



Synthesis, Spectroscopic and Antimicrobial properties of Co(II), Ni (II) and Cu(II) complexes of (*E*)-N'-(2-hydroxy -5-nitrobenzylidene)isonicotinohydrazide)

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ABSTRACT: The objective of this study is to investigate the antimicrobial activity of novel Schiff base metal complexes. The resistance of micro-organisms to classical antimicrobial compounds poses a challenge to effective management and treatment of some diseases. In line with this, copper (II), nickel (II) and cobalt (II) complexes of the Schiff base ligand (*E*)-N'-(2-hydroxy-5-nitrobenzylidene) isonicotinohydrazide) were synthesized and characterized based on elemental analysis, conductivity measurements, infrared and electronic absorption spectroscopy. The results suggest a 1:2 [M:L] stoichiometry for the copper and cobalt complexes and a 1:1 [M:L] ratio for the nickel complex. The electronic absorption spectral data support octahedral geometry for cobalt(II) and copper(II) complexes and square planar for nickel (II). *In-vitro* antimicrobial properties of the compounds evaluated against five pathogenic bacteria using the agar-well diffusion method revealed that the activity was in the order Ni>Co>Cu. These compounds can be used as substrates for development of new antimicrobial agents. © JASEM
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Keywords: isonicotinohydrazide, Schiff base metal complexes, *in-vitro*, antimicrobial activity, electronic spectra, 2-hydroxy-5-nitrobenzaldehyde

Hydrazones are Schiff bases containing two adjacent nitrogen atoms in which both nitrogen atoms are nucleophilic and the azomethine carbon possesses both electrophilic and nucleophilic character. These compounds have received much attention as a result of their interesting properties and important applications. They are used widely in organic synthesis (Brehme *et al.*, 2007), analytical chemistry (Tezcan *et al.*, 2008) and in medical biotechnology (Sigel *et al.*, 2004). Hydrazones have been reported to demonstrate broad spectrum of biological activities including antimicrobial, antituberculosis, anti-inflammatory, anticancer, anticonvulsant and analgesic activities. (Wang *et al.*, 2014; Kaushik *et al.*, 2010; Jordão *et al.*, 2011)

Schiff bases of aroyl, acyl and heteroacyl hydrazones are more versatile and flexible as a result of the carbonyl group present which introduces an additional donor site in the molecule. These compounds exhibit high and selective anti-proliferative activities (Ommis *et al.*, 2016). For example, salicylaldehyde benzoylhydrazone has been shown to be a potent inhibitor of DNA synthesis and cell growth in some cultured human and rodent cells. (Johnson *et al.*, 1982). Furthermore, 2-hydroxynaphthaldehyde-trimethoxybenzoylhydrazone is an efficient chelator of intracellular trace metals with high anticancer properties. Hydrazones of isonicotinic hydrazide are used in treatment of iron

overload and as pharmacophore in design of anti-convulsant drugs (Kim *et al.*, 2010).

There is a growing interest in the coordination chemistry of aroylhydrazones based on reports of formation of stable bioactive metal chelates with various transition metal ions (Ogunniran *et al.*, 2015; Rakesh *et al.*, 2009).

In particular, Schiff bases of isonicotinic hydrazide (INH) a first line anti-tuberculosis drug, the isonicotinoyl hydrazones have been shown to form bioactive metal chelates with many bivalent ions (Chohan *et al.*, 2006; Sharma *et al.*, 2010).

In this paper, we report the synthesis, spectroscopic and antimicrobial properties of the cobalt, nickel and copper complexes of a Schiff base, (5-nitro-2-hydroxybenzylidene) isonicotinoyl hydrazide derived from 5-nitro-isonicotinic hydrazide and 2-hydroxy-5-nitrobenzaldehyde.

MATERIALS AND METHODS

Chemicals used were of highest purity available and purchased from Sigma Aldrich Chemicals Ltd, Germany.

The solvents were of analytical grade and used without further purification. The complexes were

synthesized using the method reported by Fernandez *et al.*, (2010).

