Characterization, Biological Activity and DNA Studies of Atomoxetine and Ortho hydroxy benzaldehyde Imine Metal Complexes

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ABSTRACT

The rapid increasing applications and anti-bacterial and anti hyperactive properties of different drug the author have synthesized Imine complexes of Atomoxetine with O-hydroxy benzaldehyde. These were depicted with various techniques via Elemental Analysis, UV, FT-IR, NMR, ESR, VSM, Conductivity and TG-DTA. These ligands and metal complexes were also screened for biological activity and DNA Studies.

Keywords: Atomoxetine, Copper chloride, Ruthenium chloride and Biological activity.

INTRODUCTION

Imines are the condensed products of Carbonyl compounds and Amines¹. These are also known as Schiff Bases. The Scientist Hugo Schiff was introduced in 1864². Imines show a unique property in the participation of covalent bond with transition metal ions, since the easy formation of ligands act as intermediates for the formation of complexes with transition metal ions. Imine complexes perform biological activities like anti inflammatory, anti fungal, anti bacterial, anti viral, anti diabetic, anti cancer and in pharmaceuticals etc³⁹. Biological activity of the Ligands were recorded by the presence of >C=N moiety of the Schiff bases. These are also having uncountable appliances in technical fields like automobiles, electro plating, printing technology, textile and detergents¹⁰­¹¹.

The author has reported characterization, biological activity and DNA studies of the ligands and their metal complexes. The synthesis of Imine metal complexes has been carried out by conventional method and characterization with various techniques like FT-IR, H¹-NMR, ESR, UV-Vis and Conductometry. Thermal stability of the complexes was identified by TG-DTA with various temperatures. Biological activity of Imines and their Cu and Ru complexes was performed in In vitro conditions and DNA binding mode with UV-Vis Spectroscopic technique.
MATERIALS AND METHODS

Atomoxetine (AT), O-hydroxy benzaldehyde (OHB), methanol, DMF, chlorides of Copper and ruthenium.

Instrumentation

IR Spectral data with KBr Pellets, H\textsuperscript{1}-NMR Spectral data with BRUKER 400MHZ SUPERCON Spectrometer, ESR Spectral data with Bruker ESP 300E spectrometer, UV-Vis Spectral data with Schimadzu UV-1800 model Spectrometer, XRD on Panalytical X’pert3 diffractometer, conductivity measurements with digital conductivity meter DCM-900, VSM with EG and G-155 magnetometer were recorded.

Synthesis of OHB-AT Ligand

The equal concentration of AT and OHB was dissolved separately, this mixture was heated for 2 h by adding few drops of concentrated HCL, pink colored solution was obtained and this was cooled to room temperature. Then pink color precipitate was obtained. These crystals were washed with methanol and dried in micro wave. The percentage yield of ligand was 80.

Synthesis of OHBAT-Cu(II) & Ru(III) metal complexes

The complexes were prepared by mixing 1:2 ratio of an aqueous solution of metal ions with the methanolic solution of Imine separately and stirred with magnetic stirrer for proper mixing of contents. Then the contents in the flask were reflexed for six hours and kept in ice bath for cooling, bluish green color sharp needle like crystals were obtained. These were washed and recrystallized with methanol. The yield of copper and ruthenium complexes was 75% and 78%.

RESULTS AND DISCUSSIONS

The main purpose of the present work is to study the characterization of metal complexes with various techniques, Biological activity in \textit{In vitro} conditions with different micro-organisms and DNA studies on UV-Vis Spectrophotometer.

IR spectral data

The nature of Imine metal complexes were identified by obtaining strong band at 1627 cm\textsuperscript{-1} indicated the development of Imine group, which on complexing with the metals viz. Cu (II) and Ru (III) the bands appeared at 1605 cm\textsuperscript{-1} and 1622 cm\textsuperscript{-1} indicated the coordination between Imine group of nitrogen with electron deficient metal ions because of decreased electron density on Nitrogen atom\textsuperscript{12,13}. The another strong bands at 420 cm\textsuperscript{-1} and 429 cm\textsuperscript{-1} for copper and ruthenium expressed coordination between metal and ligand\textsuperscript{14,15}. IR data of the ligand and complexes were represented in the Table 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ν OH Water</th>
<th>ν OH Phenolic</th>
<th>ν C=N</th>
<th>ν M-O</th>
<th>ν M-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>OHBAT</td>
<td>-</td>
<td>3363</td>
<td>1627</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>OHBAT-Cu</td>
<td>3304</td>
<td>-</td>
<td>1605</td>
<td>620</td>
<td>420</td>
</tr>
<tr>
<td>OHBAT-Ru</td>
<td>3413</td>
<td>-</td>
<td>1622</td>
<td>649</td>
<td>429</td>
</tr>
</tbody>
</table>

