



Antibacterial Activity and DNA Binding Properties of Bivalent Metal Complexes of Cuminaldehyde Acetoylhydrazone

Y. B. NAGAMANI², K. HUSSAIN REDDY^{1*}, K. SRINIVASULU¹,
D. DHANALAKSHMI¹ and K. ANUJA¹

¹Department of Chemistry, Sri Krishnadevaraya University, Ananthapuramu-515 003, India.

²Department of Chemistry, Govt. Degree College (W), Hindupur-515 201, India.

*Corresponding author E-mail: khussainreddy@yahoo.co.in

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ABSTRACT

Metallo-hydrazones having the formula $[M(\text{IBAH})_2]$ (where, M = Ni(II), Cu(II) and Zn(II); IBAH is p-Isopropylbenzaldehyde acetoylhydrazone) are prepared and confirmed on the basis of physico-chemical and spectral analyses. Conductivity data revealed that the complexes are non-electrolytes. Metal-DNA interactions are investigated using absorption spectrophotometry. Binding constant (K_b) data revealed that the copper complex interact DNA more strongly than other complexes. Antibacterial activity studies indicated higher activity for complexes than the metal free hydrazone ligand. The copper compound displays higher activity. DNA binding constants are correlated with the activity of metal compounds in this article.

Keywords: New metallo-hydrazones, Spectral characterization, DNA binding constants, Antibacterial activity.

INTRODUCTION

Hydrazones exhibit a broad spectrum of biological activities such as antibacterial¹, antitubercular¹, antioxidant², antiviral³ and anticancer⁴ activities. Hydrazones constitute an important class of compounds for new drug development⁵. Hydrazones derived from acetichydrazide are named as acetoylhydrazones. Compared to simple hydrazone Schiff bases, acyl and aroyl hydrazones have an extra donor site in $>C=O$. This presents a wide range of properties in them. The discovery that acetoylhydrazones show higher activity than

benzoylhydrazones evoked considerable interest^{6,7} to investigate metal complexes of former type of ligands.

Survey of literature revealed that a very few acetoylhydrazones are used as chromogenic reagents⁸ and in the study of transition⁹⁻¹¹ and lanthanide^{12,13} metal complexes. Hydrazones derived from aldehydes contain hydrogen atom on azomethine carbon atom. It is reported¹⁴ that hydrazones having azomethine hydrogen atom shows higher activity. Hence it is of interest to investigate hydrazones derived from aldehydes rather than ketones.



Our customary foods contain carbonyl compounds¹⁵ showing beneficial effects to human health. The chemical name of cuminaldehyde is *p*-Isopropylbenzaldehyde (IB). It is known to present in cummin, essential oils of eucalyptus, myrrh, cassia etc. The aromatic aldehyde, viz. *p*-Isopropylbenzaldehyde (IB) is an important ingredient of cummin. It has medicinal properties. For example, it is known to inhibit the fibrillation of alpha-synuclein¹⁶ present in cummin. Hence it is of interest to use *p*-Isopropylbenzaldehyde in the synthesis of new hydrazone ligand and its metal complexes.

Studies on Metal-DNA interactions are expected to predict biological activity of compounds. The compound which binds DNA strongly may find application in the drug development. Survey of literatures revealed that metal complexes with organic ligands showed higher biological activity than metal free organic compounds. Hence it is considered worthwhile to investigate novel hydrazone ligand originated from *p*-Isopropylbenzaldehyde and its metal complexes. We have studied¹⁷⁻²⁰ nucleic acid binding of various metal complexes in the past to develop antimicrobial agents. To renew our interests, herein we communicate

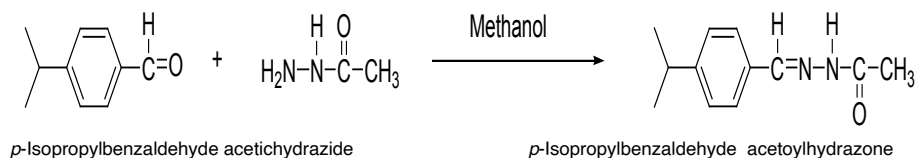
our results on spectral analysis and biological applications of bivalent metal complexes with *p*-Isopropylbenzaldehyde acetoxyhydrazone (IBAH)

EXPERIMENTAL

Acetichydrazide and *p*-Isopropylbenzaldehyde were bought from Sigma Aldrich and utilized without further purification. Metal salts (ZnCl₂, NiCl₂·6H₂O and CuCl₂·2H₂O) were of Merck AR quality. Solvents were distilled before use.

