Cu(II) COMPLEXES WITH AN NNO FUNCTIONALIZED HYDRAZONE LIGAND: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES

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ABSTRACT

We report the synthesis of Cu(II) complexes with hydrazone ligands, 2-[(6-Nitro-benzothiazole-2-yl)-hydrazonomethyl]-4,6-dichloro-phenol (BHD5) and 2-[(6-Nitro-benzothiazole-2-yl)-hydrazonomethyl]-4-chlorophenol (BH5). The synthesized compounds were characterized using elemental analyses, FT-IR, UV-Vis, \textsuperscript{1}H-NMR, mass spectra, molar conductance and TGA. The stoichiometry of the copper complexes as 1:1\[M:L\] ratio and ligand behave as tridentate moiety with NNO donor atoms toward the metal ion. The complexes have been assigned a tetrahedral geometry. The synthesized ligands and their copper complexes were screened for antibacterial activities against Gram-positive bacteria (\textit{Bacillus subtilis}), Gram-negative bacteria (\textit{Salmonella typhi}) and two funguses (\textit{Candida tropicalis} and \textit{Kluyveromyces marxianus}). The DPPH antioxidant activity shows that the copper complexes have a promising biological activity than their parent ligand.

Keywords: Hydrazone Ligand, Cu(II) Complexes, Biological and Antioxidant Activity.

INTRODUCTION

Thiazole and benzothiazole possess effective antimicrobial and antioxidant activity. Thiazoles are known as antioxidant and neuroprotective agent.\textsuperscript{1,3} Benzothiazole containing a 5-membered 1,3-thiazole ring which is fused into the benzene ring. They show excellent biological properties.\textsuperscript{4, 5} Hydrazones are the azomethines, described by the tri-nuclear gathering >C=N‒N< in their structure.\textsuperscript{6,7} Hydrazones contain azomethine linkage which is used as intermediates to synthesis some biologically active compounds. The hydrazone metal complexes are known for their excellent application in the biological and pharmaceutical fields as antimicrobial, antiviral, antitumor, antiparasitic and anti-inflammatory agents.\textsuperscript{8,10} In this work, we synthesized hydrazones ligand by condensation of 6-nitro-benzothiazole-2-yl-hydrazine with substituted different salicylaldehyde and study their coordination behavior, antimicrobial and antioxidant activity.

EXPERIMENTAL

Material and Methods

All organic solvents and inorganic substances employed in the present investigation is pure and analytical grade. Melting points take on Kumar KI-11-02 electrical melting point apparatus using one end seal capillary. The elemental contents CHN and S were carried out on Thermo- scientific (FLASH 2000) analyzer. The metal contents of the transition metal complexes were analyzed by gravimetric method.\textsuperscript{5} The IR spectrum was recorded on Nicolet iS\textsuperscript{5} FT-IR. The UV-Vis spectrum was taken on a Shimadzu UV-1800 spectrophotometer in DMF using 10\textsuperscript{-4} M solution. The \textsuperscript{1}H-NMR spectral data were recorded in DMSO for 400 MHz at SAIF, Panjab University. The mass spectrum was carried out on Waters micro-
mass Q-TOF. The molar conductance carried out on CM model ELICO, 162 conductivity cell. Thermogravimetric studies were carried out in the temperature range RT-785 °C on a TGA 55, TA instrument, USA.

**Synthesis of Ligands**

The hydrazone ligands were synthesized by under reflux of 6-Nitro-benzothiazole-2-yl-hydrazine (0.01 mmol) and substituted salicyaldehyde (0.01 mmol) in the present of 2-3 droop glacial acetic acid using 20 ml ethanol as reaction solvent §. Obtain precipitated was filtered, washed, recrystallized in ethanol and dried (Scheme-1).

![Scheme-1: Synthesis of Hydrazone Ligands.](image)

**BHD5**

Yield 79%; m. p. 248 °C; Yellow solid; Anal. Caled for C_{14}H_{7}Cl_{3}N_{4}S: C, 43.88; H, 1.57; N, 11.64; S, 6.54; Cu, 13.21. Experimentally found (%): C, 43.50; H, 1.82; N, 11.90; Cu, 13.42. FT-IR (KBr, cm⁻¹): ν(-NH) 3434, exocyclic ν(C=N) 1606, endocyclic ν(C=N) 1577, aromatic ν(C=N) 1285, phenolic ν(C-O) 1341, v(M-O) 617, v(M-N) 428. ΛM (Ω · m⁻¹ cm² mol⁻¹): 9.48. Mass (ESI) [M⁺] = 481.9 amu.

