DNA Binding and Antimicrobial Studies on Bivalent Transition Metal Complexes with 2-Acetylpyridine Isonicotinoylhydrazone

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ABSTRACT

Bivalent transition metal [Co(II), Ni(II), Cu(II) and Zn(II)] complexes with 2-acetylpyridine isonicotinoyl hydrazone(APINH, LH) have been prepared and analyzed based on mass spectra, molar conductivity, FT-IR and UV-Vis spectral data. The ML2complexes are found to be non-electrolytes. From FT-IR data it is evident that the APINH acts as uni-negative tridentate ligand. Electronic spectral data confers octahedral structure for the complexes. The copper complex is also studied using ESR spectroscopy. The Spin Hamiltonian, orbital reduction and bonding parameters are determined for the complex. DNA binding properties of the complexes uncovered by using UV-Visible spectrophotometry. Of all the compounds, the nickel complex binds more strongly than either. The metal complexes are screened for their antibacterial activity against pathogenic bacterial strains viz. Gram–ve bacteria (Escherichia coli, Klebsiella pneumonia) and Gram +ve bacteria (Bacillus cereus, Staphylococcus aureus). The copper complex shows more activity than other complexes under investigation.

Keywords: DNA binding; antibacterial activity; Transition Metal complexes; 2-acetylpyridine isonicotyinoylhydrazone

INTRODUCTION

Schiff base ligands occupy pivot position in coordination chemistry. The ligands have numerous applications. Because of their novel properties, the Schiff base metal complexes have gained lot of importance in Biology and Medicine. Important types of Schiff base ligands are thiosemicarbazones, semicarbazones, oximes, hydrazones. These ligands have been frequently used in Inorganic and Analytical chemistry. Among them, hydrazones have received special attention from researchers due to their excellent chelating capability and structural diversity, rigidity and stability that can provide to the prepared metal complexes. Hydrazones have been investigated for new drug development. Analogous compounds of hydrazones act as anti-tumour agents with the support of suitable carrier proteins. The real impetus towards studying coordination compounds of hydrazones has been due to their remarkable biological activities [1].

Isonicotinoylhydrazones (INHS) occupy distinct position in the domain of hydrazones due to their biological pharmacological applications. INHS have been used in the treatment of tuberculosis, cancer and diseases due to the iron overload. Isonicotinoylhydrazones were first prepared by Sah and Peoples by reacting isoniazid with various aldehydes/ ketones. These compounds showed inhibitory activity in mice infected with various strains of M. Tuberculosis. Due to the synthetic facility, biological potency and pharmacological activates of isoniazid group, several isonicotinoyl hydrazones have been synthesized and their transition metal complexes are investigated. The heterocyclic isonicotinoylhydrazones constitute an important class of hydrazones. These hydrazones have attracted the attention of medicinal chemists due to their wide-ranging pharmacological properties including iron scavenging and anti-tubercular activities. Biological activities of many of these compounds were shown to be related to their. Chelating abilities [2].

Deoxyribonucleic acid (DNA) is the storage site of cellular information that is accessed continuously for storing and dispensing information required for existence. It acts as the main intracellular target for those who thrive to develop a new drug

for innumerable diseases, especially cancer. Coordination compounds can bind and react with specific DNA sites. It provides a means to access and manipulate cellular information and functions. Metal-DNA interaction is an exciting area of research due to their potential use as drugs, tools for biochemical and biomedical applications in gene regulation. Considerable efforts are being made to investigate Metal - DNA interactions and to develop drugs. Thus, looking to the importance of DNA binding agents with complexes of essential metal ions for successful development of antimicrobial agents, herein we report synthesis, characterization, DNA binding properties and anti bacterial activity of Cu(II), Ni(II), Co(II) and Zn(II) complexes of 2-acetylpyridine isonicotinoylhydrazones (APINH) [3].

MATERIALS AND METHODS

The reactants employed in the synthesis of 2-acetylpyridine isonicotinoyl hydrazone(APINH) were procured from Aldrich company and were used without further purification. Metal salts used were of reagent grade (Merck). Solvents were distilled before use. Calf thymus DNA was purchased from Genie Bio labs, Bangalore, India. All other chemicals were of AR grade and used without purification.

