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SPECTRAL CHARACTERIZATION, DNA BINDING PROPERTIES AND ANTIBACTERIAL ACTIVITY OF NEW METALLO-HYDRAZONES

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ABSTRACT

Metallo-hydrazones having the formula $[M(DABH)_2]$ (Where, M = Co(II), Ni(II), Cu(II) and Zn(II); DABH = 4-dimethylaminobenzaldehyde benzoylhydrazone) are prepared and characterized based on physicochemical and spectral analyses. Molar conductivity data revealed that the complexes are non-electrolytes. Metal-DNA interactions are investigated using absorption spectrophotometry. Binding constant (Kb) data revealed that the cobalt complex interacted DNA more strongly than other complexes. Antibacterial activity studies indicated higher activity for complexes than the metal-free hydrazone ligand. The cobalt compound displays higher activity. DNA binding constants are correlated with the activity of metal compounds in this article.

Keywords: Synthesis, New Metallo-hydrazones, Spectral Characterization, DNA Binding Constants, Antibacterial Activity.

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INTRODUCTION

Metal complexes of hydrazones show various antimicrobial activities such as antibacterial, anticonvulsant, anti-platelet, analgesic, anti-inflammatory, anti-tuberculosis, anti-tumor, anti-proliferative, antiviral, and anti-tumor anti-malarial actions.[1] These observations have been directed toward the development of new Meallo-hydrazones. Compared to simple hydrazones, acyl and aroyl hydrazones have an extra donor site in >C=O to exhibit flexibility in metal chelation.[2] In some instances, the reactivity of hydrazone depends on the substituent in *para*- position to azomethine (>C=N-) group.[3] Aroylhydrazones, as well as their metal complexes, showed higher microbial activities due to increased liphophilicity.

Hydrazones derived from aldehydes are more potential than the ligands obtained from ketones. The activity of aldehydic hydrozones is due to the presence of aldehydic (α) proton. In recent years, we have reported synthesis, spectral characterization, structural determination, deoxyribonucleic acid (DNA) binding and antibacterial activities of transition and lanthanide metal complexes of benzoyl and acetoylhydrazones.[4,5] Although transition metal complexes of hydrazones derived from *ortho* substituted benzaldehyde were made and analyzed, but their nucleic acid binding and antibacterial activities were not studied.[6–10] Transition metal complexes with benzoylhydrazones of *p*-aminoacetophenone and *p*-chloroacetophenone were reported.[10] Copper(II) complexes with ligands derived from *para*-isopropylbenzaldehyde (viz. cuminaldehyde) were synthesized, characterized and their DNA binding and nuclease activities have been investigated and reported[11] recently by us from our laboratories. The complexes showed higher nuclease activity than the ligands. Very recently, we have reported preparation, spectral analysis and antibacterial tasks of metal derivatives with benzoylhydrazones of 4-substituted aldehydes.[12, 13]

Among aromatic carbonyl compounds, 4-dimethylaminobenzaldehyde is an interesting organic carbonyl compound containing amino and aldehyde moieties. It has been used in Ehrlich reagent and Kovac's reagent for testing indole. It has diverse applications in analytical, synthetic, pharmaceutical and biological chemistry.[14-20] Survey of the literature revealed that studies on bivalent metal complexes with

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hydrazones of *para*-substituted aldehydes are minimal. Hence it is of interest to investigate **DNA** binding and antibacterial activity of bivalent metal complexes of new hydrazone derived from *para*-dimethylaminobanzaldehyde.[21]

EXPERIMENTAL

Benzhydrazide & *para*-dimethylaminobenzaldehyde were bought from Sigma Aldrich and utilized without further purification. Metal salts were of Merck AR quality. Solvents were distilled before use.

Synthesis of DABH ligand

Round bottom flask(100 mL capacity) was charged with 20 mL of methanolic solution of benzhydrazide (2.0g; 0.013mol) and 20 mL of methanolic solution of 4-(dimethylamino) benzaldehyde (1.82g, 0.013mol). Glacial acetic acid (a few drops) was added as a catalyst to the ingredients of the flask. The total ingredients were warmed up on a water bath for 3 hrs and chilled. The product was collected and treated repeatedly with hot H_2O & dried. Methanol solvent was used in the recrystallization of ligand. Yield: 86.38%; M.P: 106-108°C. Molecular formula: $C_{16}H_{17}N_3O$.

