# Synthesis, Structural Characterization, Anti-Microbial and DNA Binding Studies Of Novel Schiff Base Metal(Ii) Complexes Derived From Isoniazid And 2-(4-Nitro-Phenyl)-2h-Isoquinolin-1-One

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*Abstract*- **Novel complexes of Cu(II), Co(II), Zn(II) and Ni(II) with isoniazid derivative (derived from 2-(4-nitro-phenyl)-2H-isoquinolin-1-one and isoniazid) were synthesised and characterized by spectroscopic techniques like elemental analysis, magnetic susceptibility measurements, molar conductivity, IR, $^{1}$H-NMR, $^{13}$C-NMR, UV-Vis., EPR, thermal analysis and FAB Mass spectra. Further, the synthesized metal complexes were subjected to DNA binding study using electrochemical method. The synthesized metal complexes exhibited significant antimicrobial activity against the organisms Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhii and candida albicans when compared with the standard antibiotic (Streptomycin). The ligand and its metal complexes were screened for antioxidant activity and all the complexes showed favorable free radical scavenging activity. Among the synthesized metal complexes, the copper complex marked the highest activity. The in vitro antimycobacterial activity against Mycobacterium tuberculosis was also assessed and summarized.**

Key words: Isonizaid, Antioxidant, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhii, candida albicans, mycobacterium tuberculosis.

## 1. INTRODUCTION

The progress in the field of medical inorganic chemistry has upshot the development of all kinds of medical implants and is chiefly concerned with metal ions in the human body [1]. Its vivid and comprehensiveness in medicinal field are stupendous and indispensable. This growing scientific discipline has to its credit of inclusions like pharmacopeia of metal–based therapeutic and diagnostic agents. Isoniazid and its derivatives form an important class of heterocyclic compounds in an extensive manner. They are pertained to have diversified therapeutic uses such as anti-carcinogenic, antioxidant, anti-inflammatory, and anti-bacterial activity [2-5]. In order to minimize the severe side effects induced by commercially available drugs a great deal of structurally similar analogues has been synthesized.

The present research work probes into the synthesizing of hybrid compounds through the combination of different metal ions with an isoniazid derivative for obtaining potent antimicrobial lead compounds and it will be scrutinized using spectral and analytical studies. Further, they were screened for different biological activities.

## 2. EXPERIMENTAL

### 2.1 Materials

All the solvents and chemicals used in the synthesis were purchased from Sigma-Aldrich suppliers and purified when necessary. The completion of the reaction was monitored by thin layer chromatography and performed on Merck percolated silica gel plates. Silica gel (60–120 mesh size, Merck) was used for column chromatography for purification purpose.

### 2.1. Synthesis of Ligand (L)

Equimolar quantity of 4-chloro-4-nitrobenzene (0.01 mmol) and 2H- Isoquinolin 1-one (0.01mmol) in ethanol medium (15-20 mL) containing few crystals of CuCl2 was refluxed for 6 hrs. The resultant solution was cooled and poured in cold water. The separated solid was filtered and recrystallized from ethyl alcohol. The resultant solid (0.01 M) and isoniazid (0.01 M) were mixed in hot ethanol and stirred for 2 hrs. Then, the

resulting mixture was refluxed for 6 hrs. It was poured in cold water to obtain solid product was filtered and recrystallized.

**L**: Yield: 74%. Mol. Formula: $C_{22}H_{18}N_4O_2$. CNH analysis Calcd: C 71.34, N 15.13, H 4.90. Found: C 71.22, N 15.04, H 4.82. $^1$H-NMR (ppm): 7.64-7.82 (6H m, isoquinoli-1-one moiety Ar-H), 7.42–7.86 (8H m, Ar-H phenyl), 3.75 (3H, s, -$OCH_3$), 10.6 (1H, s, -OH). $^{13}$C-NMR (ppm): 160.4 (C1, C=O**)**, 116.8 (C2), 109.0 (C3), 172.6 (C4), 120.6 (C5), 123.0 (C6), 126.2 (C-7), 136.0 (C-8), 116.2 (C-9), 155.4 (C10), 132.8 (C11), 126.4 (C12), 128.4 (C13), 127.0 (C14), 128.6 (C15), 170.2 (C16, C=N, imine), 128.0 (C17), 154.2 (C18), 148.8 (C19), 144.0 (C20) and 128.8 (C21). Mass spectra: *m/z* 371 [M+1]. IR (KBr, cm$^{-1}$): 3620-3250, 3120-3000, 1650.

