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Structural Investigation and Antibacterial Activity of Cu(II), Co(II), Ni(II) and Zn(II) Complexes of a Schiff Base Derived from Salicylaldehyde and Thiosemicarbazide

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Authors' contributions

This work was carried out in collaboration among all authors. Author TP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MFH, MKEZ, MMH and RZ managed the analyses of the study. Author MAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The metal (II) complexes, Na₂[ML₂] [M = Cu, Co, Ni, and Zn], of salicylaldehyde thiosemicarbazone (L) containing a trifunctional ONS-donor system have been synthesized and characterized on the basis of physicochemical data by elemental analysis, magnetic moment, molar conductance, and spectroscopic (electronic, IR and ESI-MS). The ligand functions as ONS tridentates producing octahedral complexes. All compounds showed significant antibacterial activity in different ranges against gram positive (*Bacillus subtillus*) and gram negative (*Escherichia coli*) bacteria.

Keywords: Schiff bases; thiosemicarbazone; complexation; antibacterial activity.

1. INTRODUCTION

Thiosemicarbazones have been the subject of several studies because of their variable bonding

modes, encouraging biological implications and structural variety [1,2]. The preparation of the transition metal complexes with thiosemicarbazone ligands has been gaining

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substantial attention due to the potentially chemotherapeutic properties of both ligands and complexes as antitumor and antibacterial agents [3]. It was reported that their dibasic tridentate fashion with ONS donors are of enormous importance as they own a wide spectrum of medicinal properties [4]. Thiosemicarbazone derivatives are of specific chemical and medicinal importance for the possession of a number of different biological activities: antiviral, anticancer, antibacterial, antifungal activity, etc. Pharmacological activity studies of [5.6]. thiosemicarbazone as antitumor agent is one of the most favorable areas of its research [7]. Salicylaldehyde thiosemicarbazone and its derivatives are a class of flexible tridentate ONS donors capable of stabilizing the higher and lower oxidation state of transition metal ions [8]. Thiosemicarbazone metal complexes differs from the free ligand with respect to their biological properties [9]. Although the thiosemicarbazone, produced by the condensation method with ohydroxyl carbonyl compounds, show biological activity, the metal complexes often manifest effect of a larger order of magnitude compared to the corresponding ligand [10]. A predominantly significant feature is the reduction of the cytotoxic action of thiosemicarbazone, which may be reduced by complexing to the metal cation [11]. Preparation of transition metal complexes with thiosemicarbazone ligands received considerable attention of researchers because of the potentially valuable chemotherapeutic properties of thiosemicarbazone itself, which may be possible by complexing in terms of anticancer and antibacterial activity [1]. Salicylaldehyde thiosemicarbazone is typically expected to bind to a metal center via dissociation of two acidic protons, as a dianionic tridentate ONS donor forming stable chelate [12]. Here, we report the syntheses, as well as the spectral and structural characterization of Cu(II), Co(II), Ni(II) and Zn(II) complexes with salicylaldehyde thiosemicarbazone. Antibacterial activities of the synthesized compounds are also tested and reported.

2. EXPERIMENTAL

2.1 Materials and Methods

All chemicals used were commercially available with analytical grade of purity and used without further purification. FT-IR spectra were obtained on a FT-IR spectrophotometer [JASCO, FT-IR/4100 Japan], using KBr pellets. ESI-MS spectra were recorded with an Agilent

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Technologies MSD SL Trap mass spectrometer with ESI source coupled with an 1100 Series HPLC system for the confirmation of molecular formulas of compounds. The UV/Visible spectra were obtained from PerkinElmer Lambda 25 spectrophotometer. Elemental analyses for CHN were performed using a Vario EL cube [Germany elements (Elemental) analysis system]. The melting points of prepared compounds were determined using a digital melting point apparatus (METTLER TOLEDO). A Sherwood Scientific MX Gouy magnetic susceptibility apparatus was used to determine the magnetic moments of the metal complexes. Molar conductivities of freshly prepared 1.0×10⁻³ mol/dm⁻³ DMSO solutions of the synthesized compounds were measured using Jenway 4010 conductivity meter.

2.2 Synthesis of Ligand, Thiosemicarbazone, L

Thiosemicarbazide (0.01 mol; 0.91 g) was dissolved in ethanol (40 mL) by refluxing at 90°C and ethanolic solution (40 mL) of the salicylaldehyde (0.01 mol; 0.89 mL) was added. The reaction mixture was refluxed for five hours at 90°C. The volume of reaction mixture was reduced and then cooled on ice water bath. The final products of salicylaldehyde thiosemicarbazone were precipitated out. The products were recrystallized in ethanol.

