

Synthesis, spectral characterisation and biological evaluation of tridentate incorporated isoniazid Schiff base metal complexes

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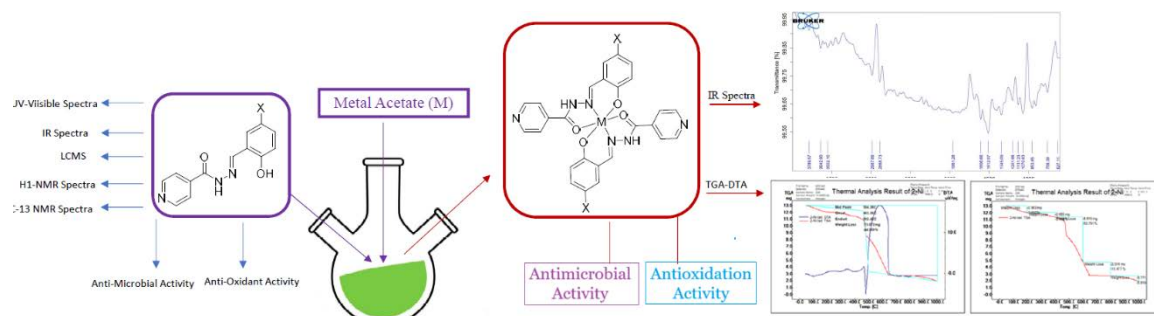
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Article

ABSTRACT

The tridentate Schiff base ligand results from reaction of isoniazid and substituted 2-



hydroxybenzaldehyde and its series of eight metal complexes (C1-C8) Co(II), Ni(II), Cu(II) and Zn(II) were prepared by conventional stirring method. The structural features of synthesized metal complexes were confirmed by IR, UV, DTA-TGA, XRD and ESR spectroscopic techniques. From the data, it illustrates that the ligand coordinates with metal ions in neutral form through -N, -O, -O donor atoms. All complexes have an octahedral skeleton with different crystal systems and $P4_1/P4_3$ and P_1 space groups for C1 and C6 complexes. The TGA study revealed that prepared metal complexes are stable at room temperature and almost show no weight loss at high temperature. Further, all synthesized complexes were evaluated for *in-vitro* antimicrobial activity against *E. Coli*, *S. aureus*, *C. albicans* and *A. niger*. The activity index of prepared complexes revealed good to moderate growth inhibitory activity against all tested pathogens. The metal complexes display excellent potent activity compared to the ligand. The perception of this activity has been made on the basis of the pharmacophore structure of metal complexes and the groups present in it.

Keywords: Synthesis, Schiff bases, Metal complexes, Antimicrobial activity, Antioxidant activity.

INTRODUCTION

Schiff bases are a core of numerous heterocyclic compounds that have growing interest over decades for the synthesis of important drug molecules¹⁻³. Schiff bases play a crucial role in various biochemical processes to evaluate the activity of proteins and key intermediates in enzymatic reactions.^{4,5} Schiff bases are imines in which an aromatic or heteroaromatic moiety is attached to the -CH=N- group. The presence of a double bond is feasible for the synthesis of various heterocyclic compounds with a broad spectrum of biological activities.⁶⁻⁸ The distinctive combination of functional groups makes imines the most versatile as organic ligands for effective metal-ion complex formation.^{9,10} A variety of biological

activities, including antiproliferative, antitubercular, antibacterial, antioxidant, antimalarial, anti-inflammatory, and urease inhibitory properties, have been documented for metal complexes generated from imines in recent decades.¹¹⁻¹⁴ In addition to these characteristics, they exhibit DNA-binding, fluorescence, non-linear optical (NLO), and electroluminescent properties.¹⁵⁻¹⁸

However, isoniazid is a heterocyclic structural motif found in natural and pharmaceutical products.¹⁹ Schiff base ligands derived from isoniazid with suitable substitution of C=O and OH offer coordination with metal ions to give stable complexes.¹⁸ Antidiabetic, antineurogenerative, antipsychotic, anticonvulsant, depressive, anticancer, antibacterial, anti-inflammatory, antitubercular, and antihypertensive pharmacological actions are linked to the metal complexes derived from isoniazid. Numerous compounds based on isoniazid have received regulatory approval and are being sold as medicines. In view of these concerns and the continuation of research work,²⁰⁻²² we report here the preparation of ternary metal ion complexes derived from imines containing an isoniazid scaffold and evaluation of their antimicrobial potency.