Physical measurements and analyses: Melting points ($^{\circ}\text{C}$) were determined on a Stuart SMP3 melting point apparatus and are uncorrected. Elemental analyses were performed with a Perkin-Elmer 2400 CHNS/O analyzer. Solution electronic absorption spectra were recorded in DMF on a Cecil Super Aquarius 9000 series UV-vis spectrophotometer. The infrared (IR) spectra were recorded on an FTS 7000 series Digilab Win-IR Pro spectrometer equipped with a diamond ATR accessory in the range 4000 to 400 cm^{-1} . The Raman spectroscopic analysis was carried out using MultiRam FR-Raman Spectrometer with Nd-YAG laser which lasers in the near infrared at 1064 nm equipped for the collection of fluorescence-free Raman spectra. ^1H and ^{13}C NMR spectra were recorded in DMSO- d_6 on a Varian Mercury 300 MHz NMR spectrometer and the chemical shifts quoted relative to the solvent peaks. Conductivity measurements were obtained in DMF solutions using a DDS-307 conductivity meter.

Synthesis of (E)-N'-(2-hydroxy-5-nitrobenzylidene)isonicotinoylhydrazide (DF): 2-hydroxy-5-nitrobenzaldehyde (300 mg, 1.8 mmol.) was added to a homogenous solution of isonicotinic acid hydrazide (246 mg, 1.8 mmol.) in hot DMSO (20 mL) and the mixture refluxed for 8 h. The mixture was allowed to cool to room temperature, the solvent removed under reduced pressure and the yellow solid obtained was recrystallized from DMSO and dried in a desiccator.

Yield: 89% (0.49 g). m.p.: 310 $^{\circ}\text{C}$; IR (ATR, cm^{-1}): 3305, 3069, 1664, 1608, 1582, 1550, 1480, 1439, 1373, 1338, 1296, 961, 839, 750, 685. Raman (Solid/1064nm): 3074, 1617, 1600, 1585, 1518, 1340, 1299, 1245, 996, 964, 908, 834, 666, 639; ^1H NMR (300 MHz, DMSO- d_6) δ_{H} : 7.13 (d, J 9.3Hz, 1H), 7.86 (d, J 6.3Hz, 2H), 8.20 (d, J 9.0Hz, 1H), 8.61 (d, J 3.0Hz, 1H), 8.76 (s, 1H), 8.81 (d, J 4.8Hz, 2H), 12.20 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6) δ_{C} : 117.68, 120.59, 122.07, 123.85, 127.44, 140.40, 145.41, 150.94, 162.17, 163.22. Anal. Calcd for $\text{C}_{13}\text{H}_{10}\text{N}_4\text{O}_4$: C, 54.24, H, 3.80, N, 19.37. Found: C, 54.55, H, 3.52, N, 19.57.

General synthesis of metal complexes: The metal chloride (1.1 mmol) in 1:2 ethanol/water mixture (30 mL) was added to a degassed mixture of isonicotinic acid hydrazide (274 mg, 2 mmol) and 2-hydroxy-5-nitrobenzaldehyde (334 mg, 2 mmol) in ethanol (20 mL), was refluxed under nitrogen for 6 h. The mixture was allowed to cool to room temperature and

the solid obtained was filtered, washed with water, recrystallized from methanol and dried in a desiccator.

Dichlorobis-(2-(E)-N'-(2-hydroxy-5-nitrobenzylidene)isonicotinoylhydrazide)copper(II) (DF1) Green solid; Yield = 4.65 mg (66%); mp: >349 $^{\circ}\text{C}$. IR (cm^{-1}): 3066, 1602, 1496, 1365, 1321, 1242, 1191, 1129, 1100, 946, 896, 795, 731, 692, 597, 518, 467, 446. Raman (Solid/1064nm): 3051, 1616, 1571, 1552, 1419, 1378, 1326, 1217, 1194, 1156, 1052, 923, 843, 799, 654. Anal. calcd. for $\text{C}_{26}\text{H}_{20}\text{Cl}_2\text{CuN}_8\text{O}_8$: C, 44.17, H, 2.85, N, 15.85, Cu, 8.99. Found: C, 44.16, H, 2.40, N, 15.23, Cu, 9.91.