NMR spectral data

A singlet at 5.6 ppm revealed aromatic-\textit{OH} protons of OHB, this was absent in complex given the coordination between oxygen moiety of OHB and metal ions of Cu (II) and Ru (III). A singlet at 6.26 ppm specified the presence of Imine group of ligand\textsuperscript{16}, this was shifted to 7.6 ppm and 7.26 ppm by the coordination of ligand with metal ions. Another singlet at 4.1 ppm and 4.65 ppm appeared only by the coordination of water molecules with metal ions. NMR data of the ligand and complexes were represented in the Table 2.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compound</th>
<th>H-C=N</th>
<th>Ar-H</th>
<th>OH-Phenilic</th>
<th>OH-H\textsubscript{2}O -CH\textsubscript{3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OHBAT</td>
<td>6.26</td>
<td>6.7-7.3</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>2</td>
<td>OHBAT-Cu</td>
<td>7.2</td>
<td>7.4-7.9</td>
<td>-</td>
<td>4.1</td>
</tr>
<tr>
<td>3</td>
<td>OHBAT-Ru</td>
<td>7.26</td>
<td>7.3-7.7</td>
<td>-</td>
<td>4.65</td>
</tr>
</tbody>
</table>

ESR spectral data

The value of G is >4 shows mononuclear nature of complex. This value can be calculated as, \( G = \frac{g^\parallel}{g^\perp} = \frac{2.0023}{-2.0023} \).

\( \alpha^2 \) value of Copper and Ruthenium complexes(0.4518 and 0.4794) specified the covalent nature of complexes. The values of \( g^\parallel \)
> \( g_{\text{ave}} \) values were greater than 2.0023 said that unpaired electrons of \( d_{x^2-y^2} \) and \( d_{z^2} \) orbital is delocalized for Cu and Ru ions respectively. ESR spectral data of the complexes were represented in Table 3.

### Table 3: ESR Spectral data of OHBAT-Cu and OHBAT-Ru

<table>
<thead>
<tr>
<th>Parameters</th>
<th>OHBAT-Cu</th>
<th>OHBAT-Ru</th>
</tr>
</thead>
<tbody>
<tr>
<td>( g^\parallel )</td>
<td>2.5864</td>
<td>2.6885</td>
</tr>
<tr>
<td>( g^\perp )</td>
<td>2.2038</td>
<td>2.3698</td>
</tr>
<tr>
<td>( g_{\text{ave}} )</td>
<td>2.3313</td>
<td>2.4968</td>
</tr>
<tr>
<td>( A^\parallel )</td>
<td>0.00869</td>
<td>0.00981</td>
</tr>
<tr>
<td>( A^\perp )</td>
<td>0.0097</td>
<td>0.0168</td>
</tr>
<tr>
<td>( K^\parallel )</td>
<td>0.0762</td>
<td>0.0865</td>
</tr>
<tr>
<td>( K^\perp )</td>
<td>0.0336</td>
<td>0.0418</td>
</tr>
<tr>
<td>( \alpha^2 )</td>
<td>0.2798</td>
<td>0.4198</td>
</tr>
</tbody>
</table>

### UV-Vis spectral data

The transition of ligand was 277 nm, which on complexation with Cu (II) and Ru (III) metal complexes change in transitions at 289 nm and 306 nm signified the charge transfer transition from \( L \rightarrow M \). UV-Vis Spectral data were represented in Table 4.

### Table 4: UV-Vis Spectral of OHBAT and ITS Cu (II) and Ru (III) Metal complexes

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compound</th>
<th>( \lambda_{\text{max}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OHBAT</td>
<td>277</td>
</tr>
<tr>
<td>2</td>
<td>OHBAT-Cu</td>
<td>289</td>
</tr>
<tr>
<td>3</td>
<td>OHBAT-Ru</td>
<td>306</td>
</tr>
</tbody>
</table>

### XRD of OHBAT-Cu and OHBAT-Ru metal complexes

The diffracto grams, calculated miller indices \((h k l)\) values, \(2\theta^\parallel\) and \(d\) values were represented in the Table 5 suggested a good agreement between \(2\theta^\parallel\) and \(d\) values. \(2\theta\) values recommended poor crystallinity of the complexes.

