Metal-based biologically active compounds: Synthesis, spectral and antioxidant studies of transition metal complexes with hydrazone derivatives

Asad Khan Tanoli*, Zahid Khan1, Tuba Kamal1, Mohsin Ali1, Mehreen Latif2 and Zahida Tasneem Maqsood1
1Department of Chemistry, University of Karachi, Karachi, Pakistan
2Multidisciplinary Research Laboratory (MDRL), Bahria University Medical and Dental College, Bahria University, Karachi, Pakistan

Abstract: New series of Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} complexes have been synthesized using a bio-active hydrazone compound 4-hydroxybenzaldehyde-N-(5-chloro-2-oxo-1,2-dihydro-3H-indol-3-ylidene), abbreviated as [HL]. Complexes were characterized using elemental analysis, FT-IR, UV-visible spectroscopy conductivity and magnetic susceptibility measurements. Results showed that the bi-dentate [HL] coordinated in a square planner manner with Cu^{2+} while for the rest of the M^{2+} ions, it coordinated in a octahedral fashion. Free ligand and its metal complexes were also studied for their antioxidant potential by employing two methods i.e. DPPH radical scavenging and reducing power assay. It was observed that the metal complexes were considerably more potent free radical scavenger and had better reducing abilities compared to the free ligand, furthermore, for both in vitro assays, metal complexes turned out to be better DPPH scavengers and had better reducing abilities than the standards used during biological assays.

Keywords: Hydrazone, spectroscopy, magnetic susceptibility, antioxidant potential, DPPH radical scavenging

INTRODUCTION

Drugs with some degree of antioxidant potentials are generally considered as a good starting point in the context of seeking new therapeutic agents for several pathological disorders associated with oxidative damage caused by reactive oxygen species (ROS) (Wu 2006). Recently, synthetic antioxidants are widely used because they are effective and easy to obtain than the availability of natural antioxidants. In recent years, a number of hydrazone based metal complexes have been synthesized and investigated as effective scavengers of ROS. Literature on hydrazone based metal complexes showed that this is a subject of increased interest for the people working in this domain in order to come up with new possibilities (Sathyadevi et al., 2012).

Hydrazones (\(\text{C}=\text{N}^\text{−} \text{−} \text{N}=\text{R}\)) forms an important class of organic compounds and are widely studied for various biological applications over the decades (Khan et al., 2009, Thakar et al., 2010). The structural analogue of hydrazones can easily form stable complexes with most transition metal ions (Khan et al. 2016). Literature search reveals that a number of hydrazones have been synthesized as bi-dentate as well as multi-dentate ligands (Stadler et al., 2009). Studies shows that the coordination behaviour of these compounds is dependent on pH of the medium, nature of substituents and the position of C≡N group in their structures (Ara et al., 2007). Literature assessment further revealed that metal complexes of hydrazones are well studied but those of hydrazones (\(\text{C}≡\text{N}^\text{−} \text{−} \text{N}=\text{R}\)) have been studied prominently less so far (Suvarapu et al., 2012).

The aim of the present study was to synthesize the metal complexes with the hydrazone ligand, 4-hydroxybenzaldehyde-N-(5-chloro-2-oxo-1, 2-dihydro-3H-indol-3-ylidene) hydrazine, [HL]. Metals from the first transition series in the periodic table were selected in their +2 oxidation states, i.e. Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}. Newly synthesized complexes were characterized using elemental analysis, FT-IR, UV-visible spectroscopy conductivity and magnetic susceptibility measurements. All compounds were also screened for their antioxidant potential using two different methods i.e. DPPH radical scavenging and reducing power assay.

MATERIALS AND METHODS

All the chemicals used were of analytical grade (BDH and Sigma-Aldrich) and used without further purification. All the solvents were distilled before use.

Physical measurements

Metal contents of the synthesized complexes were analysed using Perkin Elmer series 200 atomic absorption. Carbon hydrogen and nitrogen analysis was performed on Perkin Elmer 2400 series II CHNS/O analyser. Proton NMR spectrum were recorded on Avance Bruker AM 300 MHz. Infrared spectrum of the [HL] and its complexes were recorded in KBr on Shimadzu IR-prestige-21. Molar conductance measurements were made

*Corresponding author: e-mail: asad.tanoli@uok.edu.pk
at room temperature in DMSO using JENWAY 4701 conductivity meter. Electronic spectrum of [HL] and complexes were recorded on Perkin Elmer Lambda-5 UV/Vis spectrometer and magnetic moment was carried out by using Sherwood MSB Mk1.

