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Synthesis, Characterization, In-Vitro Antimicrobial and Antioxidant Activities of Co^{+2}, Ni^{+2}, Cu^{+2} and Zn^{+2} Complexes of 3-(2-(2-hydroxy-3-methoxybenzylidene)hydrazono)indolin-2-one

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Abstract: Four novel complexes of "3-(2-(2-hydroxy-3-methoxybenzylidene)hydrazono)indolin-2-one" have been synthesized with Co^{+2}, Ni^{+2}, Cu^{+2} and Zn^{+2}. Physical and analytical techniques including CHN, IR, UV-Vis, AAS, molar conductivity values and magnetic susceptibility data were used to characterize all complexes. The *bis* Schiff base ligand i.e. 3-(2-(2-hydroxy-3-methoxybenzylidene)hydrazono)indolin-2-one, acted as a tridentate ligand and coordinated through phenolic oxygen, azomethine nitrogen and carbonyl group. Low values of molar conductance suggested the non-electrolyte nature of all complexes. Elemental analysis of complexes indicated the 1:1 metal to ligand mole ratios for [Cu(Inh)(OAc)] and [Zn(Inh)(OAc)] metal complexes and 1:2 metal to ligand mole ratios for $[Co(Inh)_2]$ and $[Ni(Inh)_2]$ metal complexes. Square planner geometry is proposed for [Cu(Inh)(OAc)] and distorted tetrahedral geometry is proposed for [Zn(Inh)(OAc)] while octahedral geometries are proposed for $[Co(Inh)_2]$ and $[Ni(Inh)_2]$ metal complexes. Antimicrobial and antioxidant studies were performed for all compounds and it was discovered that the complexes are more potent antibacterial and antifungal agents while the ligand exhibited comparatively more DPPH (*1,1-diphenyl-2-picryl-hydrazil*) radical scavenging activity than the complexes.

Keywords: Bis Schiff base, Transition metal complexes, Antimicrobial, Antioxidant.

1. INTRODUCTION

Schiff bases forms an important class of organic compounds and are widely studied for various biological applications over the decades [1-3]. Schiff bases have been synthesized as bi-dentate as well as multi-dentate ligands [1, 4, 5]. Studies shows that the coordination behavior of these compounds is dependent on pH of the medium, types and nature of substituents and the position of C=N group in their structures [2]. Literature assessment revealed that metal complexes of *Schiff bases* are well studied but those of *bis Schiff bases* have been studied prominently less so far [2, 3, 6].

In a previous work we have reported the synthesis of a series of *bis* Schiff bases of isatin [6], for this study the "3-(2-(2-hydroxy-3-methoxybenzylidene)hydrazono)indolin-2-one" *bis* Schiff base has been selected in an attempt to synthesize metal complexes and study the impact of coordination on the overall biological activity of the ligand molecule. Metals from the first transition series in periodic table are selected in their +2 oxidation states, i.e. Co^{+2}, Ni^{+2}, Cu^{+2} and Zn^{+2}. Selection of these metals is merely based upon their importance in biological systems and on literature review that suggests that complexes of these metals with Schiff base exhibits a range of biological activities such as anti-bacterial, anti-fungal, antioxidant, antitumor and enzyme inhibition etc. [7-10].

2. EXPERIMENTAL

All chemicals including isatin, solvents, metal salts and hydrazine hydrated were of analytical grade and were purchased from Sigma-Aldrich.

2.1. Synthesis of *bis* Schiff Base Ligand

Ligand compound was synthesized in two steps. In first step 2.94 g of isatin (0.02 mol) was added to 20.0 mL of hydrazine hydrated solution (0.02 mol) with constant stirring in a 50 mL beaker, the reaction mixture was then transferred to a 250 mL round bottom flask that contained 50 mL hot methanol. This reaction mixture was set on a sand bath for about an hour to reflux that produced a yellow solid of "isatin monohydrazone". The flask was allowed to cool and the solid product was filtered, washed successively, dried in vacuum and recrystallized using methanol.

In the second step 1.2 g 2-hydroxy-6-methoxybenzaldehyde (0.01mol) in 10.0 mL methanol

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was added to 1.61 g of isatin monohydrazon solution in 50 mL hot methanol. The mixture was moved to a 250 mL round bottom flask and was set on sand bath to reflux for another 3 hours. After some time the bright orange colored product started separating in the flask, the progress of reaction was monitored using TLC. On completion of reaction the flask was allowed to cool and solid product was filtered, washed, dried in vacuum and recrystallized from methanol. High yields were obtained in both steps of the synthesis.