### Table 5: XRD studies of OHBAT-Cu metal complex

<table>
<thead>
<tr>
<th>S.No</th>
<th>d exp</th>
<th>d Cal.</th>
<th>(2\theta) exp</th>
<th>(2\theta) Cal.</th>
<th>h k l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0354</td>
<td>0.0349</td>
<td>5.2805</td>
<td>5.2801</td>
<td>1 1 1</td>
</tr>
<tr>
<td>2</td>
<td>0.03917</td>
<td>0.03912</td>
<td>5.8303</td>
<td>5.8299</td>
<td>1 1 1</td>
</tr>
<tr>
<td>3</td>
<td>0.04428</td>
<td>0.04422</td>
<td>6.5921</td>
<td>6.5915</td>
<td>1 1 1</td>
</tr>
<tr>
<td>4</td>
<td>0.04925</td>
<td>0.04919</td>
<td>7.3326</td>
<td>7.3321</td>
<td>2 1 1</td>
</tr>
<tr>
<td>5</td>
<td>0.05915</td>
<td>0.05910</td>
<td>8.8085</td>
<td>8.8081</td>
<td>2 2 1</td>
</tr>
<tr>
<td>6</td>
<td>0.0623</td>
<td>0.0619</td>
<td>9.2791</td>
<td>9.2785</td>
<td>2 2 1</td>
</tr>
<tr>
<td>7</td>
<td>0.0965</td>
<td>0.0959</td>
<td>14.3999</td>
<td>14.3994</td>
<td>5 4 4</td>
</tr>
<tr>
<td>8</td>
<td>0.1039</td>
<td>0.1033</td>
<td>15.5905</td>
<td>15.5901</td>
<td>5 5 2</td>
</tr>
<tr>
<td>9</td>
<td>0.1088</td>
<td>0.1081</td>
<td>16.2419</td>
<td>16.2414</td>
<td>5 5 3</td>
</tr>
<tr>
<td>10</td>
<td>0.1255</td>
<td>0.1250</td>
<td>18.7663</td>
<td>18.7659</td>
<td>6 6 3</td>
</tr>
<tr>
<td>11</td>
<td>0.1311</td>
<td>0.1308</td>
<td>19.6003</td>
<td>19.6000</td>
<td>6 6 4</td>
</tr>
<tr>
<td>12</td>
<td>0.1466</td>
<td>0.1459</td>
<td>21.9204</td>
<td>21.9199</td>
<td>6 6 6</td>
</tr>
<tr>
<td>13</td>
<td>0.1800</td>
<td>0.1796</td>
<td>27.0421</td>
<td>27.0415</td>
<td>9 8 4</td>
</tr>
<tr>
<td>14</td>
<td>0.1891</td>
<td>0.1886</td>
<td>28.4342</td>
<td>28.4339</td>
<td>9 9 4</td>
</tr>
<tr>
<td>15</td>
<td>0.1948</td>
<td>0.1944</td>
<td>29.2495</td>
<td>29.2489</td>
<td>9 9 5</td>
</tr>
<tr>
<td>16</td>
<td>0.2165</td>
<td>0.2161</td>
<td>32.6565</td>
<td>32.6559</td>
<td>10 10 6</td>
</tr>
<tr>
<td>17</td>
<td>0.2208</td>
<td>0.2201</td>
<td>34.8814</td>
<td>34.8809</td>
<td>11 10 7</td>
</tr>
<tr>
<td>18</td>
<td>0.2582</td>
<td>0.2579</td>
<td>39.1821</td>
<td>39.1816</td>
<td>11 11 8</td>
</tr>
<tr>
<td>19</td>
<td>0.2882</td>
<td>0.2876</td>
<td>42.9935</td>
<td>42.9931</td>
<td>12 11 10</td>
</tr>
<tr>
<td>20</td>
<td>0.2929</td>
<td>0.2924</td>
<td>44.7026</td>
<td>44.7021</td>
<td>12 12 12</td>
</tr>
<tr>
<td>21</td>
<td>0.2982</td>
<td>0.2976</td>
<td>45.5550</td>
<td>45.5546</td>
<td>13 13 10</td>
</tr>
<tr>
<td>22</td>
<td>0.3252</td>
<td>0.3249</td>
<td>49.9612</td>
<td>49.9606</td>
<td>14 14 12</td>
</tr>
<tr>
<td>23</td>
<td>0.3742</td>
<td>0.3737</td>
<td>58.1390</td>
<td>58.1384</td>
<td>16 16 14</td>
</tr>
<tr>
<td>24</td>
<td>0.4059</td>
<td>0.4055</td>
<td>63.5485</td>
<td>63.5481</td>
<td>17 17 16</td>
</tr>
<tr>
<td>25</td>
<td>0.4693</td>
<td>0.4688</td>
<td>75.0662</td>
<td>75.0658</td>
<td>20 19 19</td>
</tr>
<tr>
<td>26</td>
<td>0.5132</td>
<td>0.5129</td>
<td>83.5660</td>
<td>83.5555</td>
<td>21 21 20</td>
</tr>
</tbody>
</table>