Synthesis of IBAH ligand

Round bottom flask (100 mL capacity) was charged with 20 mL of methanolic solution of acetic hydrazide (0.91g; 0.012 mol) and 20 mL of methanolic solution of *p*-Isopropylbenzaldehyde (5 mL, 0.03 mol). Glacial acetic acid (few drops) was added as catalyst to the contents of flask. The reactants were heated on water bath for 2.5 h and cooled. The product was collected and treated repeatedly with hot H₂O & dried. Methanol solvent was used in recrystallization of ligand. Yield: 70.58%; m.p., 130-132°C. Molecular formula: C₁₂H₁₆N₂O. Formula weight, 204.1. Synthesis of IBAH is depicted in Scheme 1.



Scheme 1. Preparation of *p*-Isopropylbenzaldehyde acetoxyhydrazone (IBAH)

Preparation of metallo-hydrazones

The IBAH ligand (1.5 g; 0.007 mol) was transferred to 100-mL beaker and dissolved in 20 mL ethanol. In another 100-mL beaker, CuCl₂·2 H₂O (0.007 mol) was dissolved in 15 mL of ethanol. These two solutions were mixed in a clean R.B flask and refluxed for 2 hours. On cooling, a green stained product was formed. The compound was amassed and washed with few drops 50% methanol and de-solvated in vacuum. Nickel(II) and zinc(II) complexes of IBAH were prepared similarly. Melting points and yields of compounds are delineated in Table 1. Particulars of equipment employed in the present study, deoxyribonucleic acid (DNA) binding and antibacterial activity experiments are given in our previous articles.^{19, 20}

RESULTS AND DISCUSSION

The ligand (IBAH) is characterized on the

basis of spectroscopic studies. FT-IR spectroscopy: 3264, 3082, 2945, 1648 and 1542 are designated to secondary amine (N-H), aromatic C-H, aliphatic C-H, >C=O and azomethine (>C=N) elongating vibrations correspondingly. ¹H-NMR spectroscopy (in deuterated chloroform): δ 10.64 (s, 1H) 8.95 (s, 1H) (7.29 - 7.75) (m, 4H), 2.32 (s, 3H) 1.271 (d, 6H) 2.97 (septet 1H) are respectively assigned to azomethine (CH=N-), >NH, Ar-H, CH₃ of acetoxy, -CH₃ protons of isopropyl and -CH methine protons of isopropyl group sequentially. Mass spectrometry: Mass plot of IBAH (Fig. 1) shows a peak at m/z = 204.1 which coincides with formula (C₁₂H₁₆N₂O) weight of IBAH.

Based on spectral analysis the structure (Fig. 2) of IBAH ligand is proposed. Mass spectral fragmentation pattern of IBAH (Fig. 3) is shown below.