Both steps of synthesis are shown in Scheme **1**.

2.2. Synthesis of Metal Complexes

$Co(OAc)_2.2H_2O$, $Ni(OAc)_2.4H_2O$, $Cu(OAc)_2.2H_2O$ and $Zn(OAc)_2.4H_2O$ were used to synthesize the metal complexes. Solution of ligand (0.01 mol) in methanol was added in metal solutions (0.005 mol) and allowed to reflux on water bath for about 3 hours. The solutions were subsequently concentrated and allowed to cool. The solid product was filtered, washed with methanol and ether and dried under vacuum.

The synthesis produced metal complexes in high yields and physical and analytical data is presented in the Table **1**.

2.3. Characterization and Physical Measurements

Elemental analysis of complexes was carried out on (PerkinElmer 2400 series II CHN/S) micro analyzer. Metal content was investigated on (Perkin Elmer AAnalyst 700) atomic absorption spectrophotometer. The infrared spectra were recorded on (Shimadzu IR-Prestige-21) FTIR Spectrophotometer. Electronic spectra of metal complexes were recorded on (Shimadzu UV-1601) UV-VIS Spectrophotometer. Molar conductivity values of metal complexes of 10^{-3}M solutions in DMSO were measured on (Jenway 4701) conductivity meter. Magnetic susceptibility values were measured at room temperature with (Mark 1 Magnetic Susceptibility Balance) from Sherwood Scientific. Pascal's constants were used to apply diamagnetic corrections for complexes.

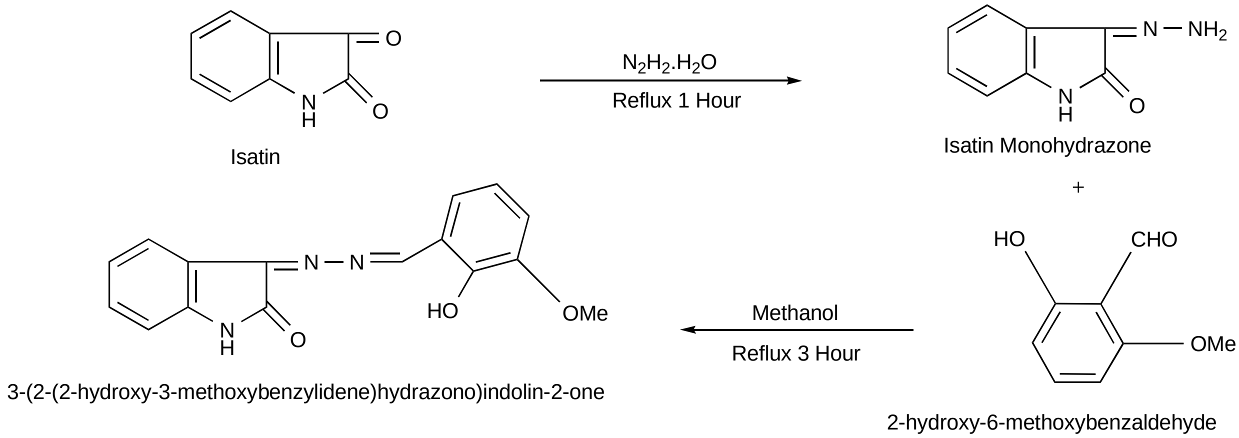
2.4. *In-Vitro* Antimicrobial Assay

The ligand and complexes were screened for anti-bacterial and anti-fungal activities using agar and potato dextrose agar diffusion methods respectively. All compounds were taken from 100, 200 and 400 μg/mL in DMSO and tested against three bacteria (*B. subtilis, E. coli,* and *S. aureus*) and two fungi (*A. niger* and *T. viride*) by MIC method [11]. Bacterial strains were incubated for 24 hours at 37°C and fungi strains were incubated for 48 hours at 37°C.

"Streptomycin" was used as standard for anti-bacterial assay while "*nystatin"* was used as standard for anti-fungal assay under similar conditions. Activity was determined by measuring the diameter of zone of inhibition in millimeter.

2.5. *In-Vitro* Antioxidant Assay

DPPH scavenging method was employed to determine the free radical scavenging potential. A 0.3 µM solution of $DPPH^+$ was prepared in ethanol. Each sample (5μL) of different concentrations (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 30 minutes. The absorbance was measured at 515 nm and percent radical scavenging activity was determined by following equation:



Scheme 1: Synthesis of *bis* Schiff base ligand.

