Synthesis, characterization, deoxyribonucleic acid binding and antimicrobial activity of bivalent metal complexes with 2-acetylpyridine isonicotinoylhydrazone

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ABSTRACT
Survey of literature revealed that bivalent metal complexes of nitrogenous heterocyles exhibit diverse structural and pharmacological features. The purpose of this study is to investigate structural and biological applications of metal complexes with 2-acetylpyridine isonicotinoylhydrazone (APINH). Hence Co(II), Ni(II), Cu(II) & Zn(II) compounds with APINH were produced and characterized according to Electrospray Ionization Mass Spectrometry (ESI-MS), Fourier Transform Infrared spectroscopy (FT-IR) and electronic spectral data. The copper complex was also studied using electron spin resonance (ESR) spectroscopy. DNA interactions of complexes were investigated by using UV-spectroscopy. The metal derivatives were examined for antimicrobial action across Bacillus cereus, Staphylococcus aureus, Escherichia coli & Klebsiella pneumonia employing agar-well diffusion process. Conductivity data suggest non-electrolytic behaviour of compounds. FT-IR results indicate uni-negative tridonor nature of ligand. UV-Visible data confirms octahedral structure for the complexes. ESR data suggest covalent nature of M─L bond. The data indicate that the nickel derivative sticks to DNA more firmly. Copper compound manifests more effectively than other investigated metal derivatives.

INTRODUCTION
Metallo-azomethines have received exceptional curiosity of inorganic chemists because of their excellent structural diversity, rigidity, and stability (Angelusiu et al., 2010; Aslan et al., 2011; Wang et al., 2009). Hydrazones have been investigated for the new drug development. Analogous compounds of hydrazones act as anti-tumour agents with the support of suitable carrier proteins (Giraldi et al., 1980). The interest in studying metallo-hydrazones has been due to their significant biotic actions.

Isonicotinoylhydrazones (INHs) occupy distinct position in the domain of hydrazones due to their biological and pharmacological applications (Rollas et al., 2007). INHs have been used in the treatment of tuberculosis (Cocco et al., 1999), cancer (Chitambar et al., 1999), and diseases due to the iron overload. INHs were first prepared by Sah and Peoples using isoniazid and suitable carbonyl compound. The composites showed action across the strains of Mycobacterium tuberculosis. Due to the synthetic facility, biological potency, and pharmacological activates of isoniazid group several INHs have been synthesized and their transition metal complexes are investigated. The heterocyclic INHs constitute an important class of hydrazones. These hydrazones have attracted the attention of medicinal chemists because of their diverse pharmacological properties covering iron scavenging and anti-tubercular activities (Krishnamoorthy et al., 2011). Biological activities of many of these compounds were explained (Patole et al., 2003) according to their DNA binding abilities.

Deoxyribonucleic acid (DNA) is the treasury of cellular intelligence that is interfaced uninterruptedly for depositing and distributing essential message for the survival. It has been regarded as an important intracellular prey for chemists who achieve success in developing a new drug for dreadful diseases, particularly for
cancer. Coordination compounds are known to interact with specific sites of DNA. These interactions have been used to access and manipulate functions and cellular information. Metal–DNA interaction is a thrilling area of research because of their potential use in the development of medicines. Significant attempts are being made to investigate Metal–DNA interactions (Moksharagni et al., 2017; Suseelamma et al., 2018) and to develop drugs. Recently we have reported (Moksharagni et al., 2016; Pragathi et al., 2013; Srinivasulu et al., 2019) structural elucidation of Ln(III)-2-acetylpyridine isonicotinoyl-hydrazine (APINH) complexes and their interaction with DNA. So far transition metal complexes of APINH are not investigated and focused as DNA binding agents and antimicrobial agents. Thus, looking to the significance of metal complexes as DNA binding agents for the successful development of antimicrobial agents, we have studied bivalent metal complexes of APINH and the results are presented in this article.

MATERIALS AND METHODS

The precursors of APINH were procured from Aldrich company and were utilized as such. LR (Merck) grades metal chlorides & CT DNA bought from Genie Bio labs used in the present study. Remaining are Analytical Reagent chemicals and utilized as such.

Preparation of ligand (APINH)

Equimolar (5 mmol) quantities of 2-acetylpyridine and isoniazid were taken in a 100-ml RB flask containing 20 ml of methyl alcohol and refluxed for 3 hours. A white colored product was appeared on chilling the flask to room temperature (RT). The product was separated by filtration, cleaned with 5 ml of ethanol, and dried. Yield, 82%, M.P., 163°C–164°C. The ligand was recrystallized from ethanol. The preparation of APINH ligand is shown in Figure 1.