Preparation of Metallo-hydrazones

The DABH ligand (1.2 g; 0.004mol) was transferred to 100-mL beaker and dissolved in 20 mL ethanol. In another 100-mL beaker, $CoCl_2.6\ H_2O$ (1.0 g; 0.004mol) was solvated in 15 mL of ethyl-alcohol. These two solutions were combined in a clean R.B flask and refluxed for 2 hrs. On cooling, a light pink stained product was formed. The compound was amassed and washed with a few drops 50% methanol and desolvated in a vacuum. Other metal [Ni(II), Cu(II) and Zn(II)] derivatives of DABH were prepared similarly. Melting points and yields of complexes are delineated in Table 1. Particulars of equipment, DNA binding and biological experiments are given in our previous articles.[4,5]

RESULTS AND DISCUSSION

Spectral Characterization of DABH ligand

Infrared Spectra

1597, 1659, 2988, 3089 and 3242 are due to ν(C=N), ν(C=O), ν(C-H, aliphatic) ν(C-H, aromatic) and ν(NH) stretching vibrations respectively.

^{1}H-NMR spectra (in deuterated chloroform)

δ 10.46 (s, 1H) 8.30 (s, 1H), 7.26-8.05 (m, 5H) (6.52-7.99) (m, 4H), 3.00 (s, 6H) are respectively assigned to (R**H**C=N-), >N**H,** Phenyl-H, Ar-**H**(benzoyl), and N-(Methyl)$_2$**─H** correspondingly.

Mass Spectrum

It shows a specific apex at m/z = 267.02 matching the molecular ($C_{16}H_{17}N_3O$) mass of the DABH. Based on spectral data, the synthesis of DABH ligand is confirmed.

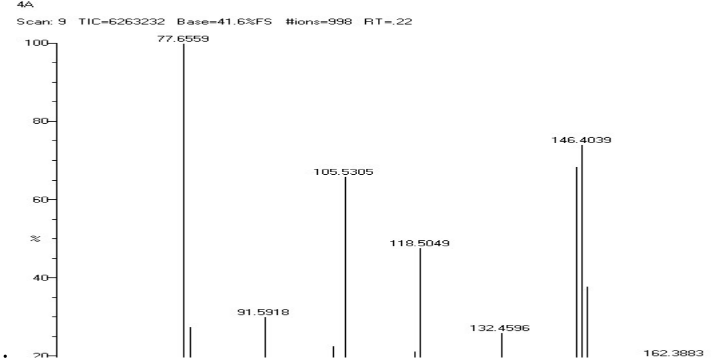


Fig.-1: Mass Spectrum of 4-dimethylaminobenzaldehyde benzoylhydrazone(DABH)

Structural Characterization of metallo-hydrazones

Analytical data of metallo-hydrazones are summarized in Table-1. Conductivity data revealed non-electrolytic nature of metal derivatives.[22]

Table-1: Physicochemical and Analytical Data of Metal Derivatives

Compound	Shade (Yield %)	Mass* Data (F.W)	Λm ($\Omega^{-1}cm^2 mol^{-1}$)
$Cu(DABH)_2$	Orange Red (71)	596.2 (595.5)	13.78
$Ni(DABH)_2$	Dark Red (74)	573.1† (590.7)	23.97
$Co(DABH)_2$	Dull pink (65)	588.3 (590.9)	17.95
$Zn(DABH)_2$	Dull Yellow (62)	591.1 (597.4)	14.85

*Obtained from ESI- Mass spectrometry, † Corresponds to the formation of $M{-}NH_3$ radical cation

Electronic Spectra

The spectrum cobalt(II) complex was displayed a prominent peak at 28,169 cm^{-1} due to CT transition. The tail of this band masks d-d bands of very low intensity. However, discernible bands are observed at 23,255 and 15,384 cm^{-1} and are respectively allocated to $^4A_{2g} \leftarrow {}^4T_{1g}$ (F) and $^4T_{1g}$ (P) $\leftarrow {}^4T_{1g}$ (F) electronic shifts in favor of octahedral structure. The peak due to $^4T_{1g}$ (F) $\rightarrow {}^4T_{2g}$ electronic migrations does not appear as it may be falling beyond 1100 nm (9,090 cm^{-1}) i.e., wavelength window of the instrument.