Table 1: Physical and Analytical Data

Compound with Melting Point	Molecular Formula	Formula Mass	%Yield / Color	Analytical Data*				Molar Conductance in DMSO (ohm^{-1} cm^{2} mol^{-1})	Magnetic Moment (B.M)
				M	C	H	N		
HInh **M.P = 237 °C**	$C_{16}H_{13}N_3O_3$	295.29	82 Orange	-	65.08 (65.68)	4.44 (4.30)	14.23 (14.55)	--	--
[Co (Inh)$_2$] **M.P > 300 °C**	$CoC_{32}H_{28}N_6O_6$	651.53	68 Dark Green	09.05 (9.21)	58.99 (59.04)	4.33 (4.22)	12.90 (13.09)	5.6	4.76
[Ni (Inh)$_2$] **M.P > 300 °C**	$NiC_{32}H_{28}N_6O_6$	651.29	66 Dark Green	9.01 (9.81)	59.01 (59.25)	4.33 (4.19)	12.90 (13.01)	5.3	3.28
[Cu (Inh)(OAc)] **M.P > 300 °C**	$CuC_{17}H_{15}N_3O_4$	388.04	69 Brown	16.34 (15.98)	52.51 (52.01)	3.89 (3.65)	10.81 (10.01)	5.9	1.79
[Zn (Inh)(OAc)] **M.P > 300 °C**	$ZnC_{18}H_{17}N_3O_5$	420.73	71 Dark Red	15.54 (15.10)	51.38 (52.01)	4.07 (4.16)	9.99 (10.29)	5.7	D

*Calculated values are given in parentheses.

$$\%\ \text{DPPH scavenging effect} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \times 100$$

Where

Ac = Absorbance of control (DMSO treated)&

As = Absorbance of sample.

The IC_{50} values were calculated using the EZ-Fit Enzyme Kinetics program [12].

3. RESULT AND DISCUSSION

Formation of the copper and zinc complexes can be presented by the following general equation.

$$\text{M(OAc)}_2 \bullet 2\text{H}_2\text{O} + \text{HInh} \rightarrow [\text{M(Inh)(OAc)}] + 2\text{H}_2\text{O}$$

While the formation of the complexes of Co^{+2} and Ni^{+2} can be presented by the following general equation.

$$\text{M(OAc)}_2 \bullet n\text{H}_2\text{O} + 2\text{HInh} \rightarrow [\text{M(Inh)}_2] + n\text{H}_2\text{O}$$

In both equations:

M = Ni^{+2}, Cu^{+2}, Co^{+2} and Zn^{+2}

HInh = 3-(2-(2-hydroxy-3-methoxybenzylidene) hydrazono)indolin-2-one

Synthesized complexes were stable, non-hygroscopic and insoluble in water but soluble in DMSO. Elemental analysis of the complexes revealed that the Cu^{+2} and Zn^{+2} complexes have a 1:1 metal to ligand mole ratio while complexes of Co^{+2} and Ni^{+2} have 1:2 metal to ligand mole ratio. For all complexes the ligand has acted as tri-dentate ligand and coordinated with the metal *via* phenolic oxygen, azomethine nitrogen and carbonyl group. Low values of molar conductance suggest the non-electrolyte nature of all complexes.

3.1. Structure of Ligand

In our previous work we have reported the detailed characterization of the *bis* Schiff base ligand i.e. "3-(2-(2-hydroxy-3-methoxybenzylidene)hydrazono)indolin-2-one"[6]. The structure of the ligand is shown in Figure **1**.

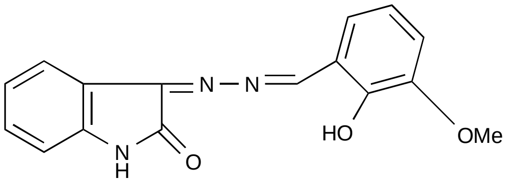


Figure 1: Structure of 3-(2-(2-hydroxy-3-methoxybenzylidene)hydrazono)indolin-2-one.

