



Structural and Biological Activity Study of (*E*)-*N'*-(5-Chloro-2-Hydroxybenzylidene)Nicotinohydrazide [H_2L] and some of its Divalent Metal Complexes

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ABSTRACT

Nicotinic acid hydrazide and 2-hydroxy-5-chlorobenzaldehyde were condensed at 80°C to form an ONO coordination pattern ligand (H_2L). The structure of the ligand was elucidated by using ¹H NMR, ¹³C NMR, COSY, HSQC, CHN analyzer, ESI mass spectrometry and IR. Thereafter, five metal complexes [Cu(II), Fe(II), Zn(II), Mn(II) and Ni(II)] of the ligand were synthesized and their structural elucidation were achieved by using elemental analysis, infrared, EPR, molar conductivity and powder X-ray diffraction studies. Structural geometries of some of the compounds were predicted. Based on the analysis, octahedral geometry was suggested for the complexes. *In vitro* anti tubercular activity study of the ligand and the metal complexes were evaluated against *Mycobacterium tuberculosis*, H37Rv, by using micro-diluted method. The results obtained (H_2L - MIC = 0.86 µg/mL), (CuL- MIC = 0.64 µg/mL), (FeL- MIC = 0.77 µg/mL), (ZnL- MIC = 0.69 µg/mL), (MnL- MIC = 1.12 µg/mL) and (NiL- MIC = 0.92 µg/mL) revealed that all the compounds except (MnL- MIC = 1.12 µg/mL) possess better activity when compared with one of the first line drug (isoniazid) (INH- MIC = 0.9 µg/mL). H_2L exhibited higher anti tubercular activity with MIC value of 0.86 µg/mL. However, the metal complexes displayed higher cytotoxicity but were found to be non-significant different ($P < 0.05$) to is on isoniazid drug.

Keywords: Hydrazones, electron spin resonance, thermogravimetric, powder X-ray diffraction, antitubercular agents.

INTRODUCTION

Tuberculosis (TB) is measured to be amongst the 10 top causes of death across the globe

(WHO, 2016). The TB widespread is greater than formerly estimated. In 2015, about 10.4 million new cases of TB was recorded of which 56% of them are men while 34% are women and 10% are children.

In the same year, the number of deaths from multi drug-resistant TB (MDR-TB) and rifampicin-resistant TB (RR-TB) increased by 0.4 million. Tuberculosis resulted to estimate 1.4 million deaths in 2015 worldwide with six countries like India, Indonesia, China, Nigeria, Pakistan and South Africa accounted for 60% of the deaths (WHO, 2015; Ian, 2016). Based on the WHO reports, the need to reduce or eradicate the occurrence of the disease before 2020 is of high priority.

Emerging transition metal complexes with anti-tubercular activity has engrossed substantial interests among the bio-inorganic chemists over the world based on the past success of cisplatin (Chen *et al.*, 2013; Dasari and Tchounwou, 2014). Hydrazones are group of compounds found with numerous biological activities which includes antibacterial, antitumor, antimalarial and antituberculosis (El-Sherif, *et al.*, 2012; Hosney, *et al.*, 2010). The reports (Vogel, *et al.*, 2008; Grande *et al.*, 2007) have revealed that hydrazides, $RCONHNH_2$ and hydra zones, $RCONHN=CHR'$, are classes of compounds that are effective as chelating agents with transition metal ions. They have readily available lone pairs of electrons useful for bind with the transition metal ions (Stadler and Harrow field, 2009). Based on these backgrounds, scientists are now directing their works on substituted hydra zones. Metal complexes of substituted hydra zones have been stated to display therapeutic activities (Angelusiu *et al.*, 2010; Hosney, *et al.*, 2010). The metal complexes bind and cleave the DNA strands. Hence, the compounds possess could be used for biomimetic applications and as models for elucidation the mechanism of enzyme inhibition (Michael, 2010; Jagvir and Prashant, 2012).

In the present work, an arylhydra zone obtained in the reaction of nicotinic hydrazide with 5-chloro-2-hydroxybenzaldehyde and its Cu(II), Fe(II), Zn(II), Mn(II) and Ni(II) complexes were characterized and tested for their *anti myco bacterium* activity. The presence of the hydrazine

pharmacophore in these compounds is expected to contribute to high *anti mycobacterium* activity.

METHODS

Chemicals

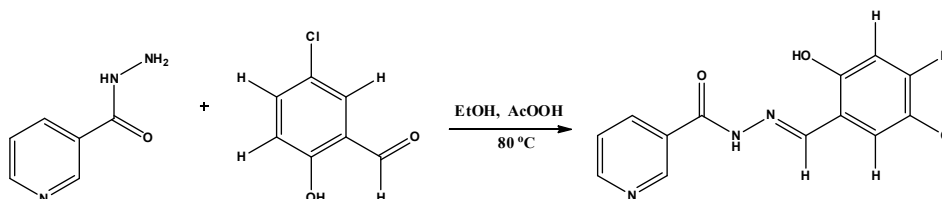
The reagents and chemicals used in this work were purchased from Sigma-Aldrich. They are analytical grade and used without further purification.

Physical measurements

The mass spectrum of H_2L was obtained on Agilent 6520 Q-TOF mass spectrometer. The % of carbon, nitrogen and hydrogen of the hydra zone and its metal complexes were determined by using Vario EL CHNS analyzer. 1H , ^{13}C and 2D NMR (COSY and HSQC) spectra H_2L were recorded by using Bruker AMX 300 FT-NMR spectrometer in $DMSO-d_6$. The IR spectral of the H_2L and its metal complexes were recorded on Perkin – Elmer Fourier Transform Infrared Spectrometer using KBr pellets in the range of $4000 - 400\text{ cm}^{-1}$. The magnetic data of some of the metal complexes were measured at room temperature by using vibrating susceptibility magnetometer. TGA/DTA thermographs of some of the metal complexes were obtained by using thermogravimetric analyzer TGA Q500 V20.8 Build 34. The EPR spectra some of the metal complexes recorded at 77 K were recorded on Varian E-112 spectrometer with TCNE used as the standard. Powder X-ray diffraction data for one of the metal complexes was recorded with a Bruker AXS D8 Advance diffractometer.

Synthesis of (E)-N'-(2-hydroxy-5 chloro benzylidene) nicotino hydrazide [H_2L]

The synthetic methods previously described [Mandewale *et al.*, 2015; Thomas *et al.*, 2011] was modified and adopted. The nicotinic acid hydrazide (10 mmole, 137 mg) was dissolved in 20 ml of absolute ethanol by heating gently on water bath. The solution obtained was mixed with ethanoic solution of 2-hydroxy-5-chlorobenzaldehyde (10 m mole) in a round bottom flask. The mixture was refluxed stirred



at 80 °C for 6