Synthesis of ligand (APINH): Equimolar (5 mmol) quantities of 2-acetylpyridine and isoniazid were taken in a 100 ml round bottom flask loaded with 20 ml of methanol. The contents were heated under reflux for 3 hours. A white colured product was formed on cooling the flask to room temperature. The product was collected by filtration, washed with a few drops of ethanol and dried in vacuum. Yield of the product is 82%, M.P-162-164$^{\text{O}}$ C. Preparation of APINH ligand is shown in Figure 1.
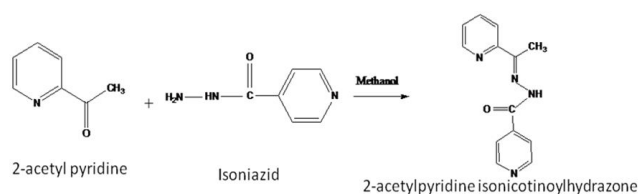

Figure 1: Preparation of APINH ligand.

Elemental analysis: C-64.00(65.02); H-5.00 (5.10); N: 23.33(24.01); IR spectra; 3189, 1668, 1581 cm-1 are assigned to ν(NH), ν(C=O) and ν(C=N) stretching vibrations respectively. 1H-NMR spectra: (in DMSO solvent). The peaks at δ (8.63-8.94) (multiplet 4$^{\text{H}}$), δ (7.36-7.62) (multiplet 4$^{\text{H}}$), δ(8.13) (singlet 1$^{\text{H}}$), δ(3.34) (singlet 3H), are respectively assigned to pyridine, isonicotine, -NH and methyl protons. GC-MS spectrum of APINH shows molecular ion peaks at (m/z) 240. It corresponds to the molecular formula C13H12N4O. Mass spectrum of APINH is shown in Figure 2.
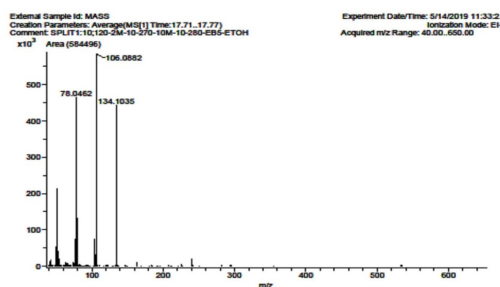

Figure 2: Mass spectrum of APINH ligand.

Synthesis of complexes: The complexes were prepared by mixing hot methanolic solution (20 ml) of APINH (1g, 0.41 mmol) with suitable metal salt (C$^{\text{O}}$Cl2.6H2$^{\text{O}}$, NiCl2.6H2$^{\text{O}}$ CuCl2.2H2$^{\text{O}}$, ZnCl2,; 0.41 mmol) dissolved in methanol (20ml) in 1:1 ratio in a clean 100- ml round bottom flask and the contents were heated under reflux on water bath for 3 hr. The resulting solution was allowed to stand at room temperature for 1 hr. On slow evaporation, coloured complexes which separated out were collected by filtration, washed with methanol followed by hexane and dried in vacuum. Physicochemical and analytical data of complexes are given in Table 1. Physical measurements: The equipment used in the present study are described by us in our recently published articles. DNA binding experiments: Experimental details are given in our recently published article. Antibacterial activity studies: The bacteria used and protocols employed in this study are given in our recently published article [4].

RESULTS AND DISCUSSION

The complexes are prepared by reacting APINH with suitable metal chloride. The reaction of The compounds are non-hygroscopic, and soluble in ethanol and freely soluble DMF and DMSO solvents. Colour, yield, ESI-MS and molar conductivity data of complexes are given Table 1. Typical ESI-Mass spectrum of Ni (APINH)2 is shown in Figure 3. A peak is observed at 537.12 due to the formation molecular ion peak in conformity with molecular formula, [Ni(C13H11N4$^{\text{O}}$)2]. Non-electrolytic nature of complexes is revealed by molar conductivity data.