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EXPERIMENTAL

Chemical material and methods

Starting materials, solvents and reagents of high purity were purchased from Thomas Baker, Loba Chemicals, Sigma and Aldrich chemicals. and used without purification. FTIR spectra were recorded as KBr pellets on a Perkin Elmer System 2000. ¹H-NMR and ¹³C-NMR spectra were acquired on a Bruker Avance NEO500 spectrometer at 500 MHz. Powdered-XRD were performed on instrument Xray diffractometer, Ultima IV, Rigaku corporation. TGA analysis was achieved on the instrument Mettler Toledo in an inert atmosphere. ESR analysis was carried on instrument ESR-JEOL, JES-FA200 ESR spectrometer with X band (8.75–9.65 GHz) at room temperature. UV–Visible spectrophotometric analysis was performed on the instrument Shimadzu UV-1800 series. Melting points of all prepared compounds were measured using a simple capillary tube method and are uncorrected. Equiptronics conductometer having magnetic stirrer (Model-Eq-664) used to measure molar conductivity by preparing solution of all compounds in DMSO (10⁻³ M) at room temperature. SES instrument's magnetic susceptibility Gouy's balance (Model-EMU50) used for measuring magnetic susceptibilities using copper (II) sulphate as a standard at room temperature. Liquid crystal mass Spectra (LCMS) and ¹³C-NMR spectrum of ligand (L₁, L₂) recorded using spectrophotometer.

Antimicrobial activity

In-vitro antimicrobial activity of ligands (L₁, L₂) and synthesized complexes (C₁-C₈) evaluated against *E. Coli*, *S. aureus*, *C. albicans* and *A. niger* using agar cup plate method. The standard antibacterial and antifungal drug streptomycin and fluconazole was used for evaluation of antimicrobial activity. The 1mg mL⁻¹ concentration of each sample were prepared from stock solution using dimethyl sulfoxide solvent. The bacterial slant was incubated in pre nutrient agar at 35 °C for 24 hrs and Streak a loopful of suspension of *C. albicans* on two slants of pre incubated sabourauds dextrose agar. Incubate the slants at 20-25°C for 72 hrs in an incubator. The suspension of *A. niger* on two slants of pre incubated sabroud dextrose agar. Incubate the slants at 30- 35°C for 24 hrs in an incubator. After incubation, the well grown slant was inoculated in saline solution and adjusted to a viable count of 10⁷ colony forming unit (CFU mL⁻¹). These culture suspensions were inoculated on Mueller–Hinton agar and 100 μL of sample solution was added to each well created on plates with a cork borer. Standard drug controls and a blank control were run for each test. Then the plates were incubated at 35 °C for 24 h for the bacteria and for the yeast and mould at 25 °C for 48 h to examine the zone of inhibition. All the experiments were performed in triplicate and the average zone of inhibition is reported.

Antioxidant activity

The prepared Schiff base ligands (L₁) was utilized to check their antioxidant activity in terms of its ability to scavenge DPPH radical. It contains odd electron and react with various electron donating species to become stable diamagnetic molecule. Due this interaction the electron gets paired off, and leads to bleaching DPPH solution with formation of colourless 2,2'-diphenyl-1-

picryl hydrazine. The reduction of DPPH solution is mainly depending on radical scavenge ability of ligands and its metal chelate complexes. The prepared 100μL of test compounds were taken in the micro titer plate. 100μL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and read the plate on Elisa plate reader at 517nm. The percentage of inhibition - scavenging activity was calculated.

$$\text{scavenging (\%)} = \frac{[(\text{Absorbance of control} - \text{Absorbance of test sample})]}{(\text{Absorbance of control})} \times 100$$

Synthesis of Isoniazid based Schiff bases

The ethanolic solution of isoniazid (0.1 mmol) and 2-hydroxybenzaldehyde/4-chloro-2-hydroxybenzaldehyde (0.15 mmol) was stirred with gentle warming. To this reaction solution the 3-4 drops of acetic acid was added and reaction set to reflux for about 4 hrs. The progress of reaction was monitored on TLC using mobile phase of ethyl acetate: pet ether: methanol, with ratio 7:2:1. After completion of reaction, the reaction solution was kept at r.t. and treated with cold water to obtain crude sample of Schiff bases (L₁ and L₂). The obtained solid was filtered off, washed with ethanol, dried and recrystallized from ethanol to get pure product of Schiff bases.

