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

Asad Khan Tanoli[1*], Zahid Khan[1], Tuba Kamal[1], Mohsin Ali[1],
Mehreen Latif[2] and Zahida Tasneem Maqsood[1]
[1]Department of Chemistry, University of Karachi, Karachi, Pakistan
[2]Multidisciplinary 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 (−C=N−N−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 (−C=N−N=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

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**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 2-3hours 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 37ºC 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.

DPPH Scavenging Effect (%) = Ac – As / Ac × 100
Where: Ac = Absorbance of control (methanol treated);
As = Absorbance of sample

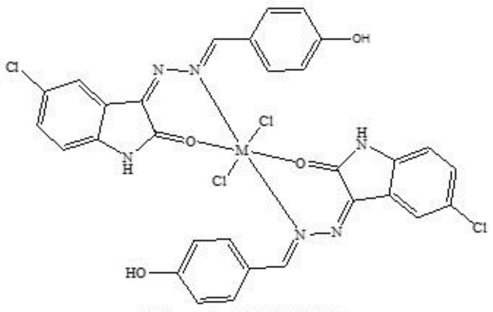
The IC_{50} value is the concentration at which 50% radicals have been scavenged by the tested compounds. The IC_{50} values of the [HL] and their metal complexes were calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, MA, USA) (Tanoli, Khan *et al*., 2014, Khan,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

Procedure for the ferric thiocyanate (FTC) assay

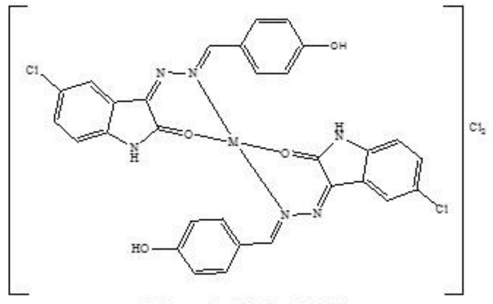
The assay was performed according to the Oyaizu method with slight modification. Newly synthesized [HL] and its metal complexes were diluted at different concentrations, 2.5 mL of 1% potassium ferricyanide solution and 2.5 mL of the 0.2 mol/L phosphate buffer of pH-7.0 were added with tested samples 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).



Mb = Co(II) & Ni(II)



Ma = Cu(II) & Zn(II)

Fig. 1: The Proposed Geometry for the Metal Complexes of [HL].

Thus, the complexes were formed according to the equation:

$MaCl_2 + 2HL \rightarrow [Ma(HL)_2]Cl_2$ (Cu^{+2}, Zn^{+2})
$MbCl_2 + 2HL \rightarrow [Mb(HL)_2Cl_2]$ (Co^{+2}, Ni^{+2})
HL = Free ligand

Table 1: Elemental analysis of metal complexes of [HL]

Compounds		[Co(HL)$_2$Cl$_2$]	[Ni(HL)$_2$Cl$_2$]	[Cu(HL)$_2$]Cl$_2$	[Zn(HL)$_2$]Cl$_2$
Formula mass		726.96	725.97	730.96	731.96
Colour		Coffee brown	Apple green	Dark blue	Yellow white
% Yield		76	74	68	57
M%	Found	8.17	7.96	8.87	8.76
	Cal	8.08	8.05	8.66	8.89
C%	Found	49.27	49.29	49.38	48.77
	Cal	49.41	49.43	49.1	48.98
H%	Found	2.61	2.76	2.89	2.81
	Cal	2.76	2.77	2.75	2.74
N%	Found	11.44	11.64	11.63	11.27
	Cal	11.52	11.53	11.45	11.42
Outer Cl%	Found	---	---	9.97	9.73
	Cal	---	---	9.71	9.70

Table 2: Magnetic moment and conductometric data of the metal complexes of [HL]

Complexes	μeff	Conductance (μs)
	(BM)	
[Co(HL)$_2$Cl$_2$]	4.65	09
[Ni(HL)$_2$Cl$_2$]	3.19	17
[Cu(HL)$_2$]Cl$_2$	1.89	169
[Zn(HL)$_2$] Cl$_2$	D	166

Table 3: Electronic spectroscopic data of the metal complexes of [HL]

Complexes	Bands maxima υ cm^{-1} (λnm)
[Co(HL)$_2$Cl$_2$]	26455 (378), 19531 (512), 11481 (871)
[Ni(HL)$_2$Cl$_2$]	25189 (397), 15552 (634), 11919 (839)
[Cu(HL)$_2$]Cl$_2$	26455 (378), 13661 (732)
[Zn(HL)$_2$] Cl$_2$	27933 (358)

Table 4: Important IR spectral bands of the [HL] and complexes

Tentative Assignments	[HL]	[Co(HL)$_2$Cl$_2$]	[Ni(HL)$_2$Cl$_2$]	[Cu(HL)$_2$]Cl$_2$	[Zn(HL)$_2$]Cl$_2$
υ (OH)	3292	3289	3297	3290	3283
υ (NH)	3180	3180	3178	3184	3175
υ (C═O)	1724	1692	1695	1701	1699
υ (C═N)	1668	1661	1663	1664	1658
υ (HC═N)	1608	1576	1574	1582	1577
υ (C─O)	1286	1285	1292	1289	1286
υ (N─N)	1010	1017	1011	1006	1015
υ (C─Cl)	812	806	807	807	813
υ (M─N)	---	455	462	419	435
υ (M─O)	---	526	524	531	519
υ (M─Cl)	---	372	377	---	---

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─π* transitions of the aldimine and ketimine moieties, respectively.