**BH5C**

Yield 68%; m.p. 239 °C; green solid; Anal. Caled for C_{14}H_{8}Cl_{2}N_{4}O_{3}S: C, 48.21; H, 1.80; N, 12.54; S, 7.18; Cu, 14.21. Experimentally found (%): C, 48.01; H, 1.89; N, 12.74; S, 6.94; Cu, 14.14. FT-IR (KBr, cm⁻¹): ν(-NH) 3447, phenolic ν(C-O) 1329, aromatic ν(C-N) 1292, v(N-N) 1129, 1042, 880, 745, 673. Mass (ESI) [M⁺] = 349.01 amu.

**Synthesis of Copper(II) Complexes**

The copper(II) complexes were synthesized by the interaction between hydrazone ligands, BHD5 and BH5C (1 mmol) in 20 ml ethanol with aqueous solution of copper(II) chloride dihydrate (CuCl₂·2H₂O, 2 mmol) under reflux for 1-2 h. Obtain precipitated were filtered, washed with aqueous ethanol and dried (Scheme-2).

**Cu(BHD5)Cl**

Yield 79%; m. p. 255 °C; greenish yellow solid. Anal. Caled for C_{14}H_{7}Cl_{3}N_{4}CuO_{3}S: C, 34.94; H, 1.57; N, 11.64; S, 6.54; Cu, 13.21. Experimentally found (%): C, 34.50; H, 1.82; N, 11.90; Cu, 13.42. FT-IR (KBr, cm⁻¹): ν(-NH) 3434, exocyclic ν(C=N) 1606, endocyclic ν(C=N) 1577, aromatic ν(C-N) 1285, phenolic ν(C-O) 1341, v(N-N) 1124, v(M-O) 617, v(M-N) 428. ΛM (Ω · m⁻¹ cm² mol⁻¹): 9.48. Mass (ESI) [M⁺] = 481.9 amu.

**Cu(BH5C)Cl**

Yield 79%; m.p. 269 °C; dark green solid. Anal. Caled for C_{14}H_{8}Cl_{2}N_{4}CuO_{3}S: C, 37.64; H, 1.80; N, 12.54; S, 7.18; Cu, 14.21. Experimentally found (%): C, 37.90; H, 1.89; N, 12.74; S, 6.94; Cu, 14.14.
13.92. FT-IR (cm⁻¹): ν(-NH) 3415, exocyclic ν(C=N) 1616, endocyclic ν(C=N) 1551, aromatic ν(C-N) 1284, phenolic ν(C-O) 1337, ν(N=N) 1104, ν(M-O) 554, ν(M-N) 424. \( \mu_{\text{eff}} \) (BM): 1.94. \( \Lambda_{\text{M}} (\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}) \): 11.06. Mass (ESI) [M⁺] = 445.9 amu.

Scheme-2: Proposed Structure for Cu(BHD5)Cl and Cu(BH5C)Cl Complexes.

**Antimicrobial Activities**

The *in vitro* antimicrobial activity was evaluated against *Bacillus subtilis* as a Gram-positive bacterium, *Salmonella typhi* as a Gram-negative bacterium and two fungal strains, *Aspergillus niger* and *Candida tropicalis* by disk diffusion method.\(^{1,12}\) Streptomycin (antibacterial) and nystatin (antifungal) were used as standard drugs. The tested compounds were previously dissolved in DMF at 100 μg/mL concentrations and soaked onto the filter paper disc (5 mm dia.). The antimicrobial activity resulting data are recorded in Table-1.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>B. subtilis</th>
<th>S. typhi</th>
<th>C. tropicalis</th>
<th>A. Niger</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHD5</td>
<td>8</td>
<td>7</td>
<td>-ve</td>
<td>7</td>
</tr>
<tr>
<td>Cu(BHD5)Cl</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>BH5C</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Cu(BH5C)Cl</td>
<td>8</td>
<td>-ve</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>20</td>
<td>19</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nystatin</td>
<td>-</td>
<td>-</td>
<td>19</td>
<td>20</td>
</tr>
</tbody>
</table>

-ve (no antimicrobial activity observed)

**DPPH Radical Scavenging Assay**

The synthesized hydrazone ligands and their copper complexes were prepared in DMF and take 1 mL of each compound solution having concentrations 100 μg/mL was added into 4 mL of 0.1 mM methanol solution of DPPH in a test tube and shaken continuously for clear solution.\(^{13}\) Then the test tubes incubated in the dark place for 30 min. The absorbance measured at 517 nm on a UV-visible spectrophotometer. Ascorbic acid was used as an internal standard.\(^{14}\) The results data of DPPH activity are summarized in Table-2. The scavenging assay percentage was calculated using following the formula:

\[
\% \text{ Scavenging} = \left[ \frac{(A_o - A_i)}{A_o} \right] \times 100
\]