### Table 6: XRD studies of OHBAT-Ru metal complex

<table>
<thead>
<tr>
<th>S.No</th>
<th>d exp</th>
<th>d Cal.</th>
<th>(2\theta) exp</th>
<th>(2\theta) Cal.</th>
<th>h k l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0407</td>
<td>0.0401</td>
<td>6.0709</td>
<td>6.0701</td>
<td>1 1 1</td>
</tr>
<tr>
<td>2</td>
<td>0.0490</td>
<td>0.0485</td>
<td>7.2553</td>
<td>7.2549</td>
<td>1 1 1</td>
</tr>
<tr>
<td>3</td>
<td>0.0563</td>
<td>0.0558</td>
<td>8.3951</td>
<td>8.3945</td>
<td>2 1 1</td>
</tr>
<tr>
<td>4</td>
<td>0.0634</td>
<td>0.0631</td>
<td>9.4552</td>
<td>9.4546</td>
<td>2 2 1</td>
</tr>
<tr>
<td>5</td>
<td>0.0855</td>
<td>0.0851</td>
<td>12.7476</td>
<td>12.7470</td>
<td>4 4 2</td>
</tr>
<tr>
<td>6</td>
<td>0.0934</td>
<td>0.0929</td>
<td>13.9398</td>
<td>13.9392</td>
<td>5 4 1</td>
</tr>
<tr>
<td>7</td>
<td>0.1246</td>
<td>0.1241</td>
<td>18.6317</td>
<td>18.6312</td>
<td>6 6 3</td>
</tr>
<tr>
<td>8</td>
<td>0.1306</td>
<td>0.1299</td>
<td>19.5272</td>
<td>19.5266</td>
<td>6 6 4</td>
</tr>
<tr>
<td>9</td>
<td>0.1328</td>
<td>0.1321</td>
<td>19.9280</td>
<td>19.9274</td>
<td>7 6 1</td>
</tr>
</tbody>
</table>

### Analysis of TG-DTA

By observing modifications at different temperature ranges one can easily explain thermal stability of the complexes. The temp ranges between 110°C-222.10°C and 215°C-280°C specified the loss of two water molecules at the first level, the second level at 261.03°C and 450°C specified the formation of stable intermediate peaks as the results of decomposition at the temperature range of 818°C and 600°C. At high temp metallic oxides formation takes by the exothermic process. Thermal data of Cu (II) and Ru (III) metal complexes were represented in the Table 7.
Table 7: Thermal Data of OHBAT-Cu & OHBAT-Ru Metal Complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Molecular weight (grams)</th>
<th>Temperature range in °C</th>
<th>Probable assignment</th>
<th>Mass loss (%)</th>
<th>Total mass loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OHBAT-Cu</td>
<td>894.68</td>
<td>110 - 222.10</td>
<td>Loss of two H₂O molecules</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Loss of two ligand molecules</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Formation of CuO</td>
<td>25.53</td>
<td>70.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Above 818.34</td>
<td>40.35</td>
<td></td>
</tr>
<tr>
<td>OHBAT-Ru</td>
<td>932.41</td>
<td>215 - 280</td>
<td>Loss of two H₂O molecules</td>
<td>5.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Loss of two ligand molecules</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Formation of CuO</td>
<td>27.35</td>
<td>88.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Above 600</td>
<td>55.63</td>
<td></td>
</tr>
</tbody>
</table>

Conductometry

Molar Conductivity value of the complexes at 58 and 60 Ohm⁻¹cm²mol⁻¹ for Cu(II) and Ru(III) specified non electrolytic in nature. Molar conductance values of the complexes were represented in the Table 8.