(*E*)-*N'*-(2-hydroxybenzylidene)isonicotinohydrazide (L₁): Yield: 93%; White solid; M.P. 252-254 °C. FTIR (KBr, cm⁻¹): 3347 (OH), 3181 (NH), 1682 (C=O), 1612 (C=N), 1567 (N-N), 1290 (C-N), 1158 (C-O). ¹HNMR (500 MHz, DMSO, δ/ppm): 12.41 (s, 1H, NH), 11.20 (s, 1H, Ar-OH), 8.59 (s, 1H, H-C=N), 8.75-7.65 (m, 7H, ArH). ¹³CNMR (500 MHz, DMSO, δ/ppm): 162 (CO), 157 (C-OH, Ar-C2), 150 (HC=N), 150 (CH₄-Py), 122 (CH₄-Py), 140 (C-C(=O)-NH), 118 (C, Ar-C1), 116 (CH₄-Ar-C3), 129 (CH₄-Ar-C6), 132 (CH₄-Ar-C4), 120 (CH₄-Ar-C5). UV (DMSO, Molar absorptivity, cm⁻¹): P/V Increases, 342.00 cm⁻¹, 4.0 Transition, n - π*. MS (m/z+1): 242.15 Elemental Analysis: Calcd: C, 64.72; H, 4.60; N, 17.42; O, 13.26. Found: C, 64.55; H, 4.57; N, 17.34.

(*E*)-*N'*-(2-hydroxy-5-chlorobenzylidene)isonicotinohydrazide (L₂): Yield: 88%; Green solid; M.P. 232 °C. FTIR (KBr, cm⁻¹): 3442 (OH), 3173 (NH), 1683 (C=O), 1619 (C=N), 1551 (N-N), 1272 (C-N), 1150 (C-O), 778, 718, 679 (C-Cl). ¹HNMR (500 MHz, DMSO, δ/ppm): 12.45 (s, 1H, NH), 11.21 (s, Ar-OH, 1H), 8.69 (s, 1H, H-C=N), 8.81-7.63 (m, 7H, ArH). ¹³CNMR (500 MHz, DMSO, δ/ppm): 162 (CO), 156 (C-OH, Ar-C2), 147 (HC=N), 150 (CH₄-Py), 122 (CH₄-Py), 140 (C-C(=O)-NH), 120 (C, Ar-C1), 118 (CH₄-Ar-C3), 127 (CH₄-Ar-C6), 131 (CH₄-Ar-C4), 123 (CH₄-Ar-C5). UV (DMSO, Molar absorptivity, cm⁻¹): P/V Increases, 271.50 and 229.00 cm⁻¹. 4.0 Transition, n - π*, π - π*. MS (m/z+1): 276.10 Elemental Analysis: Calcd: C, 56.64; H, 3.66; Cl, 12.86; N, 15.24; O, 11.61. Found: C, 56.61; H, 3.60; N, 15.20.

Synthesis of Isonicotinohydrazone based tridentate Co(II), Ni(II), Cu(II) and Zn(II) metal-complexes

A methanol solution of isonicotinohydrazone ligand (L_1/L_2) (0.50 mmol, 20mL) was prepared, adjusted at pH 7-8 by adding dilute NaOH (1N). Warm methanolic solution of metal acetate (0.25 mmol, 10mL) was added dropwise with constant stirring to ligand solution. Resulting reaction mixture was refluxed for 3 hrs. On cooling the reaction solution at room temperature gave coloured solid were separated, which was filtered washed with warmed methanol and dried and stored in desiccator. The structure of all prepared complexes C_1 - C_8 were characterized with different spectroscopic technique.

bis(E)-N'-(2-hydroxybenzylidene)isonicotinohydrazone cobalt(II) (C_1): Yield: 76 %.; Greenish brown solid; M.P. Above 400 °C; FTIR (KBr, cm^{-1}): 2992, 2882, 2824 (C-H), 1688, 1650 (C=O), 1562 (C=N), 1498 (N-N), 1335, 1427 C-O), 1199, 1135 (C-N), 1069, 990 (M-O), 899, 744 (M-N). UV (DMSO, Molar absorptivity, cm^{-1}): P/V Increases, 369, 233 cm^{-1} . 1.299, 0.395 and 4.000. Transition $n - \pi^*$, $\pi - \pi^*$.