Where, \( A_o \) is the absorbance of without compound, blank and \( A_i \) is absorbance with the synthesized compound.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>% Scavenging</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHD5</td>
<td>54.4</td>
</tr>
<tr>
<td>Cu(BHD5)Cl</td>
<td>55.0</td>
</tr>
<tr>
<td>BH5C</td>
<td>56.1</td>
</tr>
<tr>
<td>Cu(BH5C)Cl</td>
<td>60.8</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>89.1</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

The Cu(II) complexes are colored solid and soluble in DMF and DMSO. Molar conductance [Λ_m] value of Cu(II) complexes indicated that both the Cu(II) complexes are non-electrolytes in DMF.\(^1\) The magnetic moment of Cu(BH5C)Cl complex is 1.94 BM suggesting tetrahedral geometry.\(^3\)\(^,\)\(^9\) The elemental analysis data revealed the formation of 1:1 (Metal: Ligand) complexes.

IR Spectral Studies

The intermolecular hydrogen bonding vibration (O–H→N) is observed at 3440-3447 cm\(^{-1}\) into the IR spectrum of the ligand which is shifted to the lower frequency at 3415-3434 cm\(^{-1}\) into the complexes, indicated the bonding of the oxygen to the metal ions.\(^1\)\(^5\)\(^,\)\(^6\) The oxygen involves the coordination with metal ions is also conform by the upward shifting of υ(C=O) band at 5-13 cm\(^{-1}\). The exocyclic υ(C=N) of hydrazone functional group >C=N-N< shows the band at 1606-1616 cm\(^{-1}\) which is shifted downward by 6-15 cm\(^{-1}\) after the bonding with metal ions. This change in-band frequency suggesting the nitrogen is involved in coordination with metal ions.\(^1\)\(^5\)\(^,\)\(^6\)\(^7\)\(^,\)\(^16\) The oxygen involves the coordination with metal ions is also conform by the upward shifting of υ(C=O) band at 5-13 cm\(^{-1}\). The exocyclic υ(C=N) of the benzothiazole ring gives the band at 1511-1567 cm\(^{-1}\) which is shifted upward and downward in complexes, suggesting benzothiazole nitrogen is participation in bonding to the metal ions.\(^1\)\(^6\)\(^,\)\(^17\) Some importance band is observed in complexes at 424–428 cm\(^{-1}\) assigned to υ(N-N) stretch in complexes which is observed in free ligand at 1128-1129 cm\(^{-1}\). The endocyclic υ(C=N) of the benzothiazole ring gives the band at 1511-1567 cm\(^{-1}\) which is shifted upward and downward in complexes, suggesting benzothiazole nitrogen is participation in bonding to the metal ions.\(^1\)\(^6\)\(^,\)\(^17\) Some importance band is observed in complexes at 424–428 cm\(^{-1}\) assigned to υ(M-O) giving the additional support to ligand binding with metal ion.\(^1\)\(^2\)\(^,\)\(^18\)

UV–Visible Absorption Studies

In the UV–Visible spectrum of ligands, two absorption bands are observed at near 274-278 nm which is conformed the π-π* transitions of the aromatic ring.\(^1\)\(^9\) Similarly, n-π* transition of the >C=N< azomethine observed near at 381-390 nm.\(^2\)\(^0\) Due to the bonding with azomethine nitrogen to the metal ions, these bands are observed at 362–367 nm in complexes. Also, the band observed at 264–272 nm in the spectra of complexes assigned to π-π* transition.\(^2\)\(^1\) The d-d transitions bands observe at 466-475 nm which can be accepted charge transfer transitions from ligand to metal ion.\(^2\)\(^2\) UV-Visible resulting data are summarized in Table-3.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Wavelength (λ_{max}, nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHD5</td>
<td>278 π-π*, 381 n-π*</td>
</tr>
<tr>
<td>BH5C</td>
<td>274 π-π*, 390 n-π*</td>
</tr>
<tr>
<td>Cu(BHD5)Cl</td>
<td>264 π-π*, 367 n-π*, 466 d-d, LMCT</td>
</tr>
<tr>
<td>Cu(BH5C)Cl</td>
<td>272 π-π*, 362 n-π*, 475 d-d, LMCT</td>
</tr>
</tbody>
</table>

