



Synthesis, Characterization And Antibacterial Screening of Transition Metal Complexes of ω -Bromoacetoacetanilide N(4)-Methyl (Phenyl) Thiosemicarbazone

KEYWORDS

Complexes, ω -bromoacetoacetanilide N(4)- methyl(phenyl)thiosemicarbazone, antibacterial activity, zone inhibition.

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ABSTRACT *The transition metal complexes have been synthesized by refluxing the ligand, ω -bromoacetoacetanilide N(4)- methyl(phenyl)thiosemicarbazone, with metal acetates. The physico-chemical analysis of the ligands and the complexes were accomplished using magnetic-, electrical conductance-, IR-, ¹H-NMR- and electronic spectral studies. Based on magnetic measurements and various spectral studies, geometries have been assigned to the complexes. The ligand and their respective complexes were also screened for their antibacterial activity.*

INTRODUCTION

Detailed investigations on the coordination chemistry of amides and anilides are fascinating in view of their similarities to peptides and proteins, which are essential constituents of life. As ligands, thiosemicarbazones of β -keto anilides haven't received the kind of attention that they really deserve. Earlier reports on acetoacetanilide semicarbazones- and thiosemicarbazones showed that they possess antitumour activity¹. Recently, it has been shown that substitution on the 4th nitrogen of thiosemicarbazone can enhance its biological activity². We have also observed the effective substitution of acetoacetanilide changes considerably the chemical properties and ligational behavior of compounds derived from it³⁻⁵. With this view, a detailed investigation on the synthesis and characterization of ω -bromoacetoacetanilideN(4)-methyl(phenyl)thiosemicarbazone, (ω -BAacd- MPTCH₂) (L2H) and its transition metal complexes were carried out and reported.

EXPERIMENTAL

All chemicals used were of AR grade (Aldrich, E. Merck). The ligand as well as metal complexes were analyzed by standard methods. Carbon, hydrogen and nitrogen were estimated by microanalysis using Hitachi CHN-O rapid analyser at Sophisticated Test and Instrumentation Centre, Kochi.

The magnetic susceptibility measurements of the complexes were carried out on a Gouy-type magnetic balance. UV-visible spectra of the soluble complexes were recorded using their solutions (10⁻³M) in ethanol, methanol, dimethyl sulphoxide and dimethylformamide on UV-1601 Shimadzu spectrophotometer, with a scanning range of 10000 – 50000 cm⁻¹. The infrared spectra of the ligands and the complexes, in the range 4000-400 cm⁻¹, were recorded using KBr pellets on FTIR DR – 810 1A Shimadzu spectrometer. The ¹H-NMR spectra of ligands and the diamagnetic Zn(II) complexes were recorded in CDCl₃ or DMSO-d₆ by using 300 MHz, Bruker Advanced DPX spectrometer.

Preparation of ligand

A solution of N(4)- methyl(phenyl)thiosemicarbazide in ethanol (0.1 mol, 250 ml) was added to ω -bromoacetoacetanilide in ethanol (0.1 mol, 250 ml) and refluxed for 3 h. The solution obtained was evaporated, cooled and stirred several times with petroleum ether. The solid product that separated was filtered and kept in a desiccator under reduced pressure over anhydrous calcium chloride. The product was recrystallised from acetone (Yield : 40% , M.P = 110°C).

Preparation of the complexes

To the ethanolic solution of the ligand (0.005 mol in 50 ml) added a methanolic solution of metal acetate (0.0025 mol in 25 ml) and stirred for 1 h. The solid complex formed was filtered, washed with water and finally with methanol. The products were dried over anhydrous calcium chloride. Yield and M.P. of the complexes were noted.

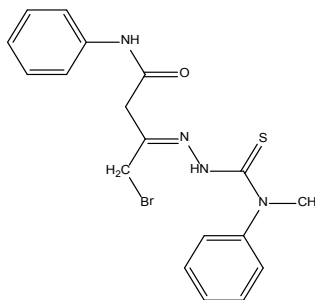
RESULTS AND DISCUSSION

Using the data obtained from the analytical and physico-chemical studies, the structure and geometries of the complexes were discussed.