### 2.2. Synthesis of Complexes

A hot ethanolic solution of the ligand (0.05 M) and metal acetate(s) (0.05 M) was stirred for 1 hr. The resulting mixture was refluxed for 6 hrs. The resultant solution was cooled and poured in cold water. The separated solid was filtered and washed successively with small amounts of distilled water, ethanol, and petroleum ether and dried in vacuum. A dark green coloured complex was obtained. The other metal complexes were prepared using similar procedure. The complexes were kept in a vacuum desiccator over anhydrous calcium chloride. The purity of the complexes was tested by thin layer chromatography using different solvent mixtures.

Copper complex of **L**: Yield: 66%. Mol. Formula: $C_{24}H_{21}CuN_4O_6$: CNH analysis Calcd: C 56.12, N 15.70, H 4.54, Cu 13.40. Found: C 56.06, N 15.54, H 4.35, Cu 13.30. $\mu_{eff}$(BM) = 1.88. $\Lambda_m$ (mhocm$^2$ mol$^{-1}$) = 14. MS: *m/z* 525 [M+1]. IR (KBr, cm-1): 3420-3650, 3450-3100, 1612, 1530, 1312, 754, 658, 556, 462.

### 2.3. DNA Binding Studies

The binding interactions of metal complexes with DNA were studied by electrochemical method using different concentrations of CT-DNA.

### 2.4. Antioxidant Assay

#### 2.4.1. DPPH Assay

The free radical scavenging activity of the ligand (L) and its complexes were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [4].

#### 2.4.2. Superoxide Dismutase Activity (SOD)

The SOD activity measurements were carried out in accordance with the procedure reported by Joseph et. al.,[6].

### 2.5. Antimicrobial Activities

Qualitative determination of antimicrobial activity was done using the well diffusion method. The synthesized ligand and their metal complexes were studied for antibacterial activities in DMSO solvent against bacterial species. The in vitro antimicrobial activity was performed against Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis and Salmonella typhii, respectively. The minimum inhibitory concentration (MIC) was determined by serial micro dilution method [7]. The standard antibiotic Streptomycin and DMSO were used as positive and negative control.

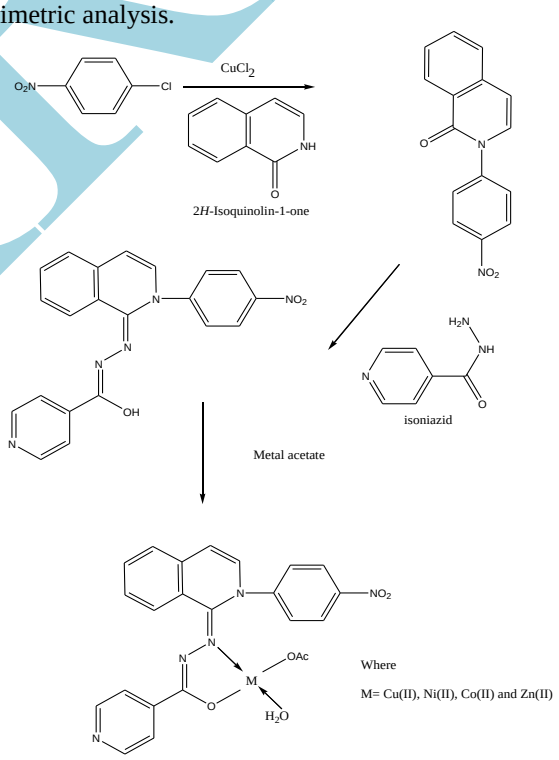
### 2.6 Antimycobacterial Activity

In vitro evaluation of the anti-tubercular activity was carried out against mycobacterium tuberculosis H37Rv by using broth dilution [8] assay method. Ciprofloxacin (MIC 9.41 µM), Pyrazinamide (MIC 25.34 µM) and Streptomycin (MIC 10.74 µM) were used as references.