\(^1\)H-NMR Spectral Studies

The \(^1\)H-NMR spectra are recording in DMSO-d\(_6\). The \(^1\)H-NMR spectrums of synthesized ligand compounds show broad signals at 12.87-13.04 ppm due to the -NH proton. The singlet peaks were observed at 10.51-10.98 ppm indicating phenolic -OH proton in ligands. The signals observed at 8.39-8.49 ppm responsible for N=C-H proton in structure. The aromatic protons Ar-H shows singlets at 6.93-8.20 ppm. The \(^1\)H- NMR spectra of ligands is full agreement with their proposed structure. \(^1\)H-NMR resulting data are summarized in Table-4.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Singlets (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHD5</td>
<td>-NH: 13.04, -OH: 10.98, -N=C-H: 8.49</td>
</tr>
<tr>
<td>BH5C</td>
<td>-NH: 12.87, -OH: 10.51, -N=C-H: 8.39</td>
</tr>
</tbody>
</table>

Mass Spectral Studies

The mass spectrum data of the ligand BHD5 show the molecular ion peak [M⁺] at m/z = 382.9 amu, which is indicated their molecular mass and The Cu(II) complex, Cu(BHD5)Cl show the molecular ion
peak [M⁺] at m/z = 481.9 which is equal to their molecular mass. Some important mass fragmentation peaks observed at m/z = 443.8 show to [M⁺- Cl], m/z = 290.9 for [C₁₁H₆ClN₄O₂S], m/z = 194.3 corresponding to [C₇H₄N₃O₂S] of benzothiazole moiety and m/z = 96.9 corresponding to [C₄H₃NS] of thiazole moiety. These molecule ion fragments result indicated 1:1 binding mode between the ligand and Cu (II) ion in complex. Similarly, the mass spectrum of the BH5C exhibited the molecular ion peak at m/z = 349.01 amu and their Cu(II) complex, Cu(BH5C)Cl shows the molecular ion peak of [C₁₄H₈Cl₂N₄CuO₃S] [M⁺] at m/z = 445.9 amu. Some important mass fragmentation peaks show at m/z = 408.9 corresponding to [M⁺- Cl], m/z = 290.9 for [C₁₁H₆ClN₄O₂S], m/z = 194.03 corresponding to [C₇H₄N₃O₂S] of benzothiazole moiety. This mass fragmentation pattern reported by many researchers.

**Thermal Gravimetric Analysis (TGA)**

Thermal studies of Cu(BHD5)Cl indicate that the complex is stable up to 185 °C. The first stage of decomposition within the temperature range 185-240 °C may be attributed to the loss of coordinated chloride ion present into the complex structure with an experimental mass loss 7.03% (theoretical mass loss = 7.25%). The next successive decomposition at 240-455 °C due to loss of remaining part of complex contains ligand with an experimental mass loss 75.93% (theoretical, 76.24%) goes to the formation of metal oxide i.e [C₁₄H₇Cl₂N₄CuO₃S] to CuO.

The thermogram of Cu(BH5C)Cl complex shows the first stage of decomposition within the temperature range 165-240 °C may be attributed to the loss of coordinated chloride ion present into the complex structure with an experimental mass loss 7.81% (theoretical mass loss = 7.98%). The remaining step of Cu(BH5C)Cl complex decomposition occurs within the temperature range 240-465 °C may be attributed to the loss of remaining part of complex includes ligand with an experimental mass loss 76.12% (theoretical, 74.93%) and remaining CuO as residue.

**Antimicrobial Activity**

The antimicrobial activity was determinate by disk diffusion method and the results are compared with standard drugs streptomycin (antibacterial) and nystatin (antifungal). The Cu(II) complexes show good antimicrobial activity than their parent ligand.

**DPPH Radical Scavenging Assay**

The resulting data of DPPH free radical scavenging assay shows the antioxidant activity of hydrazone ligands enhanced on complexation with Cu(II) ions. The Cu(II) complexes show most pronounced DPPH antioxidant activity than parent ligand but less as compared with standard ascorbic acid.

**CONCLUSION**

The hydrazone ligands have been prepared by condensation of 6-Nitro-benzothiazole-2-yl-hydrazine and substituted salicylaldehyde. The formation of complexes occurs by reaction of hydrazone ligand and CuCl₂·2H₂O salt. Based on IR spectra, ligands behaviors as NNO donor tridentate. The complexes show tetrahedral geometry. Thermal analysis reveals the thermal stability of copper complexes. The complexes are biologically active as compared to their parent ligand. The copper complexes exhibited antioxidant activity.

**ACKNOWLEDGMENT**

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**REFERENCES**

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