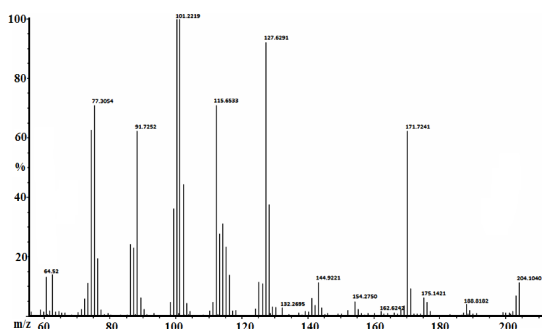


Fig. 1. Mass spectrum of IBAH

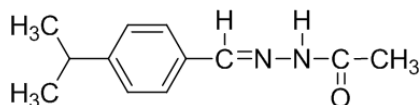


Fig. 2. Structure of IBAH ligand

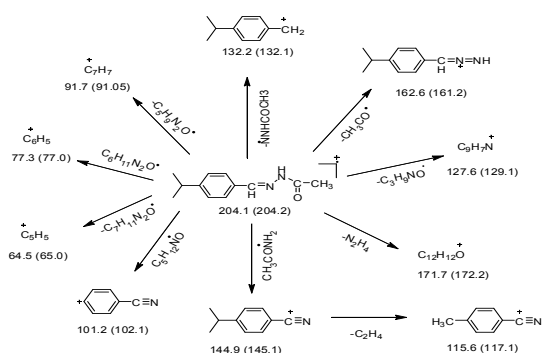


Fig. 3. Fragmentation pattern of IBAH ligand

Structural characterization of metal complexes

Metal chlorides reacted with *p*-Isopropylbenzaldehyde acetoylhydrazone (IBAH) in basic medium under reflux conditions to produce corresponding metal complexes. The complexes are stable, non-hygroscopic, moderately soluble in CH_3OH and $\text{C}_2\text{H}_5\text{OH}$ but easily soluble in dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). Color, yield, ESI-MS & conductivity data of Cu(II), Ni(II) and Zn(II) compounds are stated in Table 1. Molar conductivity outcome show that the coordination compounds are non-electrolytes²¹.

Table 1: Physicochemical and Analytical data of complexes

Compound	Colour (Yield, %)	ESI-MS (F.W)	Molar Conductivity [@]
Cu(II)IBAH ₂	Dark green (74.36%)	471.2 (469.5)	12.75
Ni(II)IBAH ₂	Parrot green (79.85%)	464.8 (464.7)	9.66
Zn(II)IBAH ₂	Yellow (75.87%)	468.3 (471.4)	15.12

*Calculated values are given in parenthesis. @ Units, $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$

Electronic spectroscopy

UV-Visible data of metal complexes and designation of peaks are summarized in Table 2. The electronic spectrum of Cu(II) complex shows (Fig. 4) peak at $14,792\text{ cm}^{-1}$ (676 nm) having shoulder peak at $16,447\text{ cm}^{-1}$ in the visible region. This band is assigned to ${}^2E_g \rightarrow {}^2T_g$ electron transfer in favour of octahedral geometry. The presence of shoulder peak at $16,447\text{ cm}^{-1}$ (608 nm) may be due to Jahn-Teller effect which is due to geometric distortion generally noticed for copper(II) octahedral compounds where the two axial bonds can be shorter or longer than those of the equatorial bonds.

Peaks are observed at 16,366 and 10,111 in the electronic spectrum of nickel complex. These peaks are related to ${}^3A_{2g} \rightarrow 3T_{2g}$ (F) and ${}^3A_{2g} \rightarrow 3T_{1g}$ (P) electron transfers respectively in support of octahedral geometry. A peak in higher energy region due to ${}^3A_{2g} \rightarrow {}^3T_{2g}$ transition is not observed due to the domination of $\pi \rightarrow \pi^*$ transition.

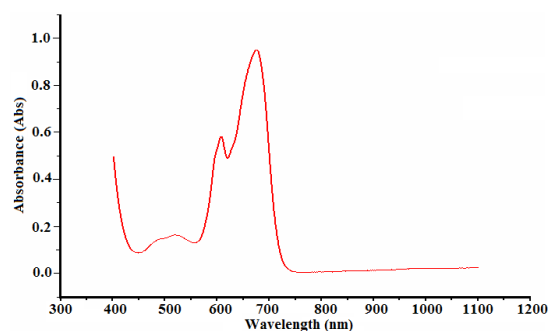


Fig. 4. UV-Visible spectrum of Cu(II)IBAH₂ complex showing Jahn-Teller effect

Table 2: UV-Visible spectral data of metal compounds with IBAH ligand

Complex	Wavelength λ_{max} (nm)	Frequency (cm^{-1})	Transition
Cu(II)IBAH ₂	285	35087	$\pi \rightarrow \pi^*$
	608	16447	$d \rightarrow d$
	676	14792	$d \rightarrow d$
Ni(II)IBAH ₂	287	34843	$\pi \rightarrow \pi^*$
	611	16366	$d \rightarrow d$
	989	10111	$d \rightarrow d$