**Synthesis of [HL] ligand**

Ligand [HL] was prepared by refluxing together 0.5M solutions of 5-chloro isatin and hydrazine hydrate in methanol for 2-3 hours at 70°C. Filter off the resulting yellow colour crystals of mono-hydrazone and further treated with the 0.5M 4-hydroxybenzaldehyde; the reaction mixture was again reflux for 3-4 hours in methanol. On cooling the solid product that obtained was filtered and washed with CCl$_4$, recrystallized with methanol and dried in vacuum to furnish the required hydrazone (Khan, Khan et al. 2009).

**Synthesis of M$^{2+}$ complexes**

Metal complexes of the [HL] were prepared by refluxing together the solution of 0.2M metal chloride (25ml) and 0.4M (30ml) solution of [HL] in methanol for 2 hours. The resulting solution was concentrated, filtered and washed with CCl$_4$ and then recrystallized by slow evaporation from methanol solution (Tanoli et al. 2014, Khan et al., 2015).

**Procedure for DPPH scavenging activity**

The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH). The ethanolic solution of DPPH of 0.3 μM was prepared. Each sample (5μL) of different concentration (62.5-500 μg) was mixed with 95μL of DPPH solution in ethanol. The mixture was dispersed in 96well plates and incubated at 37°C for 30min. The absorbance at 515nm was measured by Elisa plate reader (Spectramax plus 384 Molecular Device, USA). To determine the percent radical scavenging activity, methanol was treated as control while BHA is used as standard.

\[
\text{DPPH Scavenging Effect} \text{ (%) } = \frac{A_c - A_s}{A_c} \times 100
\]

Where: \( A_c \) = Absorbance of control (methanol treated); \( A_s \) = Absorbance of sample

The IC$_{50}$ value is the concentration at which 50% radicals have been scavenged by the tested compounds. The IC$_{50}$ values of the [HL] and their metal complexes were calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, MA, USA) (Tanoli, Khan et al., 2014, Khan, Maqsood et al., 2015).

**Procedure for the ferric thiocyanate (FTC) assay**

The assay was performed according to the Oyaizu method with slight modification. Newly synthesized [HL] and its metal complexes were diluted at different concentrations, 2.5 mL of 1% potassium ferricyanide solution and 2.5 mL of the 0.2 mol/L phosphate buffer of pH-7.0 were added with tested samples and vortexed. The mixtures were then incubated for 20 min at 50°C in a steam bath. The sample tubes were cooled at room temperature and 2.5mL of 10% trichloroacetic acid was then added and centrifuged it for 10min at the rate of 3,000 rpm. 2.5mL sample was taken and mixed with 2.5mL of distilled water and 250μL of 0.1% aqueous ferric chloride. Finally the color of the sample solutions were appeared to be fluorescent green and absorbance of the solutions were recorded at 700 nm (Liu et al., 2007).

**RESULTS**

The results of the elemental analysis of the synthesized M$^{2+}$ complexes of [HL] are reported in table 1, while respective molar conductivity and magnetic moment data have been presented in table 2. The results suggested that the [HL] acted as neutral bi-dentate ligand to form the complexes at 1:2 metal to ligand mole ratio. Furthermore, octahedral geometry was proposed for Co$^{2+}$ and Ni$^{2+}$ complexes while square planner geometry was proposed for Cu$^{2+}$ and tetrahedral geometry for Zn$^{2+}$ complexes (Maqsood et al., 2015).

![Fig. 1: The Proposed Geometry for the Metal Complexes of [HL].](Image)

Thus, the complexes were formed according to the equation:

\[
\text{MaCl}_2 + 2\text{HL} \rightarrow [\text{Ma(}\text{HL})_2]\text{Cl}_2 (\text{Cu}^{2+}, \text{Zn}^{2+})
\]

\[
\text{MbCl}_2 + 2\text{HL} \rightarrow [\text{Mb(}\text{HL})_2]\text{Cl}_2 (\text{Co}^{2+}, \text{Ni}^{2+})
\]

\[
\text{HL} = \text{Free ligand}
\]
Complexes are stable and non-hygroscopic with very little solubility in organic solvents but highly soluble in DMSO and DMF. The results shown in table 1 coincided well with the expected complexes composition, thus additionally confirming the purity of the prepared ligand and the complexes. The proposed geometry for the metal complexes has been given in fig. 1.