Table 8: Conductivity values of OHBAT-Cu (II) and OHBAT-Ru(III) metal complexes

<table>
<thead>
<tr>
<th>S.No</th>
<th>Complex</th>
<th>Conductance Ohm⁻¹cm²mol⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OHBAT-Cu</td>
<td>58</td>
</tr>
<tr>
<td>2</td>
<td>OHBAT-Ru</td>
<td>60</td>
</tr>
</tbody>
</table>

VSM analysis

The magnetic momentum values of OHBAT-Cu and OHBAT-Ru complexes at 4.28 and 5.56 BM specified octahedral geometry of the complexes due to the presence of lone pair of electrons in d-orbital of the metal ions. The magnetic susceptibility values of OHBAT-Cu and OHBAT-Ru complexes were represented in the Table 9.

Table 9: Magnetic momentum values of OHBAT-Cu and OHBAT-Ru complexes

<table>
<thead>
<tr>
<th>S.No</th>
<th>Complex</th>
<th>Magnetic momentum (BM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OHBAT-Cu</td>
<td>4.28</td>
</tr>
<tr>
<td>2</td>
<td>OHBAT-Ru</td>
<td>5.56</td>
</tr>
</tbody>
</table>

Biological studies

Biological studies of the ligands and complexes were performed in In vitro conditions; represented biological activity of Imines was less than their corresponding metal complexes because of the reduced electron density of metal ions by the transfer of charge, according to Chelating theory. The biological activity values of Imines and their metal complexes were represented in the Table 10.

Table 10: Biological studies of the metal complexes of Copper & Ruthenium metal ions

<table>
<thead>
<tr>
<th>Compound</th>
<th>E. coli</th>
<th>Klebsiella</th>
<th>Bacillus</th>
</tr>
</thead>
<tbody>
<tr>
<td>OHBAT</td>
<td>9</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>OHBAT-Cu</td>
<td>10</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>OHBAT-Ru</td>
<td>11</td>
<td>14</td>
<td>16</td>
</tr>
</tbody>
</table>

DNA binding mode of the complexes

DNA binding activity of the complexes was performed with di sodium salt of calf DNA. The spectrum was performed in the absence and presence of CT-DNA. In the presence of CT-DNA the complexes conveyed hypochromic shift because of the presence of chromophores of the ligand. The quantitative comparisons of binding parameters from the following equation

$$[\text{DNA}] / (\varepsilon_a - \varepsilon_f) = [\text{DNA}] / (\varepsilon_b - \varepsilon_f) + 1 / K_b (\varepsilon_b - \varepsilon_f)$$

The binding constants of the complexes were represented in the Table 11.

Table 11: DNA activity of OHBAT-Cu and OHBAT-Ru metal complexes

<table>
<thead>
<tr>
<th>S.No</th>
<th>Complex</th>
<th>λ_max nm</th>
<th>Δλ nm</th>
<th>H%</th>
<th>K_b (M⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>OHBAT-Cu</td>
<td>305</td>
<td>11</td>
<td>6</td>
<td>3.94 x 10⁶</td>
</tr>
<tr>
<td>4</td>
<td>OHBAT-Ru</td>
<td>313</td>
<td>17</td>
<td>6</td>
<td>3.28 x 10⁷</td>
</tr>
</tbody>
</table>

CONCLUSION

In the present article author has reported characterization, biological activity and DNA studies of the metal complexes of Cu (II) and Ru (III) metal ions. The characterization reports suggested, the complex was mono nuclear with molecular formula of [M (L₂)]. The stoichiometry of the complex was 1:2 ratios and proposed geometry of the complexes was octahedral. The complexes were exhibit more biological activity than their corresponding ligands.
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1) I would like to express my deepest appreciation to my Professor J. Sreeramulu garu部门 of chemistry, SK University, Anantapuramu for his continuous support in my research area.

2) I would like thanks to RGM college of Engineering and technology for encouraging in the area of research.

Conflicts of interest

The author has no conflicts regarding publication of this paper.

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