Preparation of complexes

The compounds were produced by combining APINH (1 g, 0.41 mmol) and suitable metal salt (0.41 mmol) in a 100-ml round bottom flask containing 40 ml of methyl alcohol. The reaction mixture was refluxed on water bath up to 3 hours. After 1 hour and on slow evaporation, chromatic complexes were formed, separated by exudation, cleaned with methyl alcohol subsequently by hexane, and dried. Preliminary data of metal derivatives are given in Table 1.

Equipment

The instruments used in the present study are described by us in our recently published articles (Moksharagni et al., 2016).

DNA binding experiments

Experimental details are given in our recently published article (Nagamani et al., 2020).

Antibacterial activity studies

The bacteria used and protocols employed in this study are given in our recently published article (Srinivasulu et al., 2019).

RESULTS AND DISCUSSION

The ligand (APINH) was easily prepared by reacting 2-acetylpyridine and isoniazid in high yield (82%). APINH is characterized as per analytical data. Elemental analysis: C-64.00 (65.02); H-5.00 (5.10); N: 23.33 (24.01); IR spectra (KBr) Peaks at 3,189, 1,668, 1,623, 1,581, 991 and 753 cm$^{-1}$ are allocated to $\nu$(NH), $\nu$(C=O) (Amide I), $\nu$(C=N), $\nu$(Py ring), $\nu$(Py bending), and $\gamma$(Py ring oop bending) vibrations, respectively. $^{1}$H-NMR spectra: (in dimethyl sulphoxide (DMSO) solvent); $\delta$ 8.60–8.94 (multiplet

![Figure 1. Preparation of APINH.](image)

| S. No. | Complex and its [Formula] | ESI-MS (F.W)$^a$ | Melting point (°C) | Color (yield gm) | (Yield %) | Molar conductivity$^a$ (Ω$^{-1}$ cm$^2$ mol$^{-1}$) |
|-------|--------------------------|------------------|--------------------|-----------------|-----------|----------------------------------|
| 1     | Co(APINH)$_2$$^b$$^b$[Co(C$_{13}$H$_{11}$N$_4$O)$_2$] | 537.11 (538.93) | 248–249            | Dark Brown (0.68) | 22.60     | 22.64                            |
| 2     | Ni(APINH)$_2$$^b$$^b$[Ni(C$_{13}$H$_{11}$N$_4$O)$_2$] | 537.12 (538.69) | 253–254            | Light Brown (0.52) | 16.10     | 36.51                            |
| 3     | Cu(APINH)$_2$$^b$$^b$[Cu(C$_{13}$H$_{11}$N$_4$O)$_2$] | 542.12 (543.54) | 267–268            | Green (0.36)      | 35.50     | 20.13                            |
| 4     | Zn(APINH)$_2$$^b$$^b$[Zn(C$_{13}$H$_{11}$N$_4$O)$_2$] | 537.12 (543.38) | 235–236            | Yellow (0.58)     | 51.60     |                                   |

$^a$Theoretical data are given in brackets.
4H), δ 8.13 (singlet 1H), δ 7.36–7.62 (multiplet 4H) δ 3.34 (singlet 3 H), are assigned to pyridine, >NH, isonicotine and –CH₃ protons. Mass spectrum (Fig. 2) of APINH shows molecular ion peak at (m/z) 240. It corresponds to chemical formula C₁₃H₁₂N₄O.

The complexes are prepared by reacting APINH with suitable metal chloride. The compounds are not hygroscopic, and solvable in ethyl alcohol and freely in dimethylformamide (DMF) and DMSO. Color, yield, Electrospray Ionization Mass Spectrometry (ESI-MS) and molar conductivity results are delineated in 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 emergence of M⁺ peak in conformity with molecular formula, [Ni(C₁₃H₁₁N₄O)₂]. Solutions (1 × 10⁻³M) of complexes were prepared using DMF solvent for measuring the conductivity. Non-electrolytic behavior of complexes is indicated by conductivity results.