2.3 Synthesis of Complexes

Ethanolic solution (30 mL) of the thiosemicarbazone ligand, L(1 mmol) was added to the solution of metal acetate salt (0.5 mmol) in ethanol (30 mL) and the reaction mixture was refluxed for 5 hours. Volume of the resulting solution was reduced to 20 mL at rotary vacuum evaporator and the solution was left overnight. The precipitation was formed by adding the equimolar amounts of aqueous solution of NaCl. The resulting products were filtered under suction, washed with ethanol and ether, and dried in vacuum.

2.4 Metal Weight Estimation

A known quantity of metal complex was put into a conical flask whose weight was known. Then, concentrated H_2SO_4 (500 µL) was added. It was fumed by heating until dry and this fuming process was repeated three times. Concentrated HNO₃ (500 µL) and HClO₄ (500 µL) were then added and the mixture was further fumed until dry. The process of adding acids and fuming was continued until there was no black material. 100 mL distilled water was added to dissolve the residue. Finally, the weight of the metal was estimated complexometrically using EDTA (Ethylenediamine tetra acetic acid) [13] and gravimetrically using DMG (Dimethylglyoxime) [14].

2.5 Antibacterial Activity

The antibacterial screening test was performed by the disk diffusion methods [15,16]. Whatmann No.1 filter paper discs (6 mm diameter) were sterilized by autoclaving for one hour at 120°C. The sterile discs were placed on the surface of the cold solid medium in Petri-dishes inoculated with the microorganism and then incubated at 28°C. The inhibition of the microbial growth was evaluated after 24 hours.

3. RESULTS AND DISCUSSION

The results of the elemental analysis and some properties of the physical synthesized compounds are given in Table 1. Condensation of salicylaldehyde with thiosemicarbazide readily gives rise to the corresponding ons donor ligand I (scheme 1) which was easily identified by ir and esi-ms spectra. Metal complexes were obtained upon reaction between metal ions with ligand I at 1:2 molar ratio. The ligandlon reaction with metal acetate m(oac)₂ where m is cu, co, ni, and zn, yield complexes corresponding to the general formula: na₂[m(l)₂]. The analytical data are in a good agreement with the proposed stoichiometry of the complexes.



Scheme 1. Synthesis of ligand, L



Scheme 2. Synthesis scheme for complexes Na₂[M(L)₂]

	Table 1. Eleme	ntal analyses and	physical data	of the ligand, L a	and its complexes
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Compounds	Formula	mp/°C	Color	Content[(calculated)found] An		Λm		
	weightg/mol			% C	% H	% N	% M	μS
L	195.24	220	White	(49.21)	(4.65)	(21.52)	-	18
C ₈ H ₉ N₃OS				49.04	4.48	21.36		
Na ₂ [Cu (L) ₂]	495.98	>300	Red-	(38.75)	(2.85)	(16.94)	(12.81)	226
$C_{16}H_{14}CuNa_2N_6O_2S_2$			Brown	38.42	2.76	16.73	12.68	
Na ₂ [Co (L) ₂]	491.36	>300	Orange	(39.11)	(2.87)	(17.10)	(11.99)	220
$C_{16}H_{14}CuNa_2N_6O_2S_2$				39.02	2.74	16.93	11.78	
Na ₂ [Ni (L) ₂]	491.12	>300	Green	(39.13)	(2.87)	(17.11)	(11.95)	212
$C_{16}H_{14}NiNa_2N_6O_2S_2$				39.03	2.76	17.01	11.86	
Na ₂ [Zn (L) ₂]	497.81	>300	White	(38.60)	(2.83)	(16.88)	(13.14)	208
$C_{16}H_{14}ZnNa_2N_6O_2S_2$				46.82	5.28	14.79	13.02	

3.1 Molar Conductivities of the Compounds

The molar conductivity values (Table 1) for the compounds in DMSO solvent $(1.0 \times 10^3 \text{ mol})$ were in the electrolytic range of 18-226 µs. Conductivity measurements have commonly been used to know the structural arrangement of metal chelates (mode of coordination) within the limits of their solubility. It is a method of testing the degree of ionization of the complexes. It is clear from the conductivity data, compared with the values of free ligand that the complexes under investigation are electrolytes.

