)</td>
<td>b (Å)</td>
<td>c (Å)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr(III)</td>
<td>8.32</td>
<td>11.2</td>
<td>14.3</td>
<td>Triclinic</td>
<td>001</td>
<td>113.62</td>
<td>α≠β≠γ≠90</td>
</tr>
<tr>
<td>Mn(II)</td>
<td>10.2</td>
<td>7.23</td>
<td>14.9</td>
<td>Monoclinic</td>
<td>003</td>
<td>104.30</td>
<td>α=β=90≠γ</td>
</tr>
<tr>
<td>Co(II)</td>
<td>19.3</td>
<td>10.2</td>
<td>10.2</td>
<td>Monoclinic</td>
<td>010</td>
<td>186.81</td>
<td>α=β=90≠γ</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>15.3</td>
<td>14.8</td>
<td>17.9</td>
<td>Orthorhombic</td>
<td>300</td>
<td>411.67</td>
<td>α=β=γ=90</td>
</tr>
</tbody>
</table>

Antibacterial and Antifungal activity

Thiosemicarbazones and their metal complexes exhibit a wide variety of biological activity.\textsuperscript{31,32} The antibacterial and antifungal activity of the newly prepared ligand 1-(2-hydroxybenzylidene)-4-ethyl-4-phenylthiosemicarbazide (HEPT) and their transition metal complexes were carried out successfully. Results of these studies are included in (Table-4). The studies were carried out on \textit{Aspergillus niger, Fusarium oxysporum} species and \textit{Staphylococcus aureus, Bacillus subtilis} species using paper disc method on appropriate medium.\textsuperscript{33} The susceptibilities of certain strains of bacteria and fungus to the thiosemicarbazone ligands and their transition metal complexes were evaluated by measuring the size of the bacteriostatic diameter. The result shows that the ligands (HEPT) as well as their metal complexes are more active against the bacteria and fungi and having high antimicrobial and antifungal activity which is almost equal to the activity of standard ciprofloxacin.

Table-4. Antibacterial and Antifungal activity of ligand(HEPT) and their transition metal complexes. [Diameter of inhibition zone in (mm)].

<table>
<thead>
<tr>
<th>Ligand/Metal Complex</th>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{Staphylococcus aureus}</td>
<td>\textit{Bacillus subtilis}</td>
</tr>
<tr>
<td></td>
<td>250 ppm</td>
<td>500 ppm</td>
</tr>
<tr>
<td>Ligand (HEPT)</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>[Cr(C2H34N6O2S2)]</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>[Mn(C2H34N6O2S2)]</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>[Co(C2H34N6O2S2)]</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>[Ni(C2H34N6O2S2)]</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>34</td>
<td>36</td>
</tr>
</tbody>
</table>
Graph-1. Comparative study of Antimicrobial and Antifungal activity of ligand (HEPT) and its transition metal complexes.

CONCLUSION
Synthesis of the Schiff base ligand 1-(2-hydroxybenzylidene)-4-ethyl-4-phenylthiosemicarbazide (HEPT) and its transition metal complexes are carried out. The X-ray diffraction studies shows Cr (III) complex having triclinic crystal system, Co (II), Mn(II) complex having monoclinic crystal system and Ni(II) complex having orthorhombic crystal system. From the analytical and spectral data it can be concluded that the synthesized complexes are stable. Ligand HEPT and its metal complexes as well show very high antibacterial and antifungal activity at both 250 ppm and 500 ppm concentration. In view of the foregoing discussions, the high melting points and insolubility in common organic solvents, also concluded that the three common coordinate sites are phenolic oxygen, azomethine nitrogen and thiol sulphur. It is observed that the Ni (II) complex of ligand (HEPT) contains two coordinated water molecules and possesses octahedral geometry.

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