IR spectroscopy

The coordination sites of IBAH ligand are uncovered by examining IR spectra of metal-free ligand and complexes. Spectral data are stated in Table 3. In the spectrum of the IBAH bands are observed due to $\nu_{\text{N-H}}$, $\nu_{\text{C=C}}$, $\nu_{\text{C=O}}$ and $\nu_{\text{C=N}}$ stretching modes. Both $\nu_{\text{N-H}}$ (3264 cm^{-1}) and $\nu_{\text{C=O}}$ (1648 cm^{-1}) bands of ligand are absent in the spectra of complexes due to enolization and subsequent deprotonation of ligand during complex formation (Scheme 2).

Table 3: Infrared Spectral data of IBAH ligand and its complexes

IBAH	Cu(IBAH) ₂	Ni(IBAH) ₂	Zn(IBAH) ₂	Assignment
3264	-	-	-	νN-H
3082	3014	3027	3024	νC-H aromatic
2945	2920	2915	2913	νC-H aliphatic
1648	-	-	-	νC=O
1648	1615	1620	1612	νC=N
1542	1528	1522	1532	νC=C (aromatic)
1465				
1378	1367	1318	1312	νC=C (aromatic)
1264	1260	1242	1248	
1185	1173	1178	1165	νC-O
	604	616	632	νM-O
	574	538	542	
	513	520	524	νM-N

You must listed one value for gM-O

The bands of $\nu_{C=O}$ and $\nu_{C=N}$ are shifted to lower wave numbers indicating involvement of acetyl $>C=O$ and azomethine ($>C=N-$) nitrogen groups in chelation. Spectral data suggest that the IBAH behaves as uninegative bidentate ligand in complexes. Bands in low energy regions, 604-632 and 513-524 cm^{-1} are related to ν_{M-O} and ν_{M-N} vibrations respectively.

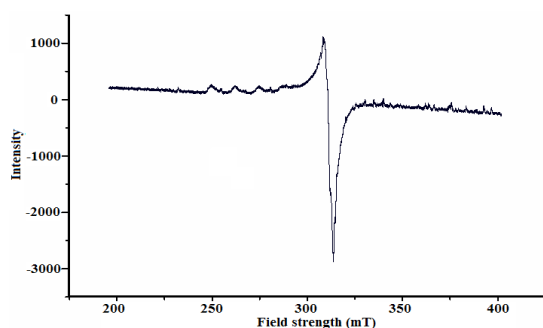
ESR spectroscopy

ESR spectrum of Cu complex in DMF at liquid nitrogen temperature (LNT) is depicted

Table 4: Spectral data of [Cu(IBAH)₂] complex in DMF at LNT

g_{\parallel}	g_{\perp}	g_{avg}	G	$A_{\parallel} \times 10^{-5}$	$A_{\perp} \times 10^{-5}$	K_{\parallel}	K_{\perp}	λ	α^2
2.38	2.07	2.17	5.64	0.00262	0.00014	0.997	1.091	520	0.3816
(2.2)	(2.12)	(2.15)	(1.62)				1		

ESR data of complex at RT are given in brackets

**Fig. 5. ESR spectrum of Cu(IBAH)₂ at LNT**

On the basis of analytical, molar conductivity, UV-Visible, FT-IR ESR spectroscopy studies a general structure (Fig. 6) is tentatively suggested.