bis(E)-N'-(2-hydroxybenzylidene)isonicotinohydrazone nickel(II) (C_2): Yield: 73 %.; Red solid; M.P. Above 400 °C; FTIR (KBr, cm^{-1}): 2990, 2880, 2820 (C-H), 1680, 1655 (C=O), 1560 (C=N), 1495 (N-N), 1333, 1429 C-O), 1209, 1125 (C-N), 1059, 980 (M-O), 888, 740 (M-N). UV (DMSO, Molar absorptivity, cm^{-1}): P/V Increases, 375, 240 cm^{-1} . 1.310, 0.396 and 4.000. Transition $n - \pi^*$, $\pi - \pi^*$.

bis(E)-N'-(2-hydroxybenzylidene)isonicotinohydrazone copper(II) (C_3): Yield: 71 %.; Green solid; M.P. Above 400 °C; FTIR (KBr, cm^{-1}): 2984, 2870, 2810 (C-H), 1678, 1654 (C=O), 1558 (C=N), 1493 (N-N), 1330, 1425 C-O), 1205, 1124 (C-N), 1054, 970 (M-O), 883, 738 (M-N). UV (DMSO, Molar absorptivity, cm^{-1}): P/V Increases, 380, 246 cm^{-1} . 1.312, 0.395 and 4.000. Transition $n - \pi^*$, $\pi - \pi^*$.

bis(E)-N'-(2-hydroxybenzylidene)isonicotinohydrazone zinc(II) (C_4): Yield: 55 %.; Yellow solid; M.P. Above 400 °C; FTIR (KBr, cm^{-1}): 2980, 2855, 2803 (C-H), 1670, 1650 (C=O), 1550 (C=N), 1491 (N-N), 1334, 1420 C-O), 1201, 1120 (C-N), 1050, 968 (M-O), 876, 734 (M-N). UV (DMSO, Molar absorptivity, cm^{-1}): P/V Increases, 384, 251 cm^{-1} . 1.315, 0.398 and 4.000. Transition $n - \pi^*$, $\pi - \pi^*$.

bis(E)-N'-(2-hydroxy-5-chlorobenzylidene)isonicotinohydrazone cobalt(II) (C_5): Yield: 75 %.; Blakish Grey solid; M.P. Above 400 °C; FTIR (KBr, cm^{-1}): 3115 (N-H), 2990, 2891 (C-H), 1675, 1610 (C=O), 1515 (C=N), 1435 (N-N), 1350 C-O), 1210, 1137(C-N), 1075, 957 (M-O), 837 (M-N), 765, 635 (C-Cl) UV (DMSO, Molar absorptivity, cm^{-1}): P/V Increases, 440.00, 380.00 & 277.00 Abs.: 1.415, 1.760 & 2.799. Transition $n - \pi^*$, $\pi - \pi^*$.

bis(E)-N'-(2-hydroxy-5-chlorobenzylidene)isonicotinohydrazone nickel(II) (C_6): Yield: 72 %.; Light White Reddish solid; M.P. Above 400 °C; FTIR (KBr, cm^{-1}): 3110 (N-H), 2987, 2888 (C-H), 1670, 1606 (C=O), 1512 (C=N), 1430 (N-N), 1345 C-O), 1201, 1131(C-N), 1070, 953 (M-O), 830 (M-N), 758, 627 (C-Cl) UV (DMSO, Molar absorptivity, cm^{-1}): P/V Increases, Wavelength: 436.50, 374.00 & 274.500 Abs.: 1.414, 1.757 & 2.797. Transition $n - \pi^*$, $\pi - \pi^*$.