S.No	Complex and its	ESI-MS	Melting	Colour	(Yield %)	Molar Conductivity
	[Formula]	(F.W)*	Point (oC)	(Yield gm)		
1	C$^{\text{O}}$(APINH)2 [Co(C13H11N4O)2]	537.11 (538.93)	248-249	Dark Brown (0.68)	22.6	22.64
2	Ni(APINH)2	537.12 (538.69)	253-254	Light	16.1	36.51

	[Ni(C13 H11N4 O)2]			Brown		
				-0.52		
3	Cu(API NH)2	542.12	267-268	Green	35.5	20.13
	[Cu(C1 3H11N 4O)2]	-543.54		-0.36		
4	Zn(API NH)2	537.12	235-236	Yellow	51.6	-
	[Zn(C13 H11N4 O)2]	-545.38		-0.58		

*Calculated values are given in parenthesis.

Table 1: Physicochemical And Analytical Data Of Complexes.

Electronic spectra: Typical electronic spectrum of Co(APINH)2 complex is shown in

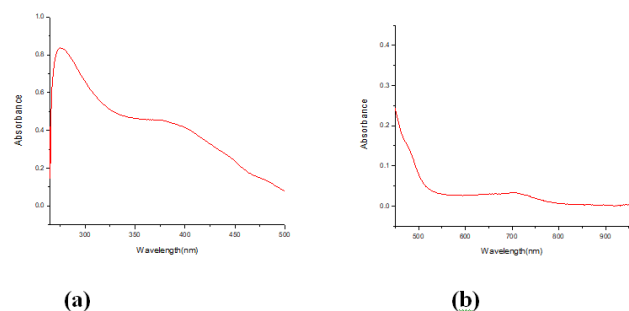Figure 3. Electronic spectral data and assignment of bands are given in Table 2.

(a)   (b)

Figure 3: Electronic spectra of Co(APINH)2 complex in a) in UV and (b) in visible regions.

S.No	Complex	Wavelength λ max (nm)	Frequency (cm-1)	Assignment
1	Co(APINH)2	275	36,363	π - π* Transition
		377	26,525	Charge Transfer Transition
		712	14,044	d-d transition
2	Ni(APINH)2	279	35,842	π - π* Transition
		307	32,573	Charge Transfer Transition
		762	13,123	d-d transition
3	Cu(APINH)2	272	36,764	π - π* Transition
		381	26,246	Charge Transfer Transition
		702	14,245	d-d transition

Table 2: Electronic Spectral Data For Co(Ii) Ni(Ii) And Co(Ii) Complexes.

In the visible spectra of Co(APINH)2, Ni(APINH)2 and Cu(APINH)2 complexes weak peaks are respectively observed at 14,044, 13,123 and 14,245 cm-1 which are assigned to $4T_{1g} \rightarrow 4T_{2g}$, $3A_{2g} \rightarrow 3T_{2g}$ and $2E_g \rightarrow 2T_{2g}$ electronic transitions respectively in favour octahedral structures for the complexes.

ESR spectral data of in solid and liquid state in DMF are given in Table 3. The g values were computed from the spectrum using tetracyanoethylene (TCNE) free radical as the g marker * ESR spectral data of Cu(APINH)2 complex in Solid and Liquid state at LNT (RT data are given in parenthesis)

Solid state ESR spectra: The g∥ and g⊥ values for Cu(APINH)2 complex are respectively found to be 2.15 and 2.05 in solid state at room temperature and liquid nitrogen temperature. According to Kivelson and Neiman the g∥ is less than 2.3 indicates covalent character and if it is greater than 2.3 suggests ionic character of the metal –ligand bonding. The g∥ value for the present complex suggests covalent character in M-L bond. The trend, g∥ > g⊥ > 2.0023 suggest that the unpaired electron predominantly in the $d_{x^2-y^2}$ orbital characteristic of octahedral geometry for copper (II) complex . The axial symmetry parameter G is defined as, $G = (g∥ - 2.003) / (g⊥ - 2.003)$

The calculated G value for the complex is found to be 3.0 which indicates the absence of exchange coupling and misalignment of molecular axes.

Liquid state ESR spectra: The typical ESR spectrum of Cu(APINH)2 complex in DMF at Liquid nitrogen temperature (LNT) and Room temperature ESR spectra of complexes in DMF at liquid nitrogen temperature (LNT) exhibit well resolved peaks at low field and at high field corresponding to g∥ and g⊥ respectively.