CHARACTERIZATION OF THE LIGAND

The ligand, ω -bromoacetoacetanilide N(4)-methyl(phenyl) thiosemicarbazone (ω BAacdMPTCH₂)(L2H) was soluble in methanol, ethanol, etc. It was characterized by elemental analysis electronic- IR- and ¹H NMR spectral studies. The ligand was subjected to elemental analysis on a CHN-O instrument .The experimentally found out- and calculated percentages of C, H, N and S were in good agreement confirming its molecular formula as C₁₈H₁₉N₂OSBr. The electronic spectrum of the ligand showed an intense band at 268 nm (37313 cm⁻¹) which can be attributed to $\pi \rightarrow \pi^*$ transition. Similarly a band at 412 nm (24271 cm⁻¹) in the ligand spectrum may be attributed to n $\rightarrow \pi^*$ transition. IR spectral data of the ligand with the probable assignments are made in comparison with the spectra of similar type of compounds⁶. The broad bands in the spectrum

of the ligand in the higher frequency region may be due to $\nu_{\text{N-H}}$ (3500-3300 cm^{-1}). A medium band at 1676 cm^{-1} in the spectrum may be assigned to $\nu_{\text{C=O(amide)}}$ and a band at 1598 cm^{-1} may be assigned to $\nu_{\text{C=N}}$. The $\nu_{\text{N-N}}$ band in the ligand spectrum appears at 1020 cm^{-1} . The strong band at 503 cm^{-1} in the spectrum of the ligand may be assigned to $\nu_{\text{C-Br}}$. The strong band at 1075 cm^{-1} in the ligand spectrum may be assigned to $\nu_{\text{C-S}}$. The ^1H NMR spectrum of the ligand shows two singlets at 10.23 and 6.8 ppm which may be assigned to NH protons of anilide ring and thiosemicarbazide moieties, respectively. The multiplets observed at 7.42-7.30 ppm and 7.20-7.10 ppm may be assigned to aromatic ring of anilide part and the phenyl ring of the substituted thiosemicarbazide moiety, respectively. The singlets observed at 3.64 and 3.42 ppm may be assigned to $\alpha\text{-CH}_2$ and $\omega\text{-CH}_2$ protons, respectively. The singlet observed at 2.72 ppm may be assigned to CH_3 group of the substituted thiosemicarbazide. The structure of the ligand is shown in Figure 1.



(Z)-4-bromo-3-(2-(methyl(phenyl)carbamothioyl)hydrazono)-N-phenylbutanamide

CHARACTERIZATION OF THE COMPLEXES

The ligand, (ω -BAacdMPTCH₂) (L2H) formed stable complexes with transition metal ions. The complexes were soluble in DMSO, DMF, etc. They were characterized as follows :

Elemental analysis

The analytical data and physical properties of the ligand (ω -BAacd MPTCH₂) (L2H) and its complexes are listed in Table 1. The complexes were found to have general formulae $[\text{Co}(\text{LH})_2]$, $[\text{NiL}(\text{H}_2\text{O})_3]$ and $[\text{ML}(\text{H}_2\text{O})]$, where $\text{M}=\text{Cu}(\text{II})$, $\text{Zn}(\text{II})$ or $\text{Cd}(\text{II})$ and LH^- is the monovalent anion- and L^{2-} is the bivalent anion of L2H.