(E)-N'-(2-hydroxy-5-nitrobenzylidene)isonicotinohydrazide)nickel(II) chloride hydrate (DF2) Yield: 2.52 mg (56%); mp: >349 $^{\circ}\text{C}$. IR (cm^{-1}): 3358, 3050, 1595, 1541, 1418, 1384, 1269, 1086, 951, 907, 834, 754, 720, 631, 587, 517. Raman (Solid/1064nm): 3069, 1611, 1552, 1508, 1374, 1312, 1194, 1049, 842, 665. Anal. calcd. for $\text{C}_{13}\text{H}_{17}\text{ClN}_4\text{NiO}_8$: C, 34.59, H, 3.80, N, 12.41, Ni, 13.00. Found: C, 34.86, H, 3.40, N, 12.38, Ni, 12.85

Bis-(2-(E)-N'-(2-hydroxy-5-nitrobenzylidene)nicotinohydrazide)cobalt(II) hydrate (DF3) Yield: 4.30 mg (65 %); mp: >349 $^{\circ}\text{C}$. IR (cm^{-1}): 3294, 3067, 1595, 1545, 1492, 1458, 1437, 1296, 1242, 1099, 961, 922, 832, 750, 686, 579, 511, 456. Raman (Solid/1064nm): 1609, 1574, 1550, 1520, 1385, 1315, 1217, 1055, 1035, 844, 810. Anal. calcd. for $\text{C}_{26}\text{H}_{24}\text{CoN}_8\text{O}_{11}$: C, 45.69, H, 3.54, N, 16.40, Co, 8.62. Found: C, 45.70, H, 3.12, N, 16.00, Co, 8.72.

Antibacterial studies: The *in-vitro* antibacterial activity of the ligand and metal complexes was evaluated using the agar-well diffusion method Valgas *et al.*, (2007). The compounds were screened against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Shigella* and *Salmonella typhi* using a solution containing 2.5 mg of each compound dissolved in 1 mL *N,N*-dimethylformamide (DMF).

A double layered aseptically prepared Muller Hinton agar plate was flooded with standardized (0.5 McFarland) test microorganism and allowed to stand for two minutes. A sterilized cork borer (1 mm) was used to make five radial wells, which were filled with the test compounds using a micropipette and then incubated at 37 $^{\circ}\text{C}$ for 24 h. During this period, the test compound diffused and the growth of the inoculated microorganism was affected. The diameter

of the zone of inhibition surrounding each well was measured and recorded. In order to clarify any participating role of the solvent in the biological screening, control test was included using the solvent alone to fill the control well.

RESULTS AND DISCUSSION

Condensation of isonicotinic acid hydrazide with 2-hydroxy-5-nitrobenzaldehyde readily gave rise to the Schiff base (*E*)-*N'*-(2-hydroxy-5-nitrobenzylidene) hydrazide (DF) (Figure 1) in good yield.

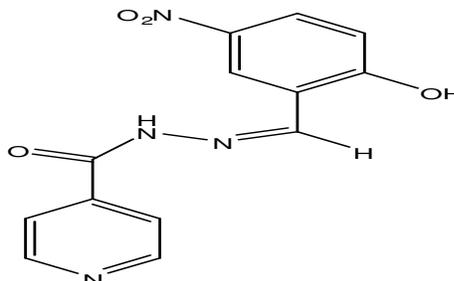


Fig 1: (*E*)-*N'*-(2-hydroxy-5-nitrobenzylidene) isonicotinoylhydrazide)

The formation of the Schiff base was confirmed on the basis of a sharp melting point, characteristic band at 1608 cm^{-1} in the FT-IR spectrum and resonance signals at 8.76 ppm in the ^1H NMR for the HC=N-group (Figure 2). The formation was further confirmed by the presence of a signal at 145 ppm in the ^{13}C NMR and elemental analysis.

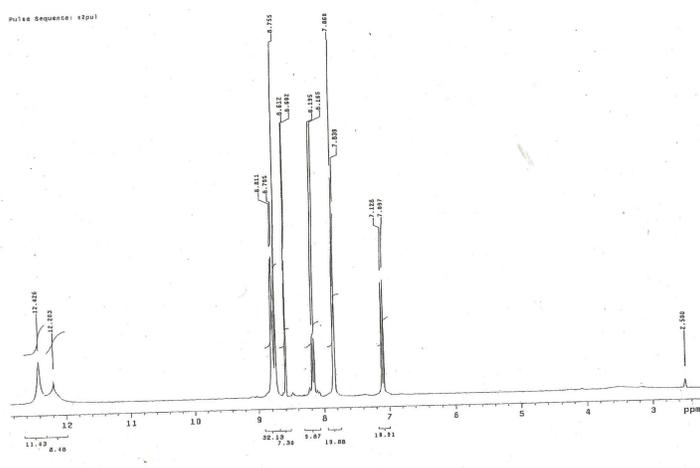


Fig 2: ^1H NMR of Schiff base DF

The reaction of the Schiff base DF with chloride salts of Cu(II), Ni(II) and Co(II) afforded the metal complexes in moderate yields. All the complexes were stable in air, insoluble in common organic solvents such as ethanol, methanol, acetone, diethyl ether, chloroform, but soluble in DMF and DMSO.