ESR Spectral parameters*										
	g∥	g⊥	g ave	G	A∥ ×10-5	A⊥ ×10-5	K∥	K⊥	λ	α2
Solid	2.15	2.05	2.08	3.09	-	-	0.98 5	0.86 6	513	-
	-2.15	-2.0 5	-2.0 8	-3.0 9						
Liquid	2.29	2.03	2.11	10.3 8	0.00 146	0.00 068	0.98 96	1.09	951	0.69 7

-2.1	-2.0 3	-2.0 5	-3.5 3

Table 3: Esr Spectral Data Of Cu(Apinh)2 At Lnt In Dmf Solvent

The spin Hamiltonian, orbital reduction and bonding parameters of complexes are incorporated in Table 3. The A|| and A$^\perp$ are the separation between two adjacent g|| and two adjacent g$^\perp$ peaks respectively (in cm-1). The orbital reduction parameters (K||, K$^\perp$) are calculated using the following equations,

g|| = ge - 8 K||2λ

ΔE(d-d)

g$^\perp$ = ge - 2 K$^\perp$2λ

ΔE(d-d)

Hathaway pointed that that for pure sigma bonding K|| = K$^\perp$ = 0.77 and for in-plane pi bonding K|| < K$^\perp$, while for out-plane pi bonding K|| >K$^\perp$. For the complex K|| and K$^\perp$ are 0.989 and 1.09 respectively. These values suggest the presence of in-plane pi bonding in the complex. The factor α2, which is usually taken as a measure of covalence is evaluated by the expression,

α2 = A|| / p + (g|| -2.0023)3/7(g$^\perp$ -2.0023) + 0.004

The α2 value (0.697) for the complex suggest the covalent nature [36] of metal ligand bond.

IR spectra: IR spectrum of APINH is compared with those of metal complexes to determine donor atoms of ligand. Important IR spectral bands and their assignment are given in Table 4.

APINH	Co(APINH)2	Ni(APINH)2	Cu(APINH)2	Zn(APINH)2	Assignment
3189	-	-	-	-	νNH
1668	-	-	-	-	ν C=O
~~	1604	1592	1618	1635	ν C=N
	1170	1062	1022	1162 1067	ν C-O
	596	569	567	554	νM-O
	491	488	461	498	νM-N

Table 4: Ir Spectral Data (Cm-1) Of Apinh And Its Metal Complexes

The IR spectra of the ligand shows prominent bands at 3189 and 1668 cm-1 due to νN-H , and νC=O stretching modes. These bands disappeared in spectra of complexes due to enolization followed by complex formation with metal ions. New band in the 1022-1170 cm-1 range is appeared in the spectra of complexes due to νC-O vibration. A strong (and new) peak is observed in the spectra of all the complexes in 1592-1635 cm-1 region due to azomethine (νC=N) involved in coordination. IR

data suggest that the ligand acts as mono basic tridentate ligands in all the complexes. The non- ligand bands in 596-554 and 498-461 cm-1 regions are tentatively assigned to ν(M-O), and ν(M-N) vibrations respectively [5].

Based on physicochemical and spectral data a general structure (Figure 4) for the complexes is proposed.
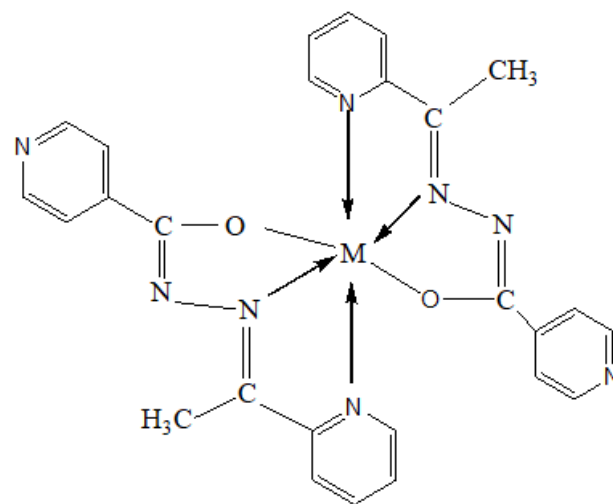

Figure 4: Proposed structure for the complexes [M = Co(II),Ni(II),Cu(II) and Zn(II)].