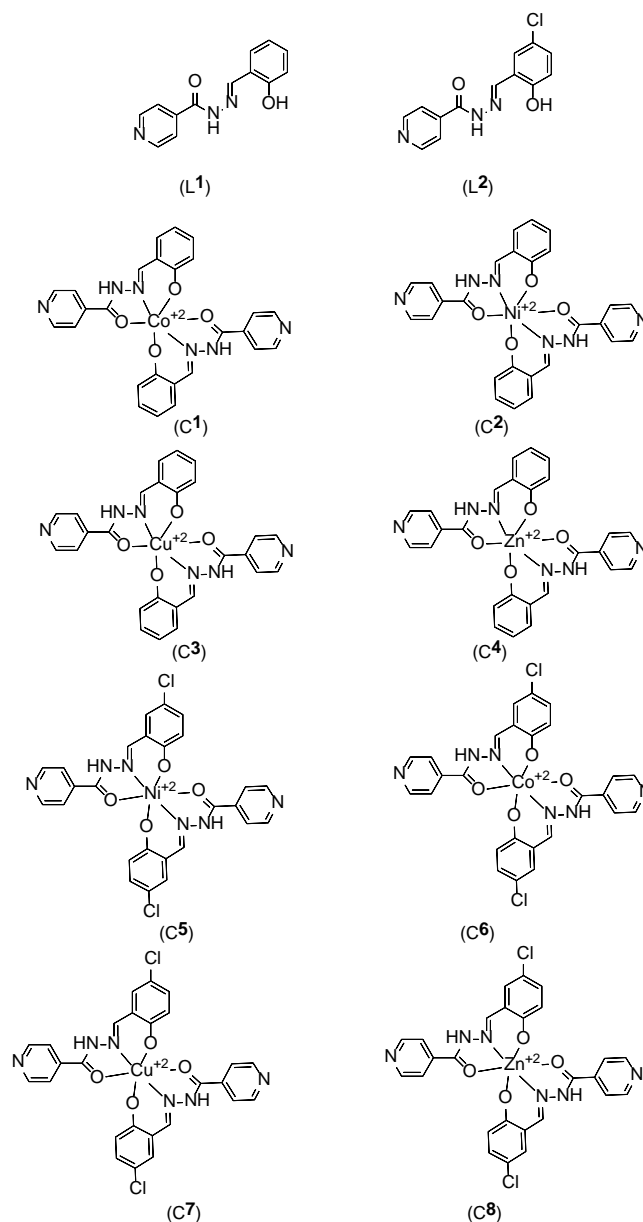


Figure 1. Structure of synthesized metal complexes from Schiff-bases (C_1 - C_8).

bis(E)-N'-(2-hydroxy-5-chlorobenzylidene)isonicotinohydrazone copper(II) (C_7): Yield: 69 %.; Yellow Green solid; M.P. Above 400 °C; FTIR (KBr, cm^{-1}): 3108 (N-H), 2970, 2867, (C-H), 1666, 1608 (C=O), 1523 (C=N), 1431 (N-N), 1344 C-O), 1203, 1130 (C-N), 1053, 952 (M-O), 831 (M-N), 749, 630 (C-Cl). UV (DMSO, Molar absorptivity, cm^{-1}): P/V Increases, Wavelength: 439.00, 376.00 & 276.00 cm^{-1} . 1.500, 1.800 & 3.120. Transition $n - \pi^*$, $\pi - \pi^*$.

bis(E)-N'-(2-hydroxy-5-chlorobenzylidene)isonicotinohydrazone zinc(II) (C_8) Yield: 50 %.; Yellow solid; M.P. Above 400 °C; FTIR (KBr, cm^{-1}): 3105 (N-H), 2949, 2852 (C-H) 1663, 1610 (C=O), 1510 (C=N), 1428 (N-N), 1340 C-O), 1206, 1125(C-N), 1056, 953 (M-O), 833 (M-N), 745, 629 (C-Cl) UV (DMSO, Molar absorptivity, cm^{-1}): P/V Increases, Wavelength: 440.10,

382.00 & 279.20 Abs.: 1.501, 1.805, & 3.007 Transition $n - \pi^*$, $\pi - \pi^*$.

RESULTS AND DISCUSSION

Chemistry

The Schiff bases derived from salicylaldehyde have been studied significantly due to their applications in medicine, dyes, agrochemicals, electrochemical, catalyst, chemo detectors and analytical techniques etc. The Schiff base contain an imine functionality ($-\text{CH}=\text{N}$), a reactive functional group which can be utilized to synthesize heterocycles such as thiazolidinedione, azetidinone, benzoxazine and formazan etc. The scaffold 2-hydroxy imines also play important role as chelating molecule in coordination chemistry. The 2-hydroxy imines provide a ligand platform due to their easier reactivity to form metal complexes, which represent an influential research field of inorganic chemistry. Therefore, in view of these significance we propose to synthesize some Co(II), Ni(II), Cu(II) and Zn(II) metal complexes derived from Schiff base containing isoniazid and halo substituted salicylaldehyde. The ligands L1 and L2 were prepared by reacting 2-hydroxybenzaldehyde with isoniazid using conventional refluxing method. The series of eight C1-C8 metal complexes were prepared by treating metal acetates with ligand L1 and L2 under similar refluxing conditions (**Figure 1**). The structure of synthesized Schiff base-metal complexes (C1-C8) are confirmed on the basis of various spectroscopic data such as FTIR, UV, powder XRD, TGA-DTA, ESR.