3.1. IR Spectra of Complexes

Infrared spectral data has been given in Table **2** with tentative assignments. The broad band of hydrogen bonded OH group 3000 to 3300 cm^{-1} disappeared in the infrared spectra of metal complexes which is an indication of the deprotonation and (C–O) bond formation. An increase in frequency from 25 to 48 cm^{-1} for (C-O) bonds is a clear indication of the coordination of phenolic oxygen to the metal ion. Stretching frequencies of (C=N) is decreased by 23 to

27 cm^{-1} from 1619 cm^{-1} which justifies the involvement of aldimin nitrogen in the coordination. The band corresponds to (C=O) shifted to lower frequency by 42 cm^{-1} in the spectra of metal complexes providing evidence for coordination by carbonyl oxygen. The vibration characteristics of the ring (NH, and C=N) however, remained unaltered which indicates that these groups did not participate in the coordination.

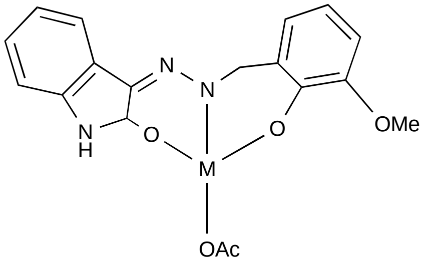


Figure 2: Proposed structure of Cu^{+2} and Zn^{+2} Complexes.

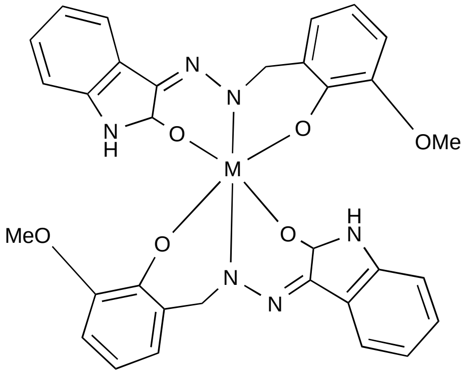


Figure 3: Proposed structure of Co^{+2} and Ni^{+2} Complexes.

The non-ligand peaks appearing in the regions of 519 – 535 cm^{-1} and 450 – 460 cm^{-1}can be assigned to (M–O) and (M–N) respectively. Therefore form the infrared spectra of metal complexes it is clear that the *bis* Schiff base ligand coordinated to the metal ions in tridentate manner though the deprotonated phenolic oxygen, aldimin nitrogen and carbonyl oxygen. The Figures **2** and **3** shows the proposed structures of the complexes.

3.2. Electronic Spectra of Complexes

Two absorption bands in between 26315 cm^{-1}– 31250 cm^{-1} are exhibited in the ultraviolet spectrum of ligand which is assignable to $n–\pi^*$ transitions of the aldimine and ketimine moieties respectively. UV spectrum of Co^{+2} complex shows bands at 9345 cm^{-1}, 20367 cm^{-1} and 29070 cm^{-1} corresponding to v_1, v_2 and v_3 transitions respectively, which can be attributed to ^4T_1g (F) → ^4T_2g (F) (v_1); ^4T_1g (F) → ^4A_2g (F) (v_2); ^4T_1g (F) → ^4T_1g (P) (v_3) transitions which are the characteristic for high spin octahedral Co^{+2} complex [2]. The magnetic moment value of Co^{+2} complex is 4.76 which is also well in agreement with the octahedral geometry[1].

UV spectrum of Ni^{+2} complex shows three bands at 10415cm^{-1}, 19569cm^{-1}, and 28986 cm^{-1} which can be attributed to the ^3A_2g → ^3T_2g (v_1); ^3A_2g → ^3T_1g (F) (v_2) and ^3A_2g → ^3T_1g (P) (v_3) respectively, which clearly indicating octahedral geometry around Ni^{+2} ion. Magnetic moment value of 3.28 of the Ni^{+2}complex is also consistent with the octahedral geometry.

UV spectrum of the Cu^{+2} complex displays two prominent bands at 18762cm^{-1}and 26954 cm^{-1} corresponding to ^2B_1g →^2A_1g and^2B_1g →^2E_1g transitions supporting square planer geometry around the Cu^{+2} ion. The Cu^{+2} complex showed magnetic moment 1.79 BM which is slightly higher than the spin-only value i.e. 1.73 BM expected for one unpaired electron and offers possibility for an square planner geometry of Cu^{+2} complex [3].

