

Microwave Assisted Synthesis, Characterisation and Biological Activities of Cu(II) Complexes of Few Thiosemicarbazones Ligands

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Abstract

Thiosemicarbazones are class of compounds which are obtained by the condensation of thiosemicarbazide with carbonyl compounds. The transition metal complexes of thiosemicarbazones are widely considered due to their broad spectrum of biological activities such as antibacterial, antitumor and antifungal activities. In present research work a series of four novel Cu(II) aryl thiosemicarbazones complexes having the general composition $[Cu(L_1)_2Cl_2]$, $[Cu(L_2)_2Cl_2]$, $[Cu(L_3)_2Cl_2]$ and $[Cu(L_4)_2Cl_2]$ {where L_1 = 4-nitroacetophenone thiosemicarbazones (4NAT), L_2 = 3-nitrobenzaldehyde thiosemicarbazone (3NBT), L_3 = 4-hydroxybenzaldehyde thiosemicarbazone (4HBT) and L_4 = 4-aminoacetophenone thiosemicarbazone (4AAT)} have been synthesized by the reaction of thiosemicarbazide with substituted aromatic aldehydes and ketones by conventional heating as well as microwave irradiations method followed by complexation with Cu(II) metal. The synthesized compounds have been characterised by elemental analysis, melting point determination, FTIR, UV-visible spectral analysis. Thiosemicarbazone ligands behave as bidentate ligand and forms octahedral complexes with Cu(II). The synthesized ligands and their new metal complexes have been screened in vitro for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* bacteria. The result shows that all the Cu(II) complexes are potent antibacterial agents against these bacteria.

Keywords: Thiosemicarbazones; Microwave irradiation; Cu(II) complexes; Antibacterial

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1. Introduction

The synthesis and chemical investigation of thiosemicarbazones and their transition metal complexes are of considerable interest due to their pharmacological properties, such as antibacterial, antiviral, antifungal, antitumor, antimalarial, trypanocidal, anti-inflammatory

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and antiparasitic activities [1-18]. Thiosemicarbazone ligands generally behave as bidentate ligands coordinating metal ions through sulphur atom and hydrazino nitrogen atom while some transition metal complexes have been reported in which they bonded to metal ion only through sulphur atom. In certain cases thiosemicarbazones also act as multidentate ligands if donor groups are also present in the parent aldehyde or ketone moiety [19]. It is well known that N and S atoms play important roles in the coordination of metal ions at active sites of numerous metallobiomolecules [20]. Interest in metal complexes with thiosemicarbazones ligands has been stimulated because biological activities are often enhanced on complexation [21]. With the growing interest of thiosemicarbazones the present work was undertaken in order to investigate the ligational behavior of the thiosemicarbazone towards Cu(II) metal ion as well as their biological activity in inhibiting the growth of some pathogenic bacteria [22].

2. Materials and Methods

All the chemicals and solvents used were of AR grade and procured from Sigma-Aldrich, USA and E Merck, Germany and used as received. Purity of synthesized compounds has been checked by thin layer chromatography. IR spectra are recorded on Bruker Optic Model Alpha (FT-IR) (Zn-Se Optics, ATR) ($4000\text{-}500\text{ cm}^{-1}$) using KBr disc. Magnetic susceptibility measurements were carried out on the vibrating sample magnetometer (VSM) model 155 at 5500 Gauss field strength. Microwave synthesis was carried out in domestic microwave oven Model KENSTAR-OM20ACF, 2450MHz, 800W and GMBR (Green Microwave Biochemical Reactor) at GCRC, P.G. Dept. of Chemistry, Govt. Dungar College (NAAC-A-Grade), MGS University Bikaner, Rajasthan. ECIL Double Beam UV-Visible Spectrophotometer, model UV 5704SS, with quartz cell of 10 mm light path was used for absorption measurement. All biological activities have been carried out with horizontal laminar at BIFR, Bikaner.

2.1. Preparation of ligands

Two different routes were used for the synthesis of ligands.