DISCUSSION

Electronic spectral and magnetic studies
The electronic spectrum of [HL] exhibited two broad absorption bands in between 320-360nm, which were assigned to $n\rightarrow\pi^*$ transitions of the aldimine and ketimine moieties, respectively.
The electronic spectra of Co\(^{2+}\) complex exhibited three absorption bands with the maximum absorbance at 11481 cm\(^{-1}\), 19531 cm\(^{-1}\) and 26455 cm\(^{-1}\), which are attributed to the \(^2T_2g\) → \(^4T_2g\) (F), \(^4T_1g(F)\) → \(^6A_2g\) (F) and \(^4T_1g\) (F) → \(^6T_1g\) (P) transitions respectively. These bands are the characteristic of high spin octahedral Co\(^{2+}\) complexes. However, the magnetic measurement of Co\(^{2+}\) complexes exhibited magnetic moment value of 4.65 BM, which is within the octahedral range of Co\(^{2+}\) (Angelusi et al. 2010).

The Ni\(^{2+}\) complex revealed three sharp bands at around 11919 cm\(^{-1}\), 15552 cm\(^{-1}\) and 25189 cm\(^{-1}\) which have been assigned to the \(^3A_2g\) → \(^3T_2g\); \(^3A_2g\) → \(^3T_1g\) (F) and \(^3A_2g\) → \(^3T_1g\) (P) transitions respectively indicating octahedral geometry. Ni\(^{2+}\) complex showed the magnetic moment value in the range of 2.96-3.28 which is within the range of 3.19 BM suggesting consistency with their octahedral environment (El-Beheri et al. 2007).

UV-Vis spectrums of the Cu\(^{2+}\) complex shown two bands, a low intensity band at around 13661 cm\(^{-1}\) and broad bands at around 26455 cm\(^{-1}\) correspond to \(^2B_1g\) → \(^2A_1g\) and \(^2B_1g\) → \(^2E_1g\) transitions, presumably arising from a square-planar Cu\(^{2+}\). Magnetic moment values of the complexes vary in the range of 1.89 BM, is higher than spin only value (1.73), also suggesting the square planer geometries and confirmed the absence metal-metal interaction in the complexes (Biradar et al. 1976).

Zn\(^{2+}\) complex exhibited single absorption maxima around 27933 cm\(^{-1}\), which can be attributed to intra-ligand transitions. Zn\(^{2+}\) ion generally forms tetrahedral complexes because of its d\(^{10}\) electronic configuration. Zn\(^{2+}\) complex does not exhibit any characteristic d-d transitions and was found to be diamagnetic in nature (Narang 1993).

Electronic data of coordination complexes of [HL] ligand with different metals have been summarized in Table 3 and overlays of UV-visible spectrum of metal complexes have been presented in fig 02.

**Table 5: IC\(_{50}\) values for DPPH radical scavenging activity of [HL] and its metal complexes**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC(_{50}) (µM) Mean ± SEM</th>
<th>% Inhibition (100µM) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL</td>
<td>&gt;500</td>
<td>7.9 ± 0.31</td>
</tr>
<tr>
<td>[Co(HL)(_2)](_2)Cl(_2)]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>[Ni(HL)(_2)](_2)Cl(_2)]</td>
<td>17.2 ± 0.61</td>
<td>94.41 ± 0.15</td>
</tr>
<tr>
<td>[Cu(HL)(_2)](_2)Cl(_2)]</td>
<td>&gt;200</td>
<td>21.21 ± 0.17</td>
</tr>
<tr>
<td>[Zn(HL)(_2)](_2)Cl(_2)]</td>
<td>85.3 ± 0.72</td>
<td>69.2 ± 0.31</td>
</tr>
<tr>
<td><em>BHA</em></td>
<td>44.3 ± 0.09</td>
<td>86 ± 0.11</td>
</tr>
</tbody>
</table>