In FTIR measurement of ligand L1 and L2 the corresponding free -OH stretching band observed around 3347, 3442 cm^{-1} . Whereas this fundamental vibrational band disappeared upon coordination of ligand with metal which confirm that the oxygen atom involved in complexation. The other stretching frequencies at 1688, 1670; 1650, 1606; 1562, 1512; 1498, 1430 cm^{-1} verified and proportional to functional group C=O; C=N and C=C respectively. However, the characteristics band for M-N and M-O were noticed around 899, 744; 830 cm^{-1} and 1069, 990; 1070, 953 cm^{-1} that revealed the metal-ligand complex formation. The X-ray powder diffraction analysis for Schiff base metal complex C1 and C6 were obtained on X-ray powder diffractometer with parameters, scanning mode; 2Theta/Theta, scanning type; continuous, x-ray; 40 kv/20 mA, fixed monochromator with 2 θ range 10–90° at a step 0.01°. To know the coordination of ligand L1 and L2 with Co(II) and Ni(II) the assessment was made between the observed pattern and reported pattern with peak search method. The results showed the peaks present at different 2 θ values. From these values calculated the grain size, dislocation density, strain and unit cell parameters and values are shown in Table 1.

The ultra-violet spectral data of Cu(II) and Zn(II) complexes (C3 and C8) with L1 and L2 has λ at around 369 – 436 nm which clearly indicate that hyperchromism with intense blue shift. These λ values were not observed in the UV spectra of ligand L1 and L2 which notified the coordination of N, C=O and O to $\text{Co}^{+2}/\text{Ni}^{+2}$ metal ions. The molar absorption coefficient (ϵ) values ranging from 1.299 - 4.00 $\text{L mol}^{-1}\text{cm}^{-1}$ and 1.414 - 2.797 $\text{L mol}^{-1}\text{cm}^{-1}$ correlated to $n - \pi^*$ and $\pi - \pi^*$ transition and charge transfer

transition respectively. The observed pattern suggested the prepared complexes display octahedral geometry with six coordination sites. Hence, these six coordination sites are indicated with donor atoms or groups such as C=N, C=O and OH of ligand L1 and L2.

Table 1: Crystallographic data collection and structure refinement of complexes C1 and C6.

Parameter	[Cu(isonicotinohydrazide) ₂ (II)] (C1)	[Cu(isonicotinohydrazide) ₂ (II)] (C6)
Empirical formula	$\text{C}_{26}\text{H}_{20}\text{N}_6\text{O}_4\text{Co}^{+2}$	$\text{C}_{26}\text{H}_{18}\text{Cl}_2\text{N}_6\text{O}_4\text{Ni}^{2+}$
Formula weight	539.42	608
Temperature, K	298	298
Crystal System	Orthorhombic	Monoclinic
Space group	$P4_1/P4_3$	$P1$
a / Å	7.7514273	3.472960
b / Å	21.76213	18.51964
c / Å	9.84627	3.70682
$\alpha / ^\circ$	90	90
$\beta / ^\circ$	103.12	90
$\gamma / ^\circ$	90	90
Volume, Å ³	12473.04	1564.76
Z	6	4
$\rho_{\text{calc}} / \text{g cm}^{-3}$	2.54126	4.71596
μ / cm^{-1}	143.05	251.21
Crystallite size, nm	25.486327	12.75
Dislocation density, nm^{-2}	1.294731	1.92436
Micro strain	5.301462	2.475621

The ¹H-NMR spectrum in DMSO solvent using TMS as internal standard. For synthesized Schiff base ligand (L1 & L2) was recorded. In the spectrum singlet at δ 9.38 ppm, δ 7.23 ppm (H-C=N), indicates presence of isocyanide proton in the ligand which indicate the formation of C=N from isoniazid and substituted halo derivatives of salicylaldehyde. A singlet signal observed at δ 12.41 ppm, δ 12.45 ppm for NH group. The broad singlet signal recorded at δ 11.20, δ 11.21 ppm for Ar-OH, ¹H NMR signal doublet at δ 8.75 – 7.81 ppm, δ 8.81 - 7.87 ppm for isoniazid aromatic proton and the signal at δ 7.65 – 6.94 ppm, δ 7.63 – 6.99 ppm observed for aromatic proton. The ¹³C -NMR spectrum in DMSO solvent using TMS as internal standard. For synthesized Schiff base ligand (L1) was recorded. The signals

recorded at δ 162 ppm for carbonyl group δ 150 ppm, δ 122 ppm for CH, δ 140 ppm for (C-C(=O)-NH), of isoniazid moiety (CO), The signal at δ 150 ppm, δ 147 ppm is due to carbon of (HC=N) group. The signals at δ 116 – 132 ppm and δ 118–131 ppm represents carbon atoms of benzene ring of substituted salicylaldehyde ring. The signal at δ 157 ppm, δ 156 ppm is due to carbon of hydroxyl group of substituted salicylaldehyde ring (C-OH). Both ^1H NMR & ^{13}C NMR provide sufficient evidences to confirmed the proposed structure of synthesized Schiff base ligand.