The copper(II) compound was exhibited a weak peak at 13,477 cm^{-1} because of $^2Eg \rightarrow {}^2T_{2g}$ transition in favor of octahedral structure. In the UV-visible gamut of nickel(II) complex, three peaks are observed in the visible region at 28,011(υ_3); 22,321(υ_2) and 13,037 (υ_1) cm^{-1}. The peaks are respectively due to $^3T_{1g}(P) \leftarrow {}^3A_{2g}(F)$, $^3T_{1g}$ (F) $\leftarrow {}^3A_{2g}(F)$ and $^3T_{2g}$ (F) $\leftarrow {}^3A_{2g}(F)$ electronic migrations. Frequency values are used in the calculation of Racah constant (B'), Cloud (orbital) expanding ratio (β) and % covalent character by using standard equations. The found values of B', β and % covalent character respectively are 748 cm^{-1}, 0.72 and 28 respectively. The electronic spectrum of Zn(II)complex was exhibited strong peaks at 38,020 and 28,090 cm^{-1} due to $\pi \rightarrow \pi^*$ and charge CT migrations, respectively.

FT-IR Spectra

Distinct group frequencies of DABH and its metal derivatives were analyzed. Bands due to ν_{N-H} and $\nu_{C=O}$ of DABH disappeared in spectra of complexes. This observation suggests that the DABH undergoes enolization, subsequently deprotonation during complexation with metal ions to act as mono-anionic ligand. The $\nu_{C=N}$ of DABH is moved to low wavenumbers in the sweeps of all metal compounds, indicating the participation of azomethine nitrogen in coordinatioin.[23, 24]

ESR Spectra

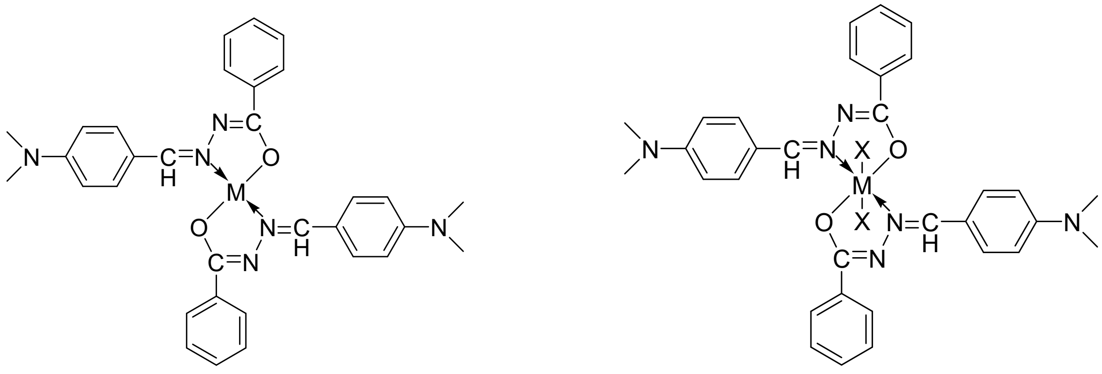
ESR spectra of $Cu(DABH)_2$ complex in DMF solvent were recorded at room temperature(RT) and at liquid nitrogen temperature (LNT). The $g_{\|}$, $g_{\perp}$ and α^2 and λ values of Cu complex suggest the electrovalent property of M ─ L bond and the existence of a single electron in $d_{x^2-y^2}$ orbital. This finding indicates the covalent nature of the metal-ligand bond.[25] The orbital reduction parameters ($K_{\|}$, $K_{\perp}$) reveal the occurrence of in-plane π-interaction in the copper derivative. Axial symmetry parameter values of complex in solid and solution states are less than 4.0. The values indicate the absence of exchange coupling.

Structures of complexes in solid [Fig.-2A] and solution states [Fig.-2B] are suggested based on spectral data.

IR spectra of solid complexes revealed that metals are four coordinate and presumably have square planar structure. The UV-Visible spectra of metal derivatives in dimethylformamide medium suggested that the metal ions are six-coordinate due to the axial ligation of solvent (DMF) molecules to give octahedral geometry for the complexes.