## 3. RESULTS AND DISCUSSION

### Characterization

Paying considerable attention to the importance of isoniazid derivative (Ligand), a novel series of metal complexes are synthesized and its reaction sequence is outlined. The prepared ligand is allowed to react with the metal salts to form different metal (II) complexes as [MLX(H2O)]. The metal complexes are quite stable in air & light and soluble in most of the common organic solvents. The formation of product was confirmed by single spot in the TLC. This confirms that the product formed, from the condensation reaction followed by cyclization. The structure of the ligand and its metal complexes are established using elemental analysis, IR, UV-Vis, NMR, electronic spectra, FAB-MS and thermo gravimetric analysis.




**Scheme 1. Synthesis of metal complexes of Schiff base ligand**

### 3.1. Molar Conductance Measurements

Molar conductance values for 10−3 M solutions of copper, nickel, cobalt and zinc complexes in DMSO are found in the range of 6–12 mho cm2 mol−1. These values are in accordance with the non-electrolytic nature of the complexes [9].

### 3.2. IR Spectral Studies

The important vibrational bands of the IR spectra of the ligand and its metal complexes are summarized in the experimental section. The FTIR spectra of ligand (L) and its metal (Zn(II), Ni(II), Co(II) &) complexes are shown in figures 1 & 2.When comparing the IR spectra of the ligand and their metal complexes, significant differences in vibrations are observed for selective frequencies [10-12]. Absence of characteristic stretching frequencies of C=O and $NH_2$ groups at 1690 $cm^{-1}$ and 3550 $cm^{-1}$ from 2-(4-nitro-phenyl)-2H-isoquinolin-1-one and isoniazid, indicates the Schiff base formation [13]. This is further evidenced by the stretching vibration around 1638 $cm^{-1}$ which is assigned to ν(C=N) vibration. On complexation, this band is shifted to a lower value of about ~30 $cm^{-1}$ indicating that imine nitrogen undergoes chelation with the central metal ion(s) in the complex(es) (Figure 4) [14]. In the IR spectra of Cu(II) complex, the band appearing at 1236-1290 $cm^{-1}$ is assigned to enolic υ(C-O) stretching vibration. This band in the corresponding free ligand is found at 1220-1232 $cm^{-1}$. The observed downward shift of this band, indicates the participation of enolic carbonyl oxygen of isoniazid moiety of the ligand in complex formation on chelation. The higher υ(C-O) frequency in metal complexes may be attributed to the transfer of σ-electron density from oxygen atom to metal ion resulting an increase in electronegativity of oxygen atom. This leads to greater ionic character of the bond and following increase in υ(C-O) vibrational frequency on complexation[15-17]. The metal complexes exhibited two new strong bands around 1480-1522 $cm^{-1}$ and 1270-1318 $cm^{-1}$ regions attributed to the asymmetric $\nu_{asy}$(COO-) and symmetric vibration $\nu_{sy}$($COO^-$) of carboxylate ion respectively. The difference, Δν, between the two stretching vibrational frequency is found to be 206 $cm^{-1}$, which indicates that there is a coordination between the carboxyl group of acetate with the central metal ion [18]. The band appears at 542-554 $cm^{-1}$, assigned to the ν(M-O) frequency, additionally supports the presence of the coordination between the carboxyl group of acetate with the central metal ion. The ligand thus coordinates to the metal ion through the imine nitrogen and the hydroxyl oxygen making its as bidentate ligand. The other coordinating sites are oxygen atom of acetate and water molecule, respectively.