2.1.1. Microwave irradiation method

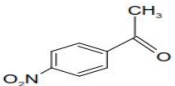
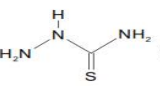
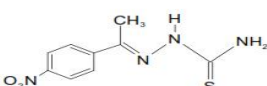
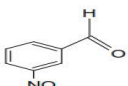
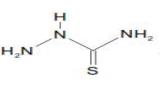
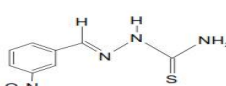
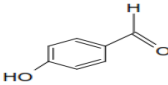
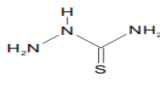
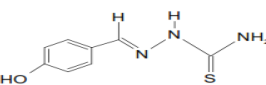
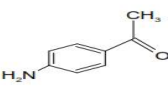
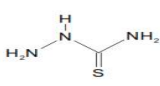
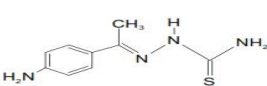
Four ligands i.e. L_1 = 4-nitroacetophenone thiosemicarbazone (4NAT), L_2 = 3-nitrobenzaldehyde thiosemicarbazone (3NBT), L_3 = 4-hydroxybenzaldehyde thiosemicarbazone (4HBT) and L_4 = 4-aminoacetophenone thiosemicarbazone (4AAT) were synthesized. In a typical preparation water or water alcohol mixture of thiosemicarbazide (0.01 mol) and aldehyde or ketone (0.01 mol) were taken in Erlen Meyer flask capped with a funnel placed in a microwave oven and irradiated at 200 watt for 2-10 min. The reaction was monitored by thin layer chromatography (TLC). After

completion the reaction, the reaction mixture was allowed to attain room temperature and solid separated was filtered. The crude product was recrystallized from redistilled ethanol.

2.1.2. Thermal method

For comparison purposes, the above ligands were also synthesized by the thermal method, where instead of a few drops of alcohol, a hot ethanolic solution (25 mL) of aldehyde or ketone (0.01 mol) was mixed to a hot ethanolic refluxing solution (30 mL) of thiosemicarbazide (0.01 mol) in a 1:1 molar ratio. The contents were refluxed for about 6-10 h in a water bath. The solution was then concentrated under reduced pressure, which upon cooling gave crystalline precipitates. The products were washed in alcohol and recrystallized in the same solvent. The structures of ligands are shown in (Table 1). A comparison between the thermal method and microwave method is given in (Table 2).

Table 1. Thiosemicarbazone ligands.

Carbonyl compound	Thiosemicarbazide	Ligands
 4-Nitroacetophenone	 Thiosemicarbazide	 4-Nitroacetophenone thiosemicarbazone
 3-Nitrobenzaldehyde	 Thiosemicarbazide	 3-Nitrobenzaldehyde thiosemicarbazone
 4-Hydroxybenzaldehyde	 Thiosemicarbazide	 4-Hydroxybenzaldehyde thiosemicarbazone
 4-Aminoacetophenone	 Thiosemicarbazide	 4-Aminoacetophenone thiosemicarbazone

2.2. Preparation of complexes

Two different methods were employed for the synthesis of the metal complexes with each of the ligands. A comparison between the thermal method and microwave method has been given in Table 1.

2.2.1. *Microwave irradiation method*

For the preparation of various complexes, a slurry of ligands (i.e. 4NAT, 3NBT, 4HBT, 4AAT) (0.2 mmol) was prepared in water or in water-ethanol mixture. In this, solution of CuCl_2 (0.01 mmol in 30 mL ethanol) was added. The resulting mixture was irradiated in a microwave oven for 2 to 10 min at medium power level (600 W) maintaining the occasional shaking. The mixture was cooled to room temperature and poured into ice chilled methanol and dried in vacuum over P_2O_5 [23].

2.2.2. *Thermal method*

The complexes were also synthesized by the thermal method. In this method hot ethanolic solution (20 mL) of the corresponding ligand (0.02 mmol) and hot ethanolic solution (20 mL) of the CuCl_2 (0.01 mmol) were mixed with constant stirring. The reaction mixture was refluxed after adding 4-5 drops of glacial acetic acid for 8-10 h at 80-90 °C. On cooling coloured complexes were precipitated out. They were filtered, washed with 50 % ethanol and dried in vacuum desiccators and recrystallized in ethanol. The obtained solid metal complexes and their colours are shown in Table 2.

3. **Results and Discussion**

Ligands and complexes were identified on the basis of elemental analysis and spectral studies. Color, yield and elemental analysis data are represented in Table 2.

Table 2. Physico-chemical data of ligands and their Cu(II) complexes (C.M. = conventional method, M.M. = microwave method).