3.2 FT-IR Spectra

In order to know the mode of coordination, the most significant ir spectral frequencies for the metal complexes are compared with those of free ligand. A peak appeared at 1616 cm⁻¹ due to the azomethine c=n stretching frequency of the free ligand, I is shifted to lower frequency in the spectra of the complexes at 1605-1612 cm⁻¹ indicating the coordination through azomethine n atom to metal ions. Strong absorption band due to phenolic c-o stretching in the spectrum of ligand positioned at 1266 cm⁻¹ after coordination is shifted to 1276 - 1330 cm⁻¹ [17], which corresponds to forming of weaker c-o(m) bond comparing to c-o(h) and confirms coordination of ligand to m(ii) via deprotonated phenolic oxygen.moreover, in the ir spectrum of the ligand, the distinctive vibration of the (oh) band is observed at 3444 cm⁻¹. The absence of this band in the ir spectra of the complexes indicates the coordination through the phenolic oxygen [2]. The ligand showed a peak at 777 cm⁻¹ for the vibration of the c=s double bond [18,19]. The c=s band isvanished in the complexes [1] and a new band due c-s bond stretching appeared at 742-759 cm⁻¹. This confirms that the other coordination site ofmetal ions is through thiolate sulphur [3]. The medium intensity band in the region 562–610 cm⁻¹ is ascribed to m–n, in the region 460–580 cm⁻¹ is attributed to m–o, and in the region 400 - 520 cm⁻¹ is attributed to m-s bonds vibration [4].

3.3 ESI-MS Spectra

ESI-MS analysis can provide important information concerning the structure, stoichiometry, and metal oxidation state of dissolved metal complexes [20,21]. The ESI-Mass spectra of the ligand and complexes are presented in Fig. 5. The obtained m/z values are similar to the formula weight (Tables 1 and 3) which further supports the proposed structure of the synthesized compounds.

3.4 Magnetic Measurements and Absorption Spectra

The electronic spectral data are often useful in the authentication of results provided by other methods of structural examination. The assignments of the important electronic spectral bands of ligand and its metal complexes are shown in Table 4. The absorption at 259 nm in the spectrum of the ligand arises from $\pi \rightarrow \pi^*$ transition [22]. Another absorption at 309 nm in the spectrum of the free ligand arises from a combination of the n→π* transitions accompanying with the azomethine group of the thiosemicarbazone moiety [23,24]. A second $n \rightarrow \pi^*$ band arises from the thioamide portion of the thiosemicarbazone moiety is shown at 340 nm in the spectrum of the ligand.

The observed magnetic moments of Cu(II) 1.86 complex is at room temperature corresponding to one unpaired electron. indicative of octahedral geometry. The UV-vis spectrum of Cu (II) complex showed three bands at 270 nm, 330 nm and 397 nm assigned to $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ and C.T (M \rightarrow L) transitions respectively. From these electronic absorption bands and paramagnetic nature, octahedral geometry [25] may be suggested for Cu(II) complex studied in the present investigation.

The Co(II) complex exhibit magnetic moment value 4.56 B.M. The Co(II) complex in the present investigation showed bands at 264, 342, 413 and 431nm for $\pi \rightarrow \pi^{*}$, $n \rightarrow \pi^{*}$ and C.T (M \rightarrow L) and d \rightarrow d transitions respectively.These transitions and observed magnetic moment value indicated high spin octahedral geometry of the complex [26,27].

In the present study, Ni(II) complex of ligand L shows magnetic moment 2.92 B.M. The observed bands in the electronic spectra at 272, 372 and 408 nm are assigned to $\pi \rightarrow \pi^{*}$, $n \rightarrow \pi^{*}$ and $d \rightarrow d$ transitions respectively.From the elemental analysis data, observed magnetic moment and electronic transitions, it is concluded that the Ni(II) complex in the present study has octahedral geometry.

The diamagnetic Zn(II) complex did not show any d→d bands. The complex showed bands at 261 nm, 312 nm and 389 nm assigned to $\pi \rightarrow \pi^{*}$,

 $n \rightarrow \pi^{-}$ and charge transfer (CT) transitions respectively suggesting octahedral geometry [28].

On the basis of the above observations, it is tentatively suggested that all the complexes show an octahedral geometry in which the ligand acts as tridentates.