Table 5: IC_{50} values for DPPH radical scavenging activity of [HL] and it's metal complexes

Compounds	IC_{50} (µM)	% Inhibition (100µM)
	Mean ± SEM	Mean ± SEM
HL	>500	7.9 ± 0.31
$[Co(HL)_2Cl_2]$	NA	NA
$[Ni(HL)_2Cl_2]$	17.2 ± 0.61	94.41 ± 0.15
$[Cu(HL)_2]Cl_2$	>200	21.21 ± 0.17
$[Zn(HL)_2]$ Cl_2	85.3 ± 0.72	69.2 ± 0.31
*BHA	44.3 + 0.09	86 ± 0.11

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 it's Metal Complexes

Compounds	IC_{50} (µM)	% Inhibition (100µM)
	Mean ± SEM	Mean ± SEM
[HL]	NA	NA
$[Co(HL)_2Cl_2]$	NA	NA
$[Ni(HL)_2Cl_2]$	21.5 ± 0.41	92.5 ± 0.62
$[Cu(HL)_2]Cl_2$	NA	NA
$[Zn(HL)_2]$ Cl_2	69.5 ± 0.91	59.2 ± 0.15
*BHT	44.3 + 0.09	86 ± 0.11

SEM standard error of mean, NA (not active), *Standard for Reducing Power,

The electronic spectra of Co^{+2} complex exhibited three absorption bands with the maximum absorbance at $11481cm^{-1}$, $19531cm^{-1}$ and $26455cm^{-1}$, which are attributed to the $^4T_1g(F) \rightarrow {}^4T_2g$ (F), $^4T_1g(F) \rightarrow {}^4A_2g$ (F) and ^4T_1g (F) $\rightarrow {}^4T_1g$ (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 4.65BM, which is within the octahedral range of Co^{+2} (Angelusiu *et al*. 2010).

The Ni^{+2} complex revealed three sharp bands at around $11919cm^{-1}$, $15552cm^{-1}$ and $25189cm^{-1}$ which have been assigned to the $^3A_2g \rightarrow {}^3T_2g$; $^3A_2g \rightarrow {}^3T_1g$ (F) and $^3A_2g \rightarrow {}^3T_1g$ (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 3.19BM suggesting consistency with their octahedral environment (El-Behery *et al*. 2007).

UV-Vis spectrums of the Cu^{+2} complex shown two bands, a low intensity band at around $13661cm^{-1}$ and broad bands at around $26455cm^{-1}$ correspond to $^2B_1g \rightarrow {}^2A_1g$ and $^2B_1g \rightarrow {}^2E_1g$ transitions, presumably arising from a square-planar Cu^{+2}. Magnetic moment values of the complexes vary in the range of 1.89BM, 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 $27933cm^{-1}$, 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.

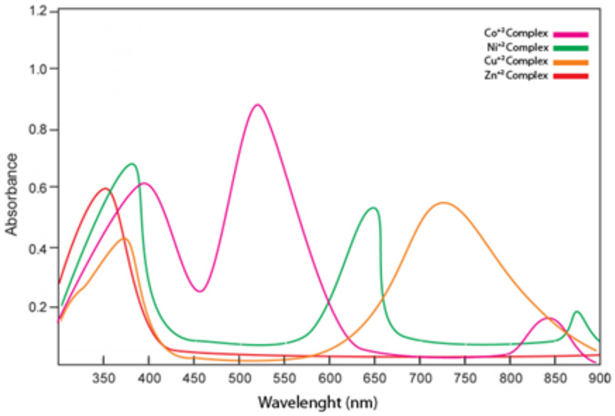


Fig. 2: Overlays of UV-visible spectrum of metal complexes of [HL]

I.R spectral studies

[HL] showed strong band with peak at $3180cm^{-1}$ attributed to indol ring (N─H) while lactonyl carbon (C═O) exhibits its characteristics band at around $1724cm^{-}$

[1]. In addition to these bands, strong bands were also observed in the region of 1608cm^{-1} attributed to aldimine HC═N group. IR spectrum of [HL] also shown some other strong bands like 3292cm^{-1},1668cm^{-1}, 1286cm^{-1}, 1010cm^{-1} and 812cm^{-1} which were assigned to hydroxyl (─OH), ketimine (C═N), phenolic (C─O), hydrazinic (N─N) and CCl groups.

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

IR spectrums of the metal complexes shown another considerable 21-32cm^{-1} shift in the frequencies of carbonyl group i.e. C═O present in the indole ring of [HL], which were observed at around 1692-1701cm^{-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 region of 519-531 cm^{-1}, 419-462cm^{-1} and 372-377cm^{-1} which can be assigned to stretching frequencies of M-O, M-N and M-Cl bonds respectively. It is interesting to notice that the stretching frequency of M-Cl was not observed in the case of Cu^{+2} and Zn^{+2} complexes. This clearly indicated that the absence of inner sphere chloride ion in the complexes of Cu^{+2} and Zn^{+2} (Liu and Yao 2007, Tanoli, Khan *et al*. 2014).

The details of the infrared absorption bands of each metal complex has been presented in table 4.

Thus, the Infrared spectral results provided strong evidences for the complexation of [HL] with the metal ions in the bi-dentate mode from its aldimine 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 aldimine (HC═N) and lactonyl carbon(C═O) in the structure of the [HL] and expedites the aromatic aldehydic side to scavenges the free radicals.

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