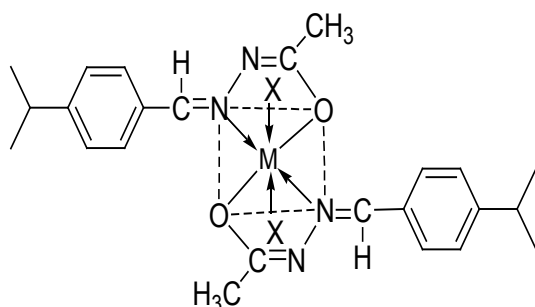
Deoxyribonucleic acid Interactions

Interactivity of complexes with CT-DNA was

in Fig. 5. Table 4 gives ESR data of copper(II) complex in solid state & in DMF solvent. The g_{\perp} and α^2 and λ values of Cu complex suggest covalent nature of Metal- Ligand (M-L) bond and the location of unpaired electron in $d_{x^2-y^2}$ orbital. This finding indicates covalent nature²² of metal-ligand (M-L) bond. The orbital reduction parameters (K_{\parallel} , K_{\perp}) reveal the occurrence of in-plane π -bonding in the complex. The axial symmetry parameter values of complex in solution state at R.T is below 4.0. The values indicate absence of quid pro quo interaction.

examined by UV-Visible spectroscopy. Spectra of copper complex are shown in Figure 7.

Metal derivatives of IBAH showed strong peak due to M→L charge transfer transitions. The binding constant (K_b) is calculated using standard equation.²³ DNA - binding constants of complexes are given in the Table 5. Metal complexes (except Cu(II) complex) showed considerable hypsochromic shift (Blue shift) ($\Delta \lambda_{\text{max}} = 0.8-1.5 \text{ nm}$) with raising amounts of DNA. High binding constants ($4.3-17.2 \times 10^6 \text{ M}^{-1}$) of present complexes are suggestive²⁴⁻²⁸ of intercalation mode of binding. The binding constant (K_b) order is $\text{Cu}(\text{IBAH})_2 > \text{Ni}(\text{IBAH})_2 > \text{Zn}(\text{IBAH})_2$. The observed trend indicates that $\text{Cu}(\text{IBAH})_2$ interacts DNA more firmly.



M=Cu(II), Ni(II), Zn(II); X=DMF

Fig. 6. Proposed Structure for complexes in DMF medium

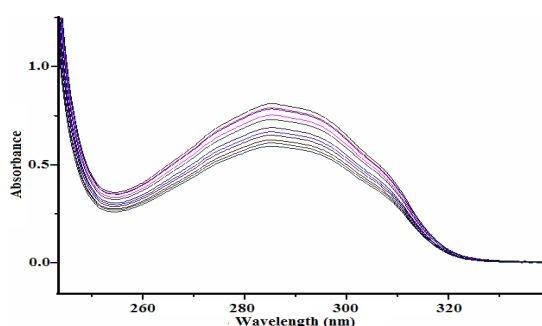


Fig. 7. UV-Visible spectra of copper complex with increasing amount of DNA. (Top most curve is spectrum of complex without DNA)

Table 5: UV-Visible Spectral Data Complexes with and without DNA

Complex	λ max, nm		$\Delta \lambda$	H%	K_b [M^{-1}] $\times 10^6$
	Free	Bound			
Cu(IBAH) ₂	285	285.5	0.5	19.32	17.2
Ni(IBAH) ₂	286	284.5	-1.5	11.26	13.4
Zn(IBAH) ₂	242.2	241.4	-0.8	16.42	4.3