The cathodic peak current function values were found to be independent of the scan rate. Repeated scans at various scan rates suggest that the presence of stable redox species in solution. It has been observed that cathodic (Ipc) and anodic (Ipa) peak currents were not equal. The E1/2 values of metal complexes are noticed at potential range of + 0.277 – 0.835 V. It may be concluded that all the bivalent metal complexes undergo one electron reduction to their respective M(I) complexes. The non-equivalent current in cathodic and anodic peaks indicates quasi-reversible behaviour. The difference, ΔEp in all the complexes be better than the Nerstian requirement 59/n mV (n = number of electrons involved in oxidation reduction), which demonstrate quasi-reversible character of electron transfer. The complexes show large separation between anodic and cathodic peaks indicating quasi-reversible character [6].

DNA binding studies: The interaction of metal complexes with calf-thymus DNA was monitored by UV-visible spectroscopy. Absorption spectra were recorded in 250-500 nm range in presence and in absence of DNA.

Metal complexes exhibit an intense absorption band in high energy region, which is attributed to metal-ligand charge transfer (MLCT) transitions. The change in absorbance values with increasing amounts of CT-DNA was used to evaluate the intrinsic binding constant Kb, for the complexes. Based on the variation in absorption, the intrinsic binding constant or association constant (Kb) of the metal complex may be calculated according to the Benesi-Hildebrand equation, modified by Wolfe et al.

[DNA]/(ϵa-ϵf) = [DNA]/(ϵb-ϵf) + 1/Kb (ϵb-ϵf)

Where, εa , εf and εb correspond to A observed /[complex], the extinction coefficient for the free metal complex, and the extinction coefficient for the metal complex fully bound to DNA, respectively, Kb represents the binding constant. The binding constants of these complexes are given in the presence of increasing amounts of CT-DNA, the UV-visible absorption spectra of metal complexes show a small bathochromic shift (Red shift) (Δ λmax = 0.5-1.0 nm). The calculated binding constants are found in the range 2.5 – 3.5 x 105 M-1.

Metal complexes binding to DNA through intercalation usually result in hypochromism and bathochromism or hypsochromism, while hyperchromism has been attributed to electrostatic attraction, hydrogen bonding and groove (minor or major) binding along the outside of DNA helix. The extent of the changes that appear in metal complex spectrum are usually consistent with strength of interaction that takes place. Such small bathochromic shifts is more inkeeping with groove binding, leading to small perturbations. The Kb values here are lower than that reported for classical intercalator for ethidium bromide and [Ru(Phen)2DPPZ]2+ complex whose binding constants have been found to be in the order 106 - 107. The observed binding constants for the present complexes are in accordance with groove binding with DNA as reported in the literature. It is pertinent to note that the binding constant for NI(APINH)2 complex is quite high. The increasing order of binding constant is given below:

Cu(APINH)2 < Zn(APINH)2 < Co(APINH)2 < Ni(APINH)2
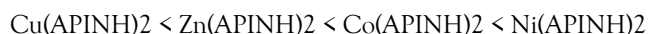
The above order suggest that the Ni(APINH)2 complex binds DNA more strongly.

Antibacterial activity studies: The metal complexes wer screened for their antibacterial activity by using agar well diffusion method against pathogenic bacterial strains such as E. coli, K. pneumonia and S. Aureus, B.cereus. Inhibition zones were estimated in the presence of varying amounts (100, 200 and 300 μg/well) of complexes with reference to the positive control viz. ciprofloxacin.

CONCLUSIONS

Spectra data suggest that the ligand, 2-acetylpyridine isonicotinoyl-hydrazone (APINH acts as monobasic tridentate ligand and complexes o have octahedral structure.

The complexes have covalent character as suggested by ESR spectral data copper(II) Complex

The cyclic voltammetric studies suggest that all the complexes undergo quasi-reversible one electron reduction. The non-equivalent current intensity of cathodic and anodic peak indicates quasireversible behaviour of these complexes.

The Kb values and variation in absorption spectra of metal complexes suggest groove binding.

The nickel complex binds DNA more strongly than other complexes while the copper complex shows more antibacterial activity than other complexes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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