Table 1.
Physico-chemical and analytical data of the ligand and its complexes

Compound	Colour	Yield (%)	MP ($^{\circ}\text{C}$)	Elemental Analysis (%) found (calculated)				
				C	H	N	S	Metal
Ligand	Colour less	40	110	48.99 (51.60)	4.22 (4.55)	12.53 (13.39)	8.01 (7.65)	--
$[\text{Co}(\text{LH})_2]$	Dark brown	65	282	49.20 (48.20)	3.10 (3.79)	13.60 (12.50)	7.91 (7.15)	6.89 (6.58)
$[\text{NiL}(\text{H}_2\text{O})_3]$	Dark green	64	274	40.70 (42.09)	4.70 (4.18)	10.55 (11.32)	12.05 (12.60)	6.03 (6.82)
$[\text{CuL}(\text{H}_2\text{O})]$	Dark green	65	278	44.60 (43.20)	4.60 (4.20)	12.04 (11.20)	6.10 (6.40)	12.90 (12.70)
$[\text{ZnL}(\text{H}_2\text{O})]$	Off white	68	272	44.11 (43.08)	5.02 (4.18)	12.21 (11.16)	7.18 (6.38)	13.90 (13.04)
$[\text{CdL}(\text{H}_2\text{O})]$	Pale yellow	70	275	40.14 (39.38)	4.12 (3.82)	10.88 (10.21)	6.12 (5.83)	21.46 (20.49)

Magnetic behaviour

The $\text{Co}(\text{II})$ complex registered a magnetic moment value of 4.64 B.M. Based on this value, we can assign octahedral geometry for this complex. The $\text{Ni}(\text{II})$ complex registered a magnetic moment value of 2.79 B.M. which is in agreement with octahedral geometry. The magnetic moment value of the copper(II) complex was 1.90 B.M. and therefore, it may be assigned a square planar structure⁷. Both the $\text{Zn}(\text{II})$ and $\text{Cd}(\text{II})$ complexes were found to be diamagnetic.

Electronic spectra

The bands shown by the $\text{Co}(\text{II})$ complex at 448, 616 and 1025 nm were assigned to $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{1g}(\text{P})$, $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{A}_{2g}(\text{F})$ and $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{2g}(\text{F})$, respectively. These bands are characteristic of octahedral geometry of the $\text{Co}(\text{II})$ complex.

The $\text{Ni}(\text{II})$ complex registered all the three bands characteristic for 6-coordinate octahedral $\text{Ni}(\text{II})$ ion at 390, 666, and 1018 nm. They may be assigned respectively to $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{1g}(\text{P})$, $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{1g}(\text{F})$ and $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{2g}(\text{F})$ transitions.

The $\text{Cu}(\text{II})$ complex, showed two weak bands corresponding to the transitions of a square planar $\text{Cu}(\text{II})$ complex, $^2\text{B}_{1g} \rightarrow ^2\text{E}_g$ and $^2\text{B}_{1g} \rightarrow ^2\text{B}_{2g}$ at 440 and 525 nm respectively.

Infrared spectra

The significant IR spectral bands of the ligand and the

complexes along with their probable assignments are given in the Table 2. The assignments were made based in comparison with the spectra of similar type of compounds⁸.

As we have mentioned earlier, a broad band in the higher frequency region, 3500-3000 cm^{-1} is present in the spectra of the complexes of $\text{Ni}(\text{II})$, $\text{Cu}(\text{II})$, $\text{Zn}(\text{II})$ and $\text{Cd}(\text{II})$. This region is found to be broad due to the presence of water coordination. The anilide carbonyl stretching frequency at 1676 cm^{-1} in the ligand is found to be lowered in the spectrum of the $\text{Co}(\text{II})$ complex, indicating the participation of carbonyl oxygen in coordination. But in the spectra of complexes of $\text{Ni}(\text{II})$, $\text{Cu}(\text{II})$, $\text{Zn}(\text{II})$ and $\text{Cd}(\text{II})$ this band disappears and a new band around 1150 cm^{-1} is observed due to the enolisation of $-\text{CH}_2\text{-C=O}$ to $-\text{CH=C-OH}$ followed by the participation of deprotonated oxygen in coordination. The stretching frequency of azomethine nitrogen is observed at 1598 cm^{-1} in the ligand spectrum. This band is found to be lowered in the complexes due to the participation of azomethine nitrogen in coordination. A strong band at 1075 cm^{-1} in the ligand spectrum, due to $\nu_{\text{(C-S)}}$ disappears and new bands around 750-850 cm^{-1} due to $\nu_{\text{C-S}}$ appears in the spectra of all the complexes. This is due to the enolization of $=\text{N-NH-C=S}$ to $=\text{N-N=C-SH}$, followed by the coordination through deprotonated sulphur.