3.5 Biological Activity

The antibacterial activity of the ligand and its complexes are summarized in Table 5. Activity of the ligand increased on coordination with different metals. Increasing in the antibacterial activities can be explained according to the formation of chelate that the less polar form of the metalloelement, which push the lipophilic character. The increased lipophilic character of chelate favors the interaction of these complexes with cell constituents, resulting in interference with normal cell processes [29]. According to overtone's concept of cell permeability, the lipid membrane that surrounds a cell favors the passage of only lipid soluble materials and liposolubility is an important factor that controls antimicrobial activity [30-32]. The widespread interaction of metal ions with cellular components is owing to the fact that the lipids and polysaccharides are vital constituents of cell wall and membranes, which are preferred for metal ion interaction. In addition to this, cell wall also contains several aminophosphates, carbonyl, and cysteinyl ligands, which maintain the integrity of the membrane by acting as a diffusion barrier and also provide suitable sites for binding. Chelation is not the only criterion for altering activities but the nature of metal ion, the nature of the ligand, the coordinating sites, and the geometry of the complex, the concentration, the hydrophilicity, the lipophilicity, and the presence of co-ligands also influence the activity [33].

Table 2. Characteristic IR spectral data of the ligand, L and its complexes (cm⁻¹)

Ligand/Complex	v (C=N)	v (C-O)	v (C=S)	v (C-S)	v (M-N)	v (M-O)	v (M-S)
L	1616	1266	777	-	-	-	-
Na ₂ [Cu (L) ₂]	1612	1276	-	742	562	548	476
Na ₂ [Co (L) ₂]	1605	1292	-	757	589	470	400
$Na_2[Ni(L)_2]$	1606	1330	-	753	610	580	520
$Na_2[Zn(L)_2]$	1609	1283	-	759	572	460	415

Base peak (m/z)	Species	Base peak (m/z)	Species	Base peak (m/z)	Species
195.1652	[C ₈ H ₉ N ₃ OS] ⁺	449.2364	[Cu (L) ₂] ²⁻	445.2364	[Co (L) ₂]
445.1216	[Ni (L) ₂] ²⁻	451.0965	[Zn (L) ₂] ²⁻		

Table 3. ESI-MS data of the compounds

Table 4. Magnetic moments and electronic spectral data of the ligand L and its metal
complexes

Compounds	λ _{max} (nm)	Wavenumber (cm ⁻¹)	μ _{eff} (Β.Μ)	Assignment
	259	38610	-	π→π_
L	309	32362		n→π [*]
	340	29411		n→π [*]
	270	37037		π→π [*]
Na ₂ [Cu (L) ₂]	330	30303	1.86	n→π [*]
	397	25188		C.T(M→L)
	264	37878		$\Pi \rightarrow \Pi^{*}$
	342	29239	1 56	n→π [*]
	413	24213	4.30	C.T(M→L)
	431	23201		d→d
	272	36764		$\pi \rightarrow \pi^{*}$
Na ₂ [Ni (L) ₂]	372	26881	2.92	n→π [*]
	408	24509		d→d
	261	38314		$\pi \rightarrow \pi^{*}$
Na ₂ [Zn (L) ₂]	312	32051	0.337	n→π [*]
_	389	25706		C.T

Compound	Zone of inhibition diameter in mm			
	Gram +ve	Gram -ve		
	Bacillus subtillus	Escherichia coli		
L	6	7		
Na[Cu (L) ₂]	16	16		
$Na[Co(L)_2]$	15	13		
Na[Ni (L) ₂]	11	10		
$Na[Zn (L)_2]$	9	9		
DMSO	0	0		

Table 5. The antibacterialactivity of salicylaldehyde thiosemicarbazone, L and its metal complexes



Fig. 1. IR spectra of salicylaldehyde semicarbazone, L



Fig. 2. IR spectra of complex,Na₂[Cu(L)₂]

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Fig. 5. IR spectra of complex, Na₂[Zn(L)₂]





Fig. 6. ESI-MS Spectra of (a) Ligand (b) Copper complex (c) Cobalt complex (d) Nickel complex and (e) Zinc complex



Fig. 7. UV-vis spectra of the synthesized compounds

4. CONCLUSION

A number of metal(II) complexes, $Na_2[ML_2]$ (M = Cu, Co, Ni, and Zn) with the trifunctional ONSdonor atoms have been synthesized and characterized on the basis of elemental analysis, magnetic moments, molar conductance and spectroscopic (electronic, IR and ESI-MS) data. The ligand behaves as ONS tridentate to form octahedral structure. The resulting antibacterial screening data indicates that the complexes are better inhibitor than the ligand against *Bacillus subtillus* and *Escherichia coli*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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