Metal-DNA Interactions

The interactivity of metal derivatives with Calf thymus DNA was examined by UV-visible spectral technique. Spectra of the copper complex are shown in Fig.-3.



(A) (B)

Fig.-2: Proposed General Structures for Metal Complexes (A) in Solid and (B) in Solution State
[M= Metal Ion, X = Dimethylformamide (DMF)]

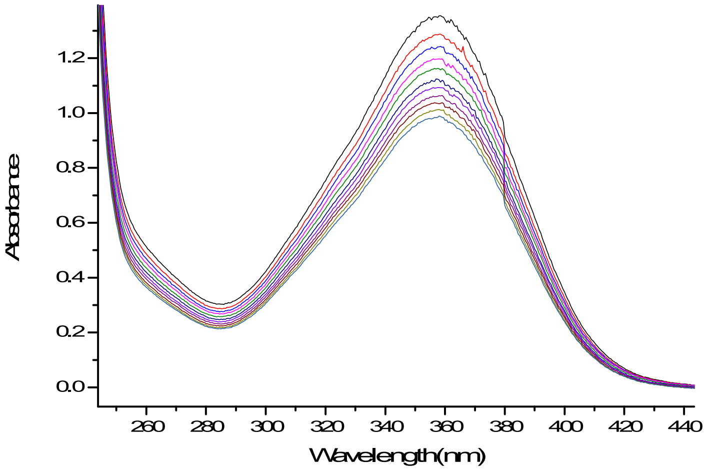


Fig.-3: UV-Visible Spectra of Copper Complex with an increasing amount of DNA.
(The top Curve is for complex without DNA)

Metal derivatives of DABH showed a strong peak due to M→L charge transfer transitions. The binding constant (Kb) is calculated using a standard equation.[26] DNA - binding constants of metal derivatives are delineated in Table-2. Metal complexes (except Zn(II) complex) showed considerable hypsochromic shift (Blueshift) ($\Delta \lambda_{max} = 1.2 – 2.5$ nm) with raising amounts of DNA.

High binding constants ($0.31- 11.81 \times 10^6 M^{-1}$) of present complexes are suggestive of intercalation mode of binding[27-31]. The binding constant (K_b) order is $Cu(DABH)_2 < Ni(DABH)_2 < Zn(DABH)_2 < Co(DABH)_2$. The observed order suggested that the $Co(DABH)_2$ interacts DNA more strongly.

Antibacterial Activity Studies

The Agar well diffusion method was applied to test the activity of compounds under investigation. The diameters of inhibition zones are given in Table-3. Activity data of compounds are shown in Fig.-4.

Table-2: Electronic Absorption Data Complexes with and without C T –DNA

Metal Derivative	Absorption Maximum (λ , nm)		Δ λ	H%	K_b [M^{-1}]
	Unbound	Bound			
$Cu(DABH)_2$	358.5	356.5	-2.0	19.68	0.31×10^6
$Ni(DABH)_2$	361.5	359.0	-2.5	28.00	1.52×10^6
$Co(DABH)_2$	359.6	358.4	-1.2	11.74	11.81×10^6
$Zn(DABH)_2$	355.4	357.2	+1.8	5.69	10.23×10^6

Table-3: Zone Inhibition Values of DABH and its Metal Derivatives

Compound	Amount (µg/µL)	*E.coli*	*P.aeruginosa*	*M.luteus*	*B.substilis*
S-Ciprofloxacin	5µg/µL	12.48±0.02	10.46±0.08	9.68±0.05	10.3±0.06
DABH	100	1.76±0.40	1.68±0.13	1.58±0.36	1.58±0.16
	200	2.48±0.32	1.48±0.48	1.64±0.28	1.60±0.44
	300	2.86±0.04	2.79±0.08	2.28±0.10	2.36±0.18
Cu-DABH	100	1.88±0.14	2.16±0.04	1.65±0.24	1.78±0.42
	200	3.76±0.44	2.92±0.35	3.66±0.18	2.84±0.26
	300	4.68±0.25	4.22±0.08	3.90±0.10	4.38±0.05
Ni-DABH	100	1.82±0.22	1.32±0.16	2.06±0.02	1.54±0.34
	200	2.68±0.35	3.46±0.25	3.44±0.38	3.05±0.16
	300	4.06±0.11	4.50±0.08	4.65±0.08	3.98±0.25
Co-DABH	100	5.24±0.05	5.01±0.13	4.82±0.06	4.16±0.10
	200	6.11±0.42	5.53±0.32	5.88±0.16	5.28±0.36
	300	7.52±0.26	6.76±0.10	6.46±0.14	7.04±0.15
Zn-DABH	100	2.35±0.22	2.08±0.24	2.52±0.36	2.78±0.24
	200	3.66±0.04	3.58±0.01	4.02±0.13	3.86±0.05
	300	5.76±0.43	4.82±0.22	5.18±0.30	4.65±0.28