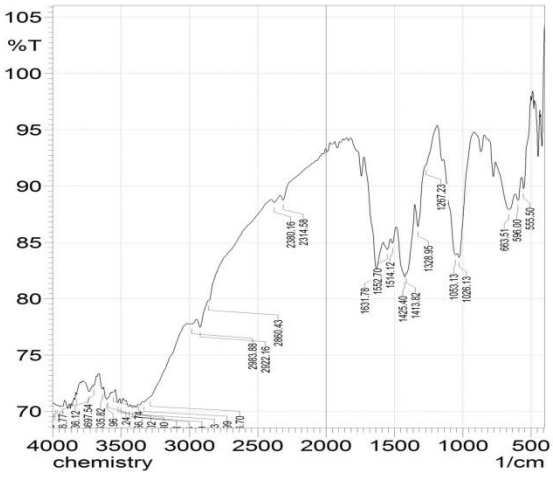



**Figure 1. IR spectrum of Isoniazid derivative**

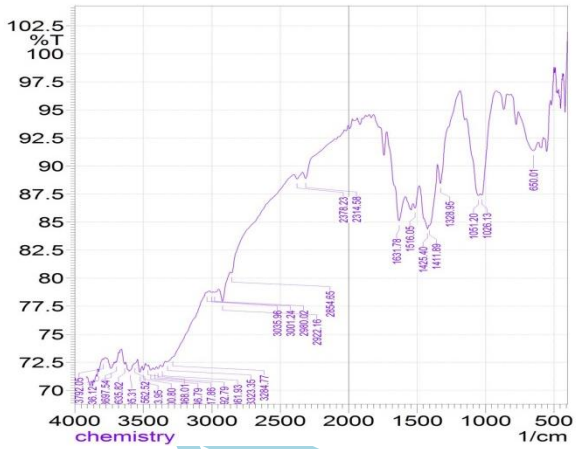



**Figure 2. IR spectrum of Zinc complex of isoniazid derivative**

### 3.3 $^1$H-NMR

The Schiff base ligand formation is further confirmed by the proton NMR spectral study. The 1H NMR of free Schiff base ligand showed a peak at 10.6 ppm which was assigned to the hydroxyl proton of the ligand. In the Zinc complex, the absence of the above peak confirms the complex formation. All the other peaks appear in the same region with trivial changes due to coordination of imine nitrogen. Similar spectra are recorded for the other complexes also.

### 3.4. Electronic Spectra and Magnetic moment

The electronic spectra of free Schiff base ligand and its metal complexes were recorded. In the electronic absorption spectrum of the ligand (L), two bands are observed at 332 and 250 nm which is due to n-π* and π-π* transitions, respectively. The band observed at 332 nm is assignable to the transition involving the imine moiety (C=N-) of isoniazid structural core. The other absorption band at 250 nm is due to π-π* transition of the phenyl ring. These transitions are slightly shifted towards higher wavelengths due to the coordinating effect of the metal ion. The electronic spectrum of the copper complex exhibited a band around 536 nm which assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition [19]. The para magnetic nature of Cu(II) ion in the complex is confirmed by magnetic moments value, 1.85 B.M [20]. The square-planar geometry is achieved by the coordination of HL as bidentate ligand, to the copper(II) ion followed by the other coordinating sites (acetate and water molecules). All other complexes showed similar geometrical features.

## 4. BIOLOGICAL STUDIES

### 4.1 DNA Binding Study:

**Electrochemical study**

The extent of DNA binding, its coordination and its interaction of metal with DNA, is determined by Cyclic voltammetric studies. The DNA binding is determined by observing the shifts in the peak potential and peak current by the addition of CT- DNA. The cyclic voltammogram of Cu(II) complex (Fig-.3) in the absence of DNA, shows a quasi-reversible character for $Cu^{2+}$ /$Cu^{+}$ couple [$E_{pc}$ at -644 mV and $E_{pa}$ at -362 mV]. The $E_{1/2}$ is taken as the average for $E_{pc}$ and

$E_{pa}$ and its value is -228 mV. In the presence of DNA, the cathodic peak potential (Epc) appears at -540 mV and the anodic peak potential (Epa) at -350 mV and the $E_{1/2}$ is -210 mV. Similarly both anodic and cathodic peak potentials are shifted to more positive values that implies Cu(II) binds to DNA by intercalation mode[21, 22].

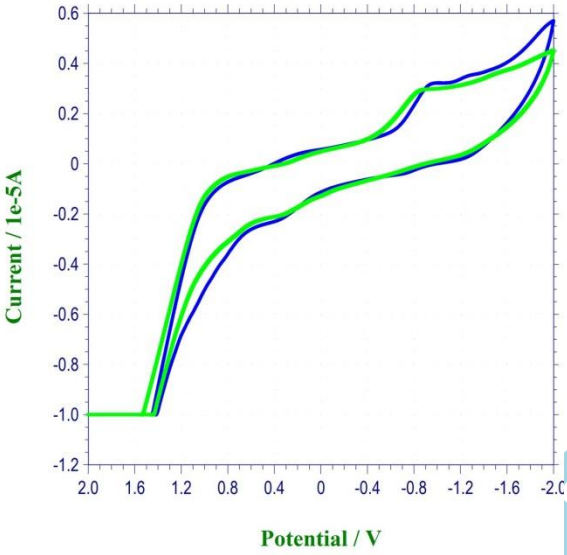



**Figure 3. DNA binding study (Electrochemical approach) in the absence and presence of DNA**

### 4.2 Antibacterial activity

The synthesised isonizaid and its metal complexes are assayed for their antimicrobial activities against Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabillis and Salmonella typhii, at a concentration of 100 mg/mL[23]. Antibacterial activity of the compounds was assessed by the MIC determination and the order of activity is shown as follows:

CuL > NiL > CoL > ZnL > L

It indicates that the complex formation enhances the antibacterial activity. Normally, the complexation of the ligand with dipositive metal ions leads to increase in lipophilicity. The enhanced growth inhibition activity of the synthesized metal complexes is may be due to their chelating abilities [24]. The redox behaviour of the copper ion in the complex might also add the higher anti-microbial activity. The higher activity of the copper (II) complex with respect to the nickel(II) complex are likely to possess higher lipophilicity of the former as compared to the latter.

### 4.3. Anti-tuberculosis evaluation

The synthesised metal complexes exhibited the antimycobacterial activities such as 7.4, 8.0, 6.8 and 7.9 [Co(II), Ni(II), Cu(II) and Zn(II)], respectively. Among the metal complexes, the copper complex exhibited unpredictable higher anti-tuberculosis activity against M. tuberculosis as its MIC value 6.8 µM. All the studied metal complexes showed different activities due to the effective barrier of an outer cell wall membrane of M. tuberculosis [25, 26].

### 4.4. Antioxidant activity

The antioxidant activity of free ligand, complexes and the standard ascorbic acid are assessed on the basis of the radical scavenging effect of the stable DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical. The comparison of the antioxidant activity of the ligand ($IC_{50}$ value is 96 µg/mL) with that of the metal complexes ($IC_{50}$ values of Cu(II), Ni(II), Co(II) and Zn(II)) are 45, 64, 78 and 72 µg/ mL, respectively. The values are found to be close to the values of standard ascorbic acid ($IC_{50}$ value is 22 µg/mL) [27]. It is suggested that Cu(II) complex possesses higher scavenging activity towards hydroxyl radical than the ligand and other complexes.

### 4.5 SOD activity

In the SOD activity, the metal complexes compete with NBT for the oxidation of the generated superoxide ions. The metal complex with lower concentration corresponds to $IC_{50}$. The metal complexes showed higher activity than ligand alone. The activity of Cu(II) complex can be rationalized by means of correlation between the redox potential of the couples $Cu^{II}/Cu^{I}$ during the catalytic cycle and the SOD-mimetic activity [28, 29].

The mechanism proposed for the dismutation of superoxide anions by both superoxide dismutase and complexes thought to involve redox cycling of metal (II) ions (eqs. 1 and 2):

$$M^{2+} + O_2^{-} \rightarrow M^{+} + O_2$$

$$M^{+} + O_2^{-} + 2H^{+} \rightarrow M^{+} + H_2O_2$$

It has been proposed that the metal complexes with two vacant sites are occupied by the superoxide anion. The binding of anion leads to change of oxidation state from Cu(II) to Cu(I ) and the conversion of superoxide to hydrogen peroxide molecule. Therefore the proposed four coordination geometry of the metal complexes gained higher activity. Among the metal complexes, copper complex explicits higher activity due to its redox nature. This observation is also confirmed by the distortion of geometry ("f" factor value). The higher distortion of geometry of copper complex is one of the features that augment the catalytic activity of the enzyme.

## 5. CONCLUSIONS

A series of metal complexes with isoniazid derivative (derived from Isonizaid and 2-(4-Methoxy-phenyl)-2H-isoquinolin-1-one) were prepared and evidenced by different spectroscopic and analytical techniques viz., Elemental analysis, IR,$^{1}$H-NMR,$^{13}$C-NMR,UV-Vis, EPR,FAB-mass spectra, TGA, magnetic moment measurements, molar conductance, and electrochemical studies. All the complexes are found to be non- electrolyte.All the synthesised complexes are found to assume square planar geometry.In the metal complexes, the ligand acts as a uninegative, bidentate, strong ligand around the metal ion. Among the different complexes synthesized, Cu(II) & Co(II) are para magnetic and Ni(II) & Zn(II) complexes are diamagnetic.The pharmacological activities of the ligand and its metal complexes are significant.Compared to the ligand and the other complexes, Cu(II) complex delineates greater pharmacological activity.

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