**Electronic spectra**

Typical electronic spectrum of Co(APINH)₂ complex is depicted in Figure 4. Electronic spectral data and assignment of bands are delineated in Table 2. In the visible spectra of Co(APINH)₂, Ni(APINH)₂, and Cu(APINH)₂ complexes, weak peaks (ε = 5–10 L·mol⁻¹·cm⁻¹) are, respectively, observed at 14,044, 13,123, and 14,245 cm⁻¹ which are assigned to ⁴T₁g → ⁴T₂g, ¹A₁g → ¹T₂g, and ²E → ²T₂g electronic transitions in favor octahedral structure for the complexes (Pragathi et al., 2013; Srinivasulu et al., 2019).

**Electron spin resonance (ESR) spectral studies**

ESR spectra of Cu(II) complex in solid and solution (in DMF) states were documented at RT and at liquid nitrogen temperature (LNT). Spectra are depicted in Figure 5 and results...
are delineated in Table 3. Using tetracyanoethylene (TCNE) radical as reference the ‘g’ values of complex were calculated.

Solid state ESR spectra

The g parallel and g perpendicular data of Cu(APINH)_2 compound are located at 2.15 and 2.05, respectively. The g_1 value (less than 2.3) of complex suggests covalent character in M-L bond (Hussain Reddy et al., 1999). The trend, g_1 > g_⊥ > 2.0023 suggest that the single electron mostly resides in d_{x^2–y^2} orbital which is considered as a trait of octahedral structure for the complex. The axial symmetry parameter G may be evaluated by applying the formula

\[ G = \frac{g_1 - 2.003}{g_⊥ - 2.003} \]

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

Solution state

Spectra of Cu(APINH)_2 compound are depicted in Figure 5. Spectrum recorded at LNT exhibits well separated peaks in low field & in high field regions matching to g_∥ and g_⊥ sequence.
Important spectral data, \( g_{||}, g_{\perp}, G, A \parallel \) (separation between two adjacent \( g_{||} \) peaks), \( A_{\perp} \) (separation between two adjacent \( g_{\perp} \) peaks), \( K_{||} \) and \( K_{\perp} \) (orbital reduction parameters), \( \lambda \) (spin-orbit constant) and \( \alpha^{2} \) (covalent factor) are calculated and data are summarized in Table 3. The \( K_{||} \) & \( K_{\perp} \) are determined utilizing equations given below

\[
g_{||} = \frac{g_{e} - 8 K_{||}^{2} \lambda}{\Delta E(d-d)}
\]

\[
g_{\perp} = \frac{g_{e} - 2 K_{\perp}^{2} \lambda}{\Delta E(d-d)}
\]

According to Hathaway, \( K_{||} > K_{\perp} = 0.77, K_{||} < K_{A} \), and \( K_{||} > K_{\perp} \) for pure \( \sigma \) binding, for in-plane \( \pi \)-binding and for out-plane \( \pi \)-binding, respectively. For the present copper compound \( K_{||} \) and \( K_{\perp} \) are 0.989 and 1.09 sequentially. For this copper compound, the \( K_{||} \) value is less than \( K_{\perp} \). This trend indicates the appearance of in-plane \( \pi \)-binding. Covalent factor \( \left(\alpha^{2}\right) \), is calculated using equation given below.

\[
\alpha^{2} = \frac{A_{\parallel}}{\rho} + (g_{||} - 2.0023)3/7(g_{\perp} - 2.0023) + 0.004
\]

Data (Table 3) advise covalent nature (Wertz et al., 1986) of metal ligand bond.

**Infrared spectra**

IR spectrum of APINH is juxtaposed with its metal compounds to reveal donating atoms. Selected IR peaks and their allocations are presented in Table 4. The IR spectrum of the APINH shows main peaks at 3,189 and 1,668 cm\(^{-1}\) due to \( \nu_{N-H} \), and \( \nu_{C=O} \) stretching modes. Such peaks are absent in spectra of metal compounds due to enolization followed by complex formation with metal ions. In the spectra of compounds, a new peak present in the 1,022–1,170 cm\(^{-1}\) range is assigned to \( \nu_{C-O} \) vibration. A strong (and new) peak is noticed in the spectra of all the compounds in 1,592–1,635 cm\(^{-1}\) range due to azomethine \( (\nu_{C=N}) \) involved in coordination. Results revealed that the APINH functions as uni-negative tridonor ligand in all the compounds. New peaks in 596–554 and 498–461 cm\(^{-1}\) range are allocated to \( \nu_{(M-O)}, \) and \( \nu_{(M-N)} \) stretching vacillations sequentially.
In accordance with preliminary and chromatic data, a common structure (Fig. 6) for the compounds is suggested.