The EPR data for synthesized Cu(II) complexes C_3 and C_7 was performed at r. t. and calculated value for g_{\parallel} and g_{\perp} are 2.0494 and 2.0513. The trend $g_{\parallel} > g_{\perp} > g_e$ observed for the synthesized complexes C_3 and C_7 , it designates to octahedral geometry with one unpaired electron present either in dx^2-y^2 or dz^2 orbital respectively, Figure 2 and 3.

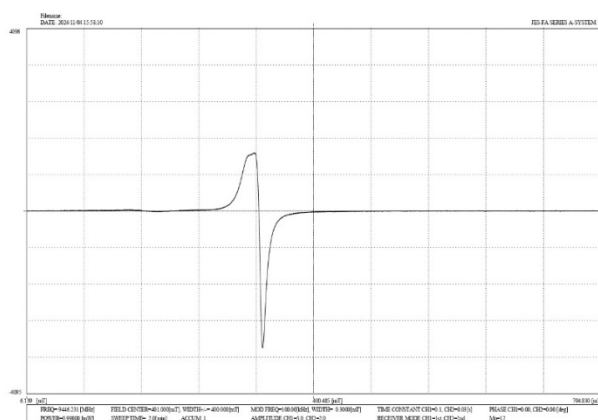


Figure 2. XRD graph for bis(E)-N'-(2-hydroxybenzylidene)isonicotinohydrazide copper(II) (C_3)

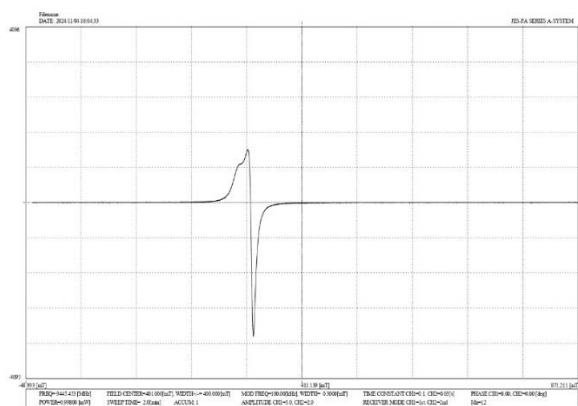


Figure 3. XRD graph for bis(E)-N'-(2-hydroxy-5-chlorobenzylidene)isonicotinohydrazide copper(II) (C_7)

Thermal studied of prepared Co(II) and Ni(II) complexes (C_1 , C_6) was performed to know thermal stability, coordination environment around metal ion and whether the presence of H_2O in coordination or outside the coordination sphere. The TGA-DTA analysis display that complexes start to decompose as temperature increases gradually. This indicate that may be due to

any loss of H_2O outside the coordination entity. The 70-80% mass loss was found at 600 and 800 $^{\circ}\text{C}$ temperature which revealed that elimination of ligand molecule. Thus, from TGA-DTA analysis it is clear that the synthesized isonicotinohydrazide Schiff base complexes are stable at high temperature. Molar conductance of all prepared complexes was carried in 10^{-3} M solutions of DMSO solvent. The non-electrolytic nature of prepared complexes observed from their low values. Out of these metal complexes Co(II), Ni(II) and Cu(II) complexes display paramagnetic and Zn(II) complex show diamagnetic properties.

BIOLOGICAL ACTIVITY

Antimicrobial Properties

The antimicrobial potency of the prepared Schiff base ligands and their metal complexes was screened against different pathogens are represented in Table 3. From the data it is clear that, the synthesized ligands and metal complexes display less to moderate inhibitory activity against *E. coli* and *S. aureus*. The ligand L_1 along with metal ion complexes C_1 , C_3 , C_4 , C_5 and C_6 exhibits excellent antibacterial activity. All the tested compounds display poor to moderate antifungal inhibitory activity against *C. albicans* and *A. niger* compare to standard drug.