S.N	Compound	Color	Reaction period		Yield %		Elemental Analysis		
			CM (h)	MM (min)	CM	MM	Calculated (Found) %		
							C	H	N
1	4NAT	Pale yellow	6	4.5	60	72	45.38 (45.19)	4.20 (4.08)	23.53 (23.29)
2	3NBT	Light Brown	6.5	4	62	69	42.86 (42.77)	3.58 (3.49)	25.00 (25.02)
3	4HBT	Dark Yellow	8	7.5	48	62	49.23 (49.14)	4.62 (4.53)	21.54 (21.46)
4	4AAT	Dark Brown	7	5	65	70	51.92 (51.88)	5.77 (5.62)	26.92 (26.83)
5	$[\text{Cu}(\text{4NAT})_2\text{Cl}_2]$	Orange	7.5	4	62	76	35.38 (35.29)	3.28 (3.17)	18.35 (18.26)
6	$[\text{Cu}(\text{3NBT})_2\text{Cl}_2]$	Light Brown	6	4.5	69	81	32.97 (32.94)	2.75 (2.64)	19.23 (19.18)
7	$[\text{Cu}(\text{4HBT})_2\text{Cl}_2]$	Off White	7	6.5	66	79	36.60 (36.83)	3.43 (3.37)	16.02 (16.01)
8	$[\text{Cu}(\text{4AAT})_2\text{Cl}_2]$	Light Brown	8.5	4.5	56	72	39.24 (39.19)	4.36 (4.23)	20.35 (20.23)

3.1. Infrared spectra

The binding mode of the ligand to metal ions was further elucidated by analysis of the IR spectra of the ligands and metal complexes (Table 3). A study and comparison of infrared spectra of free ligand and its metal complexes imply that the ligand behaves as bidentate and the metal ion is coordinated through the azomethine nitrogen and the thione sulphur. A band in the range 1660 - 1600 cm^{-1} in the IR spectra of the ligands is due to $\nu(\text{C}=\text{N})$ [24]. Coordination of azomethine nitrogen in complexes is suggested by the shift of $\nu(\text{C}=\text{N})$ band to lower frequencies. The absence of a $\nu(\text{S}-\text{H})$ absorption in the region 2700-2500 cm^{-1} is considered as evidence that the thione form of the ligands exist in the solid state [25-26]. The intensity of the medium band in the range 1160-1060 cm^{-1} assigned for $\nu(\text{N}-\text{N})$ in spectrum of the ligands is remains unchanged in all the spectra of the complexes, however, it shifted to the higher frequency. The strong band observed in the range 880-820 cm^{-1} assigned for $\nu(\text{C}=\text{S})$ in spectrum of the ligands is shifted towards lower frequency and occurred at 830-810 cm^{-1} in the corresponding spectra of the metal complexes indicating the coordination of the thione sulphur to metal atom [27,28]. The bands in the range 3200-3150 cm^{-1} in ligands are due to NH vibration. In all the complexes, the presence of a band in this region corresponds to NH vibration which indicates that the ligand is coordinated in the neutral form.

Table 3. Significant infrared spectral bands (cm^{-1}) of the ligands and its metal complexes.

Compounds	$\nu(\text{C}=\text{N})$	$\nu(\text{N}-\text{N})$	$\nu(\text{C}=\text{S})$	$\nu(\text{N}-\text{H})$	$\nu(\text{M}-\text{N})$	$\nu(\text{M}-\text{S})$
4NAT	1654	1078	830	3198	-	-
3NBT	1660	1067	818	3157	-	-
4HBT	1608	1165	881	3156	-	-
4AAT	1620	1098	868	3160	-	-
[Cu(4NAT) ₂ Cl ₂]	1585	1078	824	3197	448	413
[Cu(3NBT) ₂ Cl ₂]	1603	1067	811	3157	451	415
[Cu(4HBT) ₂ Cl ₂]	1604	1166	826	3156	464	428
[Cu(4AAT) ₂ Cl ₂]	1593	1099	831	3160	448	421

3.2. Magnetic moments and electronic spectra

The observed magnetic moments at room temperature of Cu(II) complexes fall in the range 1.85-1.91 BM. These values are typical of distorted octahedral geometry coordinated around Cu(II) which has one unpaired electrons. The electronic spectra of these complexes have been recorded in DMSO are shown in Fig. 1. The electronic spectra of all the Cu(II) complexes recorded were very similar to each other and consist of three bands in the regions 12300-14500 cm^{-1} , 18300-21100 cm^{-1} and 29000-31600 cm^{-1} which clearly indicated the octahedral stereochemistry of the complexes [29-31]. The band maxima and their assignments are presented in Table 4.