Cyclic voltammetry (CV)

Electrochemistry of metal-compounds was explored by utilizing CV in DMF using 0.1 M (CH₃CH₂CH₂CH₂)₄N(PF₆) as electrolyte. Voltammogram of Ni(APINH)₂ compound is depicted in Figure 7 and related CV results are given in Table 5.

The observed cathodic peaks currents do not depend on scan rate. Replicated recordings at 5, 10, and 20 mV S⁻¹ suggests that the metal complexes are stable even in solution state. The E¹/₂ values of metal complexes are found to be in +0.277 to −0.835 V range. Dissimilar currents of +ve and −ve peaks indicate nearly-reversible behavior. The variation, ΔEp in all the complexes be more than 59/n mV (where, n = aggregate of electrons participated in redox reaction), suggesting nearly-reversible nature of electron transfer (Pragathi et al., 2014).

DNA interaction studies

The interactivity of coordination compounds with calf-thymus DNA was tracked by absorption (UV-Visible) spectroscopy. Spectra (Fig. 8) were recorded in 250–500 nm range with and without DNA. The concentrations of CT-DNA and metal complexes utilized, respectively, are 53 × 10⁻⁶ M and 20 × 10⁻⁶ M. Metal complexes showed strong peak in UV region due to M→L CT transition. The difference in absorbance with raising quantities of CT-DNA was

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**Table 4.** Selected IR data (cm⁻¹) of APINH and its metal compounds.

|         | APINH | Co(APINH)₂ | Ni(APINH)₂ | Cu(APINH)₂ | Zn(APINH)₂ |
|---------|-------|------------|------------|------------|------------|
| ν̇NH    | 3,189 | –          | –          | –          | –          |
| ν̇C=O   | 1,668 | –          | –          | –          | –          |
| ν̇C=N   | 1,170 | 1,592      | 1,618      | 1,635      | –          |
| ν̇C-O   | 596   | 569        | 567        | 554        | –          |
| ν̇M-O   | 491   | 488        | 461        | 498        | –          |

**Figure 6.** A common proposed structure for complexes [M = Co(II), Ni(II), Cu(II) and Zn(II)].

**Table 5.** CV data of bivalent metal complexes.

| S. No | Complex   | Redox couple | Epc    | Epa    | ΔEp (mV) | E¹/₂ | i/iₐ | LogKc⁺ |
|-------|-----------|--------------|--------|--------|----------|------|------|--------|
| 1     | Co(APINH)₂| II/I         | −1.03  | −0.566 | 464      | −0.798| 1.863| 0.071  |
| 2     | Ni(APINH)₂| II/I         | −1.07  | −0.60  | 470      | −0.835| 2.120| 0.072  |
| 3     | Cu(APINH)₂| II/I         | −0.032 | 0.523  | 555      | +0.277| 1.652| 0.060  |
| 4     | Zn(APINH)₂| II/I         | −1.132 | −0.464 | 668      | −0.798| 1.872| 0.050  |

ΔG° = −2.303RTlog Kc⁺
utilized to judge inherent binding constant \( (K_b) \), for complexes. The \( K_b \) data of the metal compounds are estimated by using modified equation (Wolfe et al., 1987) and results are delineated in Table 6.

With raising amounts of CT-DNA, the spectra of metal compounds show bathochromic shift \( (\Delta \lambda_{\text{max}} = 0.5–1.0 \text{ nm}) \). The determined \( K_b \) values are found in the range \( 2.5–3.5 \times 10^5 \text{ M}^{-1} \).

Metal derivatives that cohere to DNA by intercalation generally show hypochromism and bathochromism or hypsochromism. Electrostatic attraction, hydrogen bonding and groove (minor or major) binding causes hyperchromism. Small bathochromic shifts and low \( K_b \) values of present complexes are suggestive of groove binding with DNA in analogy with previous observation (Ramakrishnan et al., 2011). The binding constant order is delineated below:

\[
\text{Cu(APINH)}_2 < \text{Zn(APINH)}_2 < \text{Co(APINH)}_2 < \text{Ni(APINH)}_2
\]

The sequence indicates that the Ni(APINH)\(_2\) complex tie up with DNA more firmly.
Table 6. Spectral data upon addition of CT-DNA to the compounds.