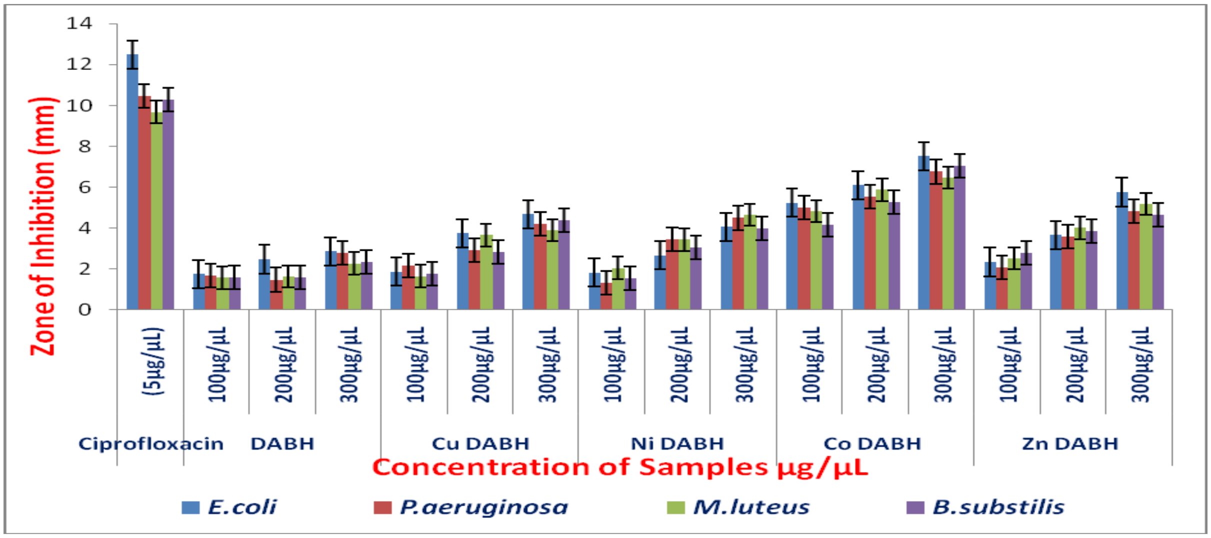


Fig.-4: Graphical Representation of Antibacterial Activity of Ligand and Metal Complexes

Metal derivatives displayed more excellent bactericidal activity[32-34] relative to DABH. Increased coordination compound activity may be explained using Tweedy's chelation theory[35] and Overtone's concept[36]. The ligand forms an organic coat around the metal ion in the formation of the complex. As a result, the complex becomes liphophilic. According to the latter concept, the cell membrane allows the passage of complexes as they are lipid-soluble. Hence lipid solubility of the compound is a principal requirement for showing this action. On complex formation, the divergence of cation is significantly decreased due to displacement of Pi-negatrons. On entering the cell, the complex releases metal ion (due to dissociation), which inhibits the enzymatic activity of microorganisms.[37]

CONCLUSION

A new organic ligand *viz*. *p*-dimethylaminobanzaldehyde benzoylhydrazone (DABH) and its metal complexes are synthesized and characterized for the first time. Structures of the metal derivatives are proposed following diagnostic and electronic spectral data. High DNA binding constants suggest that the complexes bind DNA via *intercalation*. Metal derivatives exhibited more excellent antibacterial activity than DABH. The cobalt derivative binds DNA more strongly and also inhibits bacteria more effectively among all compounds. Thus, the antibacterial activity of cobalt derivative is concomitant with its DNA binding constant.

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