SEM standard error of mean, NA (not active), *Standard DPPH scavenging activity

**Table 6: IC\(_{50}\) values for Reducing Power (F.T.C Assay) of [HL] and its Metal Complexes**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC(_{50}) (µM) Mean ± SEM</th>
<th>% Inhibition (100µM) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>[HL]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>[Co(HL)(_2)](_2)Cl(_2)]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>[Ni(HL)(_2)](_2)Cl(_2)]</td>
<td>21.5 ± 0.41</td>
<td>92.5 ± 0.62</td>
</tr>
<tr>
<td>[Cu(HL)(_2)](_2)Cl(_2)]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>[Zn(HL)(_2)](_2)Cl(_2)]</td>
<td>69.5 ± 0.91</td>
<td>59.2 ± 0.15</td>
</tr>
<tr>
<td><em>BHT</em></td>
<td>44.3 ± 0.09</td>
<td>86 ± 0.11</td>
</tr>
</tbody>
</table>

SEM standard error of mean, NA (not active), *Standard for Reducing Power,
In addition to these bands, strong bands were also observed in the region of 1608 cm\(^{-1}\) attributed to aldime HC≡N group. IR spectrum of [HL] also shown some other strong bands like 3292 cm\(^{-1}\), 1668 cm\(^{-1}\), 1286 cm\(^{-1}\), 1010 cm\(^{-1}\) and 812 cm\(^{-1}\) which were assigned to hydroxyl (\(-\text{OH}\)), ketimine (C≡N), phenolic (C–O), hydrazinic (N–N) and CCl groups.

Infrared spectra of the synthesized complexes exhibited 26-34 cm\(^{-1}\) decrease in the stretching frequencies of aldime HC≡N group when compared with the pure [HL], which were observed in between 1574-1582 cm\(^{-1}\) (already discussed above). This significant decrease in frequencies after complexation indicated the coordination of aldime nitrogen atom with the metal ions.

IR spectrums of the metal complexes shown another considerable 21-32 cm\(^{-1}\) shift in the frequencies of carbonyl group i.e. C═O present in the indole ring of [HL], which were observed at around 1692-1701 cm\(^{-1}\) in the free [HL], confirming the binding of carbonyl oxygen with the metal ion.

The position of bands in the IR spectrum of the metal complexes due to stretching frequency of N-H (indol ring) and C≡N (ketimine) does not shown any considerable shift as compare with the frequencies of [HL], this indicates that, these groups were not involved in coordination.

The significant change observed in the spectrum of metal complexes compared to [HL] is the appearance of bands in the IR spectrum of the metal ions except Cu\(^{2+}\) and Ni\(^{2+}\) ions. These results also predict the presence of aldimine nitrogen atom with the metal ions.

Thus, the Infrared spectral results provided strong evidences for the complexation of [HL] with the metal ions in the bi-dentate mode from its aldime nitrogen and carbonyl oxygen. These results also predict the presence of chloride ion in the coordination sphere, bounded with the metal ions except Cu\(^{2+}\) and Zn\(^{2+}\).

**Antioxidant activities**

Results obtain from the both methods i.e. DPPH radical scavenging and ferric thiocyanate (FTC) assay are as follows:

**DPPH scavenging**

The results of DPPH radical scavenging showed that [HL] was found to be less or inactive to scavenge the free radicals. It is a common observation that the antioxidant potential of free ligand can be altered upon complexation with the metal ion. From the given table 5, we have noticed that the metal complexes of [HL] with Zn\(^{2+}\) and Ni\(^{2+}\) ions shows excellent activities, their IC\(_{50}\) values were 85.3±0.72μM and 17.2±0.61μM respectively; this suggested that the structural modifications after complex formation redistribute the electrostatic charge, which results in improved activity.

**Ferric thiocyanate assay / reducing power assay**

The results from FTC assay given in table 6, showed that [HL] was found to be in active in its reducing power. After complexation with metal ion its reduction potential increases many folds. Metal complexes of [HL] showed excellent reducing power and the IC\(_{50}\) value for the reducing power of the Cu\(^{2+}\) and Zn\(^{2+}\) complexes of were 21.5±0.41μM and 69.5±0.91μM respectively. These results are much more comparable with the standard used i.e. BHT (IC\(_{50}\) = 44.3 ± 0.09μM).

**CONCLUSION**

The overall results suggested that the metal complexes of [HL] have better antioxidant potential than the free ligand. These pronounced increases in the scavenging ability suggested that the electrostatic charges in the metal complexes stabilizes the bonded aldime (HC≡N) and lactonoyl carbon(C═O) in the structure of the [HL] and expedites the aromatic aldehydic side to scavenge the free radicals.

**REFERENCES**