Antibacterial activity studies

The compounds under investigation are

Table 6: Zone inhibition (in mm) data of IBAH ligand and its metal complexes

Compound	Amount Taken ($\mu\text{g}/\mu\text{L}$)	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>M. luteus</i>	<i>B. subtilis</i>
Ciprofloxacin	5	11.68 \pm 0.05	10.24 \pm 0.01	9.78 \pm 0.02	10.36 \pm 0.04
IBAH	100	1.57 \pm 0.19	1.56 \pm 0.11	1.49 \pm 0.37	1.03 \pm 0.43
	200	1.82 \pm 0.35	2.48 \pm 0.01	2.73 \pm 0.25	2.87 \pm 0.04
	300	2.64 \pm 0.44	2.65 \pm 0.28	2.68 \pm 0.08	2.29 \pm 0.22
	300	2.64 \pm 0.44	2.65 \pm 0.28	2.68 \pm 0.08	2.29 \pm 0.22
Cu(IBAH) ₂	100	6.45 \pm 0.25	3.42 \pm 0.39	4.09 \pm 0.32	3.93 \pm 0.16
	200	7.05 \pm 0.36	5.46 \pm 0.05	5.92 \pm 0.42	5.84 \pm 0.38
	300	6.31 \pm 0.08	6.29 \pm 0.03	6.38 \pm 0.14	5.68 \pm 0.24
Ni(IBAH) ₂	100	2.14 \pm 0.06	1.75 \pm 0.06	1.92 \pm 0.42	2.81 \pm 0.21
	200	3.95 \pm 0.18	2.22 \pm 0.03	2.33 \pm 0.50	2.70 \pm 0.13
	300	4.09 \pm 0.22	4.86 \pm 0.36	3.57 \pm 0.27	4.58 \pm 0.11
Zn(IBAH) ₂	100	2.02 \pm 0.17	3.08 \pm 0.23	2.73 \pm 0.04	1.89 \pm 0.19
	200	4.94 \pm 0.01	4.33 \pm 0.18	4.52 \pm 0.41	2.51 \pm 0.28
	300	5.73 \pm 0.23	6.14 \pm 0.31	3.43 \pm 0.29	4.73 \pm 0.35

tested for antibacterial activity as outlined before⁶ by taking *Gram-positive* (*Micrococcus luteus*, *Bacillus subtilis*) and *Gram-negative* (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria using agar well diffusion method against ciprofloxacin as positive control. About 24 h old bacterial cultures are used to prepare bacterial lawns. Agar wells were prepared and injected with sample solutions prepared using DMF. The agar plates are incubated at 35°C for 24 h and inhibition zones are measured and expressed Table 6 in millimeter units.

Results in Table 6 indicate that the present coordination compounds show higher activity than metal-free IBAH ligand. Data are depicted in the form of bar graph (Fig. 8) which indicates that Cu(IBAH)₂ complex shows more activity possibly due to its strong binding to DNA.

Metal complexes displayed higher antibacterial activity^{29,30} than the metal free ligand. Increased activity of coordination compounds may be explained by using Tweedy's chelation theory³¹ and Overtone's concept³². The ligand forms organic coat around the metal ion in the formation of complex. As a result the complex becomes lipophilic. According to latter concept, the cell membrane allows the passage of complexes as they are lipid-soluble. Hence lipid solubility of compound is principal requirement for showing this action. On complex formation, the polarity of metal ion is significantly reduced due to delocalization of π -electrons. On entering into the cell the complex undergo dissociation and releases metal ion which inhibits enzymatic activity of microorganisms more effectively³³.

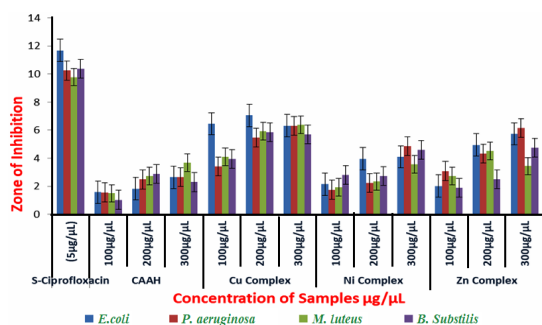


Fig. 8. Graphical representation of inhibition zones (in mm) of metal complexes

CONCLUSION

A new organic ligand viz. *p*-Isopropylbenzaldehyde acetylhydrazone (IBAH) and its metal complexes are synthesized and characterized for the first time. A general structures for the complexes is proposed based on analytical and electronic spectral data. High DNA binding constants suggest that the

complexes bind DNA via intercalation. Complexes showed higher antibacterial activity. Among all compounds, the copper complex binds DNA more firmly and inhibits bacteria effectively. The Cu(IBAH)₂ complex not only binds DNA strongly but also inhibits bacteria more effectively. The observation revealed a direct relationship between DNA binding constant and antibacterial activity. Thus antibacterial activities of metal complexes are concomitant with their DNA binding constants.

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Conflicts of interest

Regarding the publication of this article, the authors declare that there is no conflict of interests.

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