A medium band around 1020 cm^{-1} is observed both in the

case of ligand and complexes, is due to the presence of ν_{N-N} . A strong band around 505-510 cm^{-1} in the spectra of the ligand and the complexes may be assigned to ν_{C-Br} . The other bands in the spectra of the complexes around 550-450 cm^{-1} may be assigned to ν_{M-N} , and ν_{M-O} .

Table 2.
Significant infrared spectral bands (cm^{-1}) of the ligand and its metal complexes

Ligand	[Co(LH) ₂]	[NiL(H ₂ O) ₃]	[CuL(H ₂ O)]	[ZnL(H ₂ O)]	[CdL(H ₂ O)]	Assignments
3500	3695	3652	3570	3600	3414	$\nu_{(N-H)}$, $\nu_{(O-H)}$
3300	3286	3250	3315	3282	3195	$\nu_{(C-H)}$ aromatic
1676	1597	--	--	--	--	$\nu_{C=O}$
		1156	1155	1155	1155	$\nu_{(C-O)}$ anilide
1598	1546	1550	1598	1599	1549	$\nu_{(C-N)}$
1075	--	--	--	--	--	$\nu_{(C-S)}$
1020	1022	1022	1115	1027	1020	$\nu_{(N-N)}$
--	843	874	756	839	874	$\nu_{(C-S)}$ + $\nu_{(C-N)}$
503	507	502	503	507	507	$\nu_{(C-Br)}$
--	542	532	541	530	530	$\nu_{(M-N)}$
--	455	455	457	435	460	$\nu_{(M-O)}$

CONCLUSION

The Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes of ω -BAacc- MPTCH₂ (L2H) were synthesized. Based on the magnetic and spectral studies, these complexes were assigned the following structures. These are shown in Figures 2, 3 and 4.

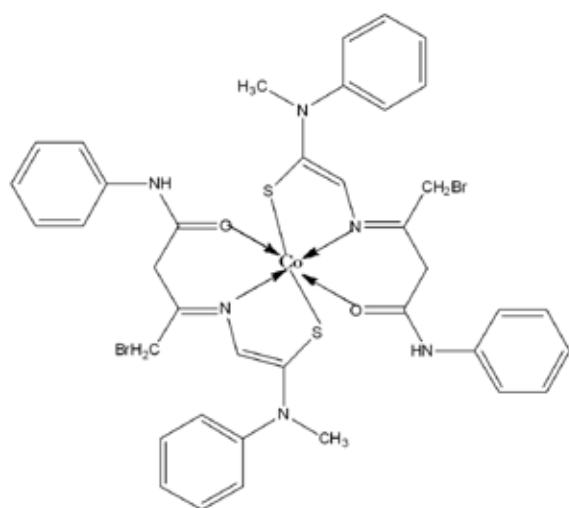


Figure 2.
Suggested structure of [Co(LH)₂]

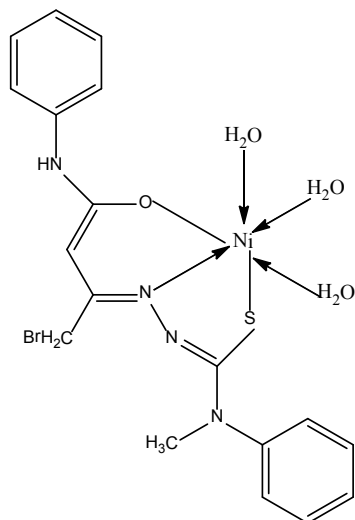


Figure 3.
suggested structure of [NiL(H₂O)₃]

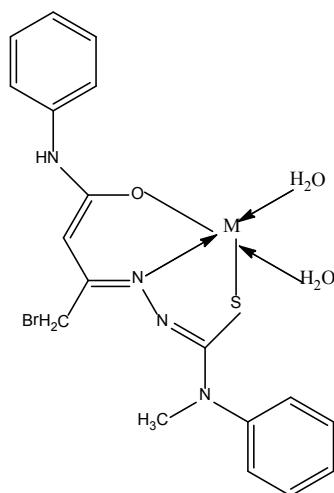


Figure 4.
Suggested structure of the complex of the formula [ML(H₂O)₂], where M = Cu(II), Zn(II) or Cd(II)