Table 2: Important Infrared Absorptions

HInh	[Co (Inh)$_2$]	[Ni (Inh)$_2$]	[Cu (Inh)(OAc)]	[Zn (Inh)(OAc)]	Tentative Assignments
3000-3300mb	-	-	-	-	(OH) group
3085m	3050m	3064m	3059m	3051m	(NH)Indol ring
1717s	1675s	1683s	1682s	1680s	(C=O)
1651m	1649m	1648m	1639m	1641m	(C=N) ring
1621s	1599m	1601m	1602s	1603m	(C=N) aldimin
1355s	1377s	1373s	1374s	1368s	(C-O) pheolic
1067m	1097m	1089m	1096m	1097m	(N-N) hydrazine
-	448w	442w	457w	455w	(M-N)
-	519w	529w	442w	521w	(M-O)

Analytical data and molar conductance values for Zn^{+2} complex sufficiently supports the tetrahedral geometry for Zn^{+2} complex because it is well known that the Zn^{+2} generally forms tetrahedral complexes because of its d^{10} electronic configuration [1]. The absorption bands at 28490cm^{-1}and 20534 cm^{-1} can be considered in association with intra-ligand transitions only. There were no *d-d* transitions seen in the visible spectrum of the Zn^{+2} complex and it was found to be diamagnetic in nature.

3.3. Antimicrobial Activity

Antibacterial and anti-fungal activity data for ligand and its complexes is presented in the Table **3a** and **3b**. The increase in the antimicrobial activity of the metal complexes can be explained on the bases of chelation theory [13-15] along with some other important factors e.g. concentration and geometry of metal complex, nature of ligands, binding sites in the ligand, lipophilic character of the ligand etc. Cell wall is composed of lipids, polysaccharides, aminophosphonates, carbonyl and cysteinyl ligands. Polarity of the metal ion is reduced with chelation which enhance the possibilities for the interactions of the metal ion with the constituents of cell wall hence interfering with the normal cell processes ultimately causing cell death [16].

3.4. Antioxidant Activity

The DPPH method was employed to study the free radical scavenging potentials of the ligand and its complexes and a decrease in the antioxidant activity was observed for nickel complex while complexes of cobalt, copper and zinc were found to be inactive. It is a well-known fact that phenolic compounds shows antioxidant potential which is mainly depend upon the degree of hydroxylation [17], this explains the antioxidant activity of the ligand since it had OH group while very less or no activity for complexes because of the deprotonation of OH group upon chelation. The antioxidant activity results are presented in Table **4**.

CONCLUSION

In this study four new complexes of a *bis* Schiff base i.e. 3-(2-(2-hydroxy-3-methoxybenzylidene) hydrazono)indolin-2-onewith Co^{+2}, Ni^{+2}, Cu^{+2} and Zn^{+2} were synthesized, characterized and were screened for their antimicrobial and antioxidant activities. It was found that for all of the complexes the ligand coordinated with the metal center in a tridentate fashion. In the case of antimicrobial activity, the metal complexes were found more potent than the ligand while for antioxidant activity the ligands showed more activity then the metal complexes.

Table 3a: Antibacterial Activity Data

Compound	Antibacterial Inhibition Zone (mm)								
	B. subtilis			*E coli.*			*S. aureus*		
	100 µM	200 µM	400 µM	100 µM	200 µM	400 µM	100 µM	200 µM	400 µM
HInh	9	8	10	9	10	10	9	8	10
[Co (Inh)$_2$]	0	11	13	11	15	14	12	12	16
[Ni (Inh)$_2$]	11	14	20	12	16	18	11	11	18
[Cu (Inh)(OAc)]	13	17	26	11	15	15	10	10	17
[Zn (Inh)(OAc)]	12	18	21	14	19	19	14	14	19
Streptomycin	19	24	35	17	22	26	18	21	29

Table 3b: Antifungal Activity Data

Compound	Zone of Inhibition against Fungi (mm)					
	T. viride			*A. niger*		
	100 µM	200 µM	400 µM	100 µM	200 µM	400 µM
HInh	6	11	13	11	17	18
[Co (Inh)$_2$]	9	14	19	13	17	24
[Ni (Inh)$_2$]	10	17	18	12	20	22
[Cu (Inh)(OAc)]	8	13	19	19	21	26
[Zn (Inh)(OAc)]	7	16	17	12	19	28
Nystatin	12	24	31	17	27	37

Table 4: Antioxidant Activity Data

Compound	IC_{50}	% Inhibition
	μM	100 μM
HInh	192	25.37 ± 0.39
[Co $(Inh)_2$]	N.A	N.A
[Ni $(Inh)_2$]	356	7.75 ± 0.41
[Cu (Inh)(OAc)]	N.A	N.A
[Zn (Inh)(OAc)]	N.A	N.A
BHA	45	87.04 ± 0.37

*N.A = not active.

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