The analytical data of the compounds reported in Table 1 are in good agreement with the calculated values and suggest a 1:2 metal: ligand ratio for the complexes. The low molar conductivity values of DF1 and DF3 indicate that the complexes are essentially non-electrolytes, however, the value for DF2 suggest it is a 1:1 electrolyte with formula $[\text{Ni}(\text{DF})_2 \cdot n\text{H}_2\text{O}]\text{Cl} \cdot n\text{H}_2\text{O}$. (Melha, 2008).

Table 1 Physical and analytical data of the ligand and its complexes

	Yield %	mp °C	$\Lambda\text{ Scm}^{-1}$	Elemental analysis Calculated (Found)				
				%C	%H	%N		
DF	89	Yellow	310	-	54.24(54.55)	3.8 (3.52)	19.37(19.57)	
DF1	[Cu(DF) ₂ Cl ₂]	66	Green	>349.0	3.75	44.17 (44.16)	2.85(2.40)	15.85(15.23)
DF2	[Ni(DF) ₂ ·3H ₂ O]Cl·H ₂ O	56	Brown	>349.0	48.20	34.59 (34.86)	3.80 (3.40)	12.41 (12.38)
DF3	[Co(DF) ₂ ·3H ₂ O]	65	Dark red	>349.0	2.44	45.69 (45.70)	3.54 (3.12)	16.40 (16.00)

In order to determine the binding mode of the Schiff base to the metal in the complexes, the IR spectrum of the ligand was compared to the spectra of the

metal complexes. The diagnostic IR spectral bands for the ligand (**DF**) and its metal complexes (**DF1** – **DF3**) presented in Table 2.

Table 2: Diagnostic infrared data of DF and its metal complexes

Complex code	$\nu(\text{H}_2\text{O})$	$\nu\text{N-H}$	$\nu\text{C=O}$	$\nu\text{N-N}$	$\nu\text{C=N}$	νOH	$\nu\text{C-O}$	$\nu(\text{M-O})$	$\nu(\text{M-N})$
DF	-	3305	1664	1554	1608	1373	1296	-	-
DF1	-	3066	-	1496	1602	1365	1321	518	446
DF2	3358	3050	1642	1541	1595	1384	1269	587	517
DF3	-	3067	-	1545	1595	1296	1246	511	456

All metal complexes exhibit a band in the region 1598 – 1605 cm^{-1} , attributed to the imine group in the Schiff base. The observed decrease in frequency compared to the corresponding band in the free ligand indicates the use of the imine nitrogen in metal-ligand coordination.

The absence of the band characteristic of carbonyl amide at 1664 cm^{-1} in DF1 and DF3 suggest that amido-imido tautomerism took place before complexation. This observation is corroborated by decrease in the NH bending vibrations of the metal complexes compared to the ligands. In DF2, the carbonyl amide band is shifted to lower frequency indicating tautomerism does not occur in the nickel complex and the carbonyl is used in coordination to the metal.

The deformation vibration of the phenolic OH group which appeared at 1339 cm^{-1} in the spectrum of the ligand was shifted to higher or lower frequencies in

the metal complexes, thus indicating its involvement in coordination.

The appearance of new bands at 446-517 cm^{-1} and 511-587 cm^{-1} characteristic of the metal-nitrogen and metal – oxygen stretching vibration supports the involvement of nitrogen and oxygen atoms in complexation.

Of interest is the broad band observed in the region 3245 cm^{-1} for DF2 indicating the presence of coordinated water molecules in the compound. The comparison of bands for outer sphere coordinated water is somewhat difficult. This could be attributed to its loss while drying the complexes in the desiccator prior to the analysis.