ANTIBACTERIAL ACTIVITIES

Thiosemicarbazones and their complexes are given immense importance due to their pharmacological- and biological activities⁹. Literature survey showed various interesting activities of thiosemicarbazones like antibacterial,¹⁰ antiviral,¹¹ antifungal,¹² and other biological properties¹³. Antibacterial activities of the samples were tested against a set of clinically important bacterial strains. The samples showed activity against all the bacterial strains studied. Some of them showed low activity.

MATERIALS AND METHODS

Bacterial cultures used

Three Gram positive bacteria; *Staphylococcus*(MTCCNO 3103), *Streptococcus* (MTCCNO 1938), *Bacillus*(MTCCNO 869) *sp* and three Gram negative bacteria; *Klebsiella* (MTCCNO 2653), *Escherichia coli* (MTCCNO 68) and *Pseudomonas* (MTCCNO 2642) *sp*. were used in this study. The pure cultures were purchased from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

MEDIA USED**Nutrient Broth**

(HiMedia Laboratories Pvt Ltd, Mumbai)

Peptic digest of animal tissue	: 5g
Sodium chloride	: 5g
Yeast extract	: 1.5g
Distilled water	: 1000ml (pH – 7.4)

Nutrient Agar

(HiMedia Laboratories Pvt Ltd, Mumbai)

Peptic digest of animal tissue	: 5g
Sodium chloride	: 5g
Yeast extract	: 1.5g
Distilled water	: 1000ml (pH – 7.4)
Agar	: 15 g

ANTIBACTERIAL ACTIVITY BY KIRBY BAUER OR DISC DIFFUSION METHOD

Antibacterial tests were carried out by disc diffusion method¹⁴ (with some modifications). The bacterial cultures (mentioned above) were maintained in nutrient broth. Each culture was uniformly distributed on Nutrient agar plates using sterile swabs. Sterile filter paper discs of 3mm diameter were placed on the surface of Nutrient agar plates at a distance of 2cm using sterile forceps. DMSO (2 %) was used to dissolve the drug, which was found to have no adverse effect on the bacterial cultures. Drugs of different concentrations [100, 250, and 500 µg/ml] were added on each disc with a micropipette. Disc with DMSO but without drug was used as a control. The plates were then incubated at 37°C for 24 hrs. After incubation, the zone diameter was measured. The greater diameters shown by the compounds than the control indicated their antifungal activity.

Table 3.**Antibacterial activity of ω-bromoacetoacetanilide N(4) methyl(phenyl)thiosemicarbazone and its complexes**

Sl. No.	Organism	[Co (LH) ₂]	Ni L (H ₂ O) ₃	[Cu L (H ₂ O)]	[Zn L (H ₂ O)]	Li-gand	S	D
1	Proteus Vulgaris	-	-	-	8	12	14	-
2	Bacillus cereus	11	9	11	9	14	15	-
3	Eschericia coli	-	-	-	-	12	10	-
4	Serratia marcescens	10	12	9	-	12	16	-
5	Pseudomonas aeruginosa	13	-	-	-	9	14	-
6	Bacillus subtilis	20	17	-	19	21	11	-
7	Salmonella typhi	-	-	-	-	-	12	-
8	Staphylococcus aureus	14	-	19	-	20	21	-
9	Klebsiella pneumonia	23	18	-	-	-	10	-

+ve control**S = Streptomyces****-ve control****D = DMSO****DISCUSSION**

It was found that the ligand, ω-bromoacetoacetanilide N(4)-methyl(phenyl) thiosemicarbazone exhibited greater activity than the complexes. Another observation is that the Co(II) complex showed greater activity for most of the samples. However, Cu(II), Zn(II) and Ni(II) complexes exhibited medium inhibition, less than that of the ligand.

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