| S. No | Complex | λmax (nm) | Δλ | H% | $K_b$ [M$^{-1}$] |
|-------|---------|-----------|----|----|-----------------|
|       |         | Free      | Bound |    |                 |
| 1     | Co(APINH)$_2$ | 375       | 375.5 | 0.5 | 21.27 | 2.8 $\times 10^5$ |
| 2     | Ni(APINH)$_2$ | 356       | 357    | 1.0 | 28.19 | 3.5 $\times 10^5$ |
| 3     | Cu(APINH)$_2$ | 357       | 357.5  | 1.0 | 18.53 | 2.5 $\times 10^5$ |
| 4     | Zn(APINH)$_2$ | 346       | 346.5  | 0.5 | 17.57 | 2.7 $\times 10^5$ |

Table 7. Antimicrobial activity data of metal complexes.

| S. No | Sample          | Treatment (concentration) | E. coli (Mean ± SE) | K. pneumonia (Mean ± SE) | S. aureus (Mean ± SE) | B. cereus (Mean ± SE) |
|-------|-----------------|---------------------------|---------------------|--------------------------|-----------------------|-----------------------|
| 1     | S-Ciprofloxacin | (5 µg/µl)                 | 10.50 ± 0.02        | 09.80 ± 0.09             | 10.03 ± 0.03          | 12.16 ± 0.05          |
| 2     | Co(APINH)$_2$   | 100 µg/µl                 | 1.18 ± 0.02         | 1.17 ± 0.03              | 1.78 ± 0.01           | 1.14 ± 0.01           |
|       |                 | 200 µg/µl                 | 1.37 ± 0.01         | 1.47 ± 0.02              | 1.80 ± 0.05           | 1.45 ± 0.02           |
|       |                 | 300 µg/µl                 | 1.62 ± 0.02         | 1.93 ± 0.04              | 1.97 ± 0.02           | 1.87 ± 0.03           |
| 3     | Ni(APINH)$_2$   | 100 µg/µl                 | 2.25 ± 0.04         | 2.27 ± 0.02              | 2.04 ± 0.02           | 2.01 ± 0.02           |
|       |                 | 200 µg/µl                 | 2.34 ± 0.04         | 2.30 ± 0.01              | 2.15 ± 0.03           | 2.13 ± 0.02           |
|       |                 | 300 µg/µl                 | 2.68 ± 0.02         | 2.47 ± 0.01              | 2.36 ± 0.04           | 2.20 ± 0.02           |
| 4     | Cu(APINH)$_2$   | 100 µg/µl                 | 5.10 ± 0.02         | 4.20 ± 0.05              | 4.10 ± 0.06           | 5.15 ± 0.02           |
|       |                 | 200 µg/µl                 | 5.47 ± 0.02         | 4.37 ± 0.05              | 4.34 ± 0.06           | 5.25 ± 0.02           |
|       |                 | 300 µg/µl                 | 5.78 ± 0.01         | 4.53 ± 0.05              | 4.50 ± 0.06           | 5.40 ± 0.02           |
| 5     | Zn(APINH)$_2$   | 100 µg/µl                 | 2.01 ± 0.01         | 2.07 ± 0.07              | 2.30 ± 0.01           | 2.30 ± 0.05           |
|       |                 | 200 µg/µl                 | 2.40 ± 0.02         | 2.13 ± 0.01              | 2.41 ± 0.01           | 2.71 ± 0.01           |
|       |                 | 300 µg/µl                 | 2.55 ± 0.03         | 2.25 ± 0.02              | 2.73 ± 0.02           | 2.83 ± 0.01           |

Values are the mean ± SE of inhibition zone in mm.

Figure 9. Graphical representation of antibacterial activity of metal complexes against pathogenic bacterial strains.
Antibacterial activity studies

The compounds were checked for antimicrobial activity across bacterial strains viz. Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, and Bacillus cereus. Inhibition zones were estimated with 100, 200, and 300 μg/well of complexes by using ciprofloxacin as a reference. The width of inhibition zones (in mm) is evaluated as described before (Srinivasulu et al., 2019). Table 7 delineates results.

Activities of coordination compounds are compared using bar graph (Fig. 9). The activity of Cu(APINH)₂ complex is more significant.

CONCLUSION

Spectral results indicate that APINH acts as mono anionic tridonor species and the coordination compounds are assigned to have octahedral structure. Voltammetric investigations suggest that all the compounds undergo quasi-reversible 1e⁻ reduction. Dissimilar currents of cathodic and anodic peaks indicate quasi-reversible conduct of compounds. DNA binding constants (Kₐ) and variation in spectra of metal-compounds suggest groove binding. Copper complex shows the higher antibacterial activity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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