Table 4. Magnetic moments and electronic spectral data of Cu(II) complexes.

S. N.	Complex	μ_{eff} (BM)	Electronic Spectral bands $\lambda_{\text{max}}(\text{cm}^{-1})$	Tentative assignments	Comments
1	[Cu(4NAT) ₂ Cl ₂]	1.91	12430, 31600	${}^2E_g \rightarrow {}^2T_{2g}, {}^2B_{1g}$ $\rightarrow {}^2A_{1g}$	Distorted octahedral Geometry
2	[Cu(3NBT) ₂ Cl ₂]	1.88	12361, 20450, 29630	${}^2E_g \rightarrow {}^2T_{2g}, {}^2B_{1g}$ $\rightarrow {}^2A_{1g}$	Distorted octahedral Geometry
3	[Cu(4HBT) ₂ Cl ₂]	1.90	14452, 31546	${}^2E_g \rightarrow {}^2T_{2g}, {}^2B_{1g}$ $\rightarrow {}^2A_{1g}$	Distorted octahedral Geometry
4	[Cu(4AAT) ₂ Cl ₂]	1.85	14380, 29112	${}^2E_g \rightarrow {}^2T_{2g}, {}^2B_{1g}$ $\rightarrow {}^2A_{1g}$	Distorted octahedral Geometry

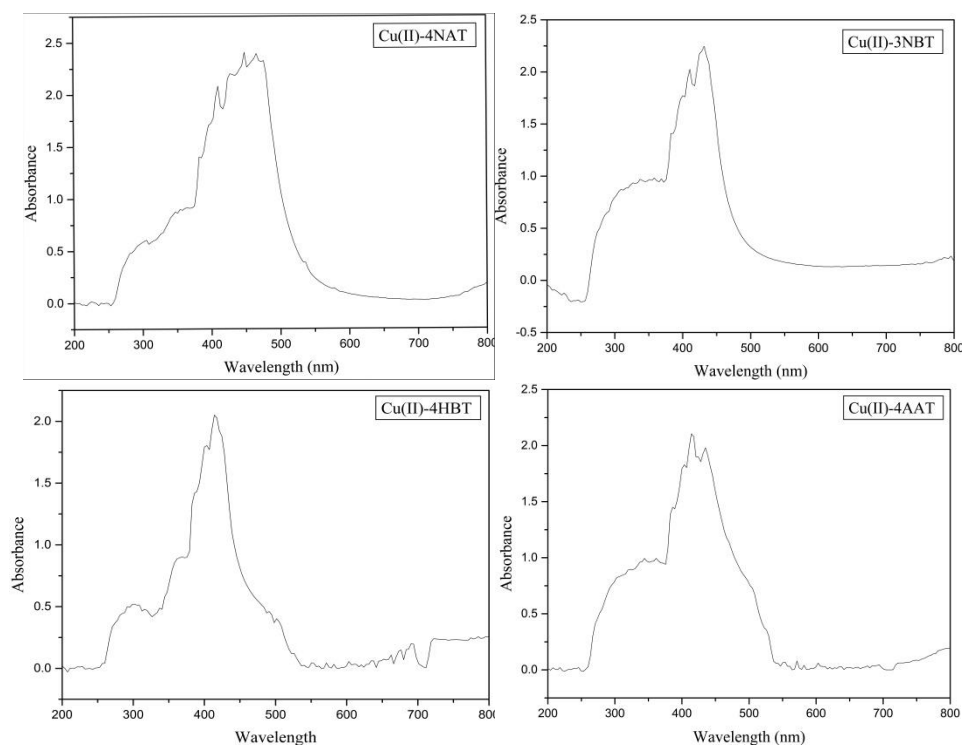


Fig. 1. Electronic spectra of Cu(II) complexes.