The minimum inhibitory concentrations of complexes were determined using concentrations of 1.0, 0.5, 0.25 and 0.12 mg mL^{-1} . The observed MIC values of complexes and respective ligands are presented in Table 4. The complexes C_3 and C_4 exhibited a significant MIC value of 0.5, 0.25 and 0.12 mg mL^{-1} against all tested pathogens. The complex C_7 and C_8 show a significant MIC value of 0.25 and 0.12 mg mL^{-1} against the pathogen *S. aureus* and *C. albicans*. The remaining complexes shows a moderate MIC value of 0.25 mg mL^{-1} against the pathogen *E. coli*, *C. albicans* and *A. niger*. All complexes showed improved MIC values with respect to their respective ligands The increased potency is due to the presence of pharmacophore isoniazid nucleus and groups like halogens substituents in the ligands.

Table 2. Antimicrobial activity of synthesized Schiff base ligands (L_1 , L_2) and metal complexes (C_1 - C_8).

Ligands and metal complexes	Zone of inhibition(mm) ^[a]			
	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>A. niger</i>
L_1	24	22	16	18
L_2	27	24	12	24
(C_1): [$\text{CO}(\text{L}_1)_2$]	34	30	17	26
(C_2): [$\text{Ni}(\text{L}_1)_2$]	30	15	20	22
(C_3): [$\text{Cu}(\text{L}_1)_2$]	36	35	20	26
(C_4): [$\text{Zn}(\text{L}_1)_2$]	40	37	24	28
(C_5): [$\text{CO}(\text{L}_2)_2$]	20	18	19	18
(C_6): [$\text{Ni}(\text{L}_2)_2$]	32	34	17	--
(C_7): [$\text{Cu}(\text{L}_2)_2$]	40	35	20	24
(C_8): [$\text{Zn}(\text{L}_2)_2$]	38	34	22	26
Streptomycin ^[b]	38	36	-	-
Fluconazole ^[b]	-	-	22	30

^[a] 5 mg/mL of compounds used for antimicrobial screening.

^[b] 1mg/mL of standard drug used for antimicrobial screening.

Antioxidant Properties

The scavenging activity of prepared ligands and their metal complexes are represented in Table 3. The antioxidant activity is expressed in terms of % inhibition. From the data it can be seen that all prepared complexes display lower inhibition value to scavenge DPPH radical than standard ascorbic acid. This indicates that these Schiff base-metal complexes are more potent scavengers in comparison with standard drug. The imine system present in prepared complexes enhances electron delocalization over metal atom, which is one of the determinants for radical scavenging potency. Additionally, the pharmacophore substituent -NH of isoniazid and -Cl group in complexes C5-C8 possess considerable level of antioxidant activity. Hence, these structural features lead to enhance antioxidant activity compared to standard drug ascorbic acid.

Table 3. Antioxidant activity of synthesized Schiff base metal complexes (C1-C8).

Compound	Absorbance	% inhibition*
(C ₁): [CO(L ₁) ₂]	0.490	60.89
(C ₂): [Ni(L ₁) ₂]	0.250	80.04
(C ₃): [Cu(L ₁) ₂]	0.243	80.84
(C ₄): [Zn(L ₁) ₂]	0.307	75.49
(C ₅): [CO(L ₂) ₂]	0.274	78.13
(C ₆): [Ni(L ₂) ₂]	0.361	71.18
(C ₇): [Cu(L ₂) ₂]	0.203	83.79
(C ₈): [Zn(L ₂) ₂]	0.292	76.69
Control	1.253	--
Ascorbic acid	0.196	84.35

*1mg/ml of concentration of complexes and standard drug used to scavenge a DPPH radical.

CONCLUSION

Summing up, the present work on synthesis of isonicotinohydrazide Schiff bases (L1, L2) and metal complexes (C1-C8) under neat and clean reaction conditions. The spectroscopic data suggested that the synthesized complexes are stable having octahedral geometry with one unpaired electron present either in $dx^2 - y^2$ or dz^2 . The biological activity profile of prepared metal complexes put on show excellent antimicrobial activity against tested pathogens. These results of biological activity attributed to pharmacophore nucleus of metal chelates and substituent present in C1 and C4. The antioxidant activity of all prepared C1-C8 complexes has been studied to scavenge DPPH radical and found significant and more radical scavenger than standard ascorbic acid. Therefore, the results of antimicrobial activity and antioxidant activity serve as preliminary screening of new pharmacophore analogues.

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CONFLICT OF INTEREST STATEMENT

Authors declare that there is no conflict of interest for the work included in this article.

SUPPLEMENTARY INFORMATION

Data given in supporting information includes detailed crystallographic refinement for complex C3 and C4, spectra of all compounds and biological study, and can be downloaded from article page.

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