The electronic spectra of the Schiff base and the metal complexes in DMF were recorded over the wavelength 250 - 700 nm at room temperature and the spectral data presented in Table 3

Table 3: Electronic absorption data the Schiff Base and its metal complexes

Compound	λ_{max} (cm^{-1})	Assignment	Geometry
DF	34722, 25773	$\pi-\pi^*$, $n-\pi^*$	
DF1	35714, 27624, 20366	$\pi-\pi^*$, $n-\pi^*$, d-d	Octahedral
DF2	35211, 29585, 22867	$\pi-\pi^*$, $n-\pi^*$, d-d	Square Planar
DF3	29767, 16482, 14352	$\pi-\pi^*$, d-d, d-d	Octahedral

The spectrum of the ligand consists of two bands in the UV region at 34722 and 25773 cm^{-1} arising from the $\pi-\pi^*$ and $n-\pi^*$ transitions respectively. In addition to the $\pi-\pi^*$ and $n-\pi^*$ transitions observed in the Ni(II) and Cu(II) complexes, a band was observed at 20366 cm^{-1} in the Cu(II) complex. This was assigned to an $E_g \rightarrow T_{2g}$ transition, thus suggesting an octahedral geometry for copper in the complex. The band at 22867 cm^{-1} in Ni(II) complex corresponds to a $A_{1g} \rightarrow A_{2g}$ transition in a square planar environment (Raman *et al.*, 2003). The absorption bands observed

at 16482 and 14352 cm^{-1} in the Co(II) complex is assigned to the ${}^4T_{1g} \rightarrow {}^4A_{2g}$, ${}^4T_{1g}(\text{F}) \rightarrow {}^4T_{1g}(\text{P})$ transitions in an octahedral field. (Raman *et al.*, 2010). The third transition expected corresponding to ${}^4T_{1g}(\text{F}) \rightarrow {}^4T_{2g}(\text{F})$ was not observed due to limitation of the spectrophotometer used.

The Schiff base and its metal complexes were screened for antibacterial activity against *S. aureus*, *E. faecalis*, *E. coli*, *Shigella* and *S. typhi* in DMF and the results summarized in Table 4.

Table 4: Antibacterial screening data of the Schiff base and its metal complexes at 2.5 mg/mL

Compound	Diameter of inhibition zone (mm)				
	<i>S. aureus</i>	<i>E. feacalis</i>	<i>E. coli</i>	<i>Shigella</i>	<i>S. typhi</i>
DF1	0	0	0	+++	++
DF2	++	+	+	++	++
DF3	+++	+++	+++	+++	+++
DF	+++	+++	++	+++	+++
DMF	0	0	0	0	0

Code: + = active; ++ = moderately active; +++ = highly active; 0 = inactive

The *in-vitro* biological activity of a compound depends on its structure, ability to survive in the condition used (temperature, solvent etc.) and morphology of the cell wall. The morphology of the cell wall is a key factor that influences the activity of antibacterial agents. The cell wall of the bacterial is composed of peptidoglycan which is thicker in the gram-negative bacteria and this usually poses a barrier to the degree of diffusion of antibacterial agent into the enzyme (Mims *et al.*, 2004).

The antibacterial study revealed that all the compounds have the capacity to inhibit metabolic growth of the bacterial species to different extents. The Schiff base was inactive against the species studied expect for *Shigella* and *Salmonella* bacterial strains. Previous reports indicate that the presence of a metal ion can enhance or suppress the biological activity of a biologically active compound. The higher activity of the metal complex may be due to the effect of metal ions on the normal cell membrane. The crucial action of a toxicant is the denaturation of one or more proteins of the cell, which impairs normal cellular processes. Delocalization of lipid layer of the bacterial cell membrane which seems to be responsible for their enhanced potent antibacterial activity (Rehder, 2003).

In this study, the Ni(II) complex was found to be more active than the ligand DF, Cu(II) and Co(II) complexes. This could be attributed to the structure of the nickel complex which allowed for easier chelate formation with the bacterial strains tested. The trend in activity of the compounds was found to be in the order Ni>Co>Cu. The compounds showed no discrimination for the type of bacterial strains, hence are suitable for development on broad spectrum antibacterial agents. Further work on the cytotoxicity of the compounds is being carried out.

Conclusion: The synthesis and bacterial study of an aroylhydrazone derived from isonicotinic hydrazide and 2-hydroxy-5-nitrobenzaldehyde and the Cu(II), Ni(II) and Co(II) complexes is described. Comparative *in-vitro* antibacterial study revealed that the metal complexes showed enhanced inhibitory activity compared to the ligand with the nickel

complex exhibiting highest activity. The results indicate the Schiff base metal complexes can be utilized in the development of new antibacterial agents making them suitable for further exploration.

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