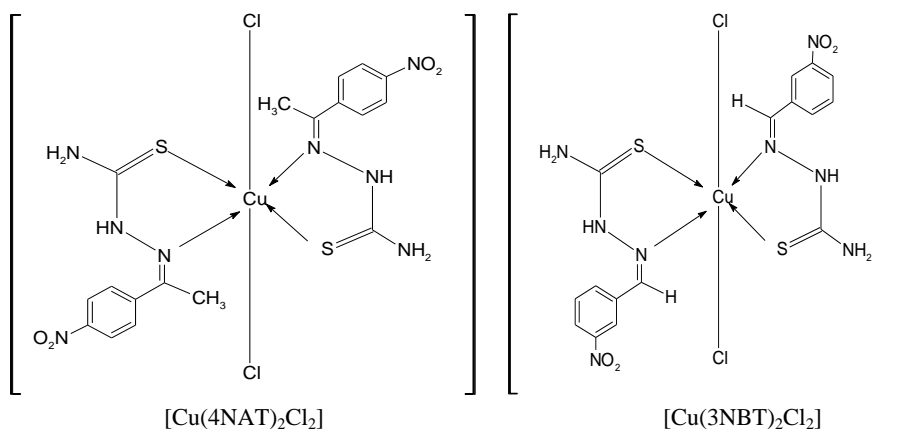
4. Biological Activity

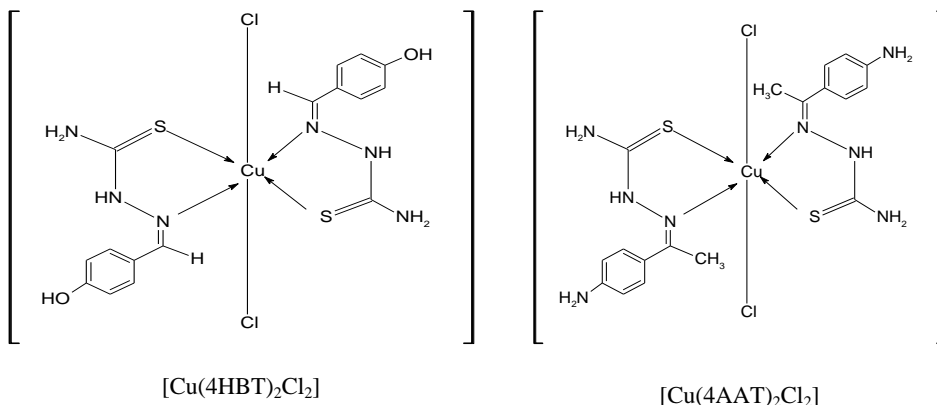
The antibacterial activities of the thiosemicarbazone ligands and their Cu(II) complexes were carried out against *E. coli*, *S. aureus* and *B. subtilis* using Muller Hinton Agar media

(Hi media) and the results were compared with standard antibiotic Kanamycin (30 µg/disc). The activity was carried out using paper disc method is represented in Table 5 which shows all the thiosemicarbazone ligands and their Cu(II) complexes have potent antibacterial activities against these bacteria. Among the various ligands 4HBT, 4AAT found most effective against all the pathogens. It was also observed that Cu(II) complexes of 4NAT and 3NBT found most effective however zone of inhibition are lesser compared to standard Kanamycin-30. Cu(II) complexes have been found more effective than their corresponding thiosemicarbazone ligands against these bacteria showing maximum clarity of zones.

Table 5. Antibacterial activity of synthesized compounds.

S. N.	Compounds	Zone of inhibition (in mm)			
		<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>	Kanamycin-30
1	4NAT	0.5	0.5	0.5	4
2	3NBT	0.5	1.0	0.5	5
3	4HBT	1.0	1.5	1.0	7
4	4AAT	0.5	5	0.5	9
5	[Cu(4NAT) ₂ Cl ₂]	1.0	2.0	3.0	10
6	[Cu(3NBT) ₂ Cl ₂]	1.0	3.0	2.5	11
7	[Cu(4HBT) ₂ Cl ₂]	2.0	1.5	2.0	9
8	[Cu(4AAT) ₂ Cl ₂]	1.5	1.5	2.0	8





5. Conclusion

Thiosemicarbazone ligands and their Cu(II) complexes were characterized by elemental analysis, spectral studies and magnetic moment measurements. On the basis of above data the thiosemicarbazone ligands appear to behave as bidentate ligand coordinating through the azomethine nitrogen and the thione sulphur atom. The results of the above studies suggest that the Cu(II) complexes probably possess a distorted octahedral geometry. The antibacterial properties of the ligands and their complexes were studied against *E. coli*, *S. aureus* and *B. subtilis* bacteria. The result shows that all the Cu(II) complexes are potent antibacterial agents against these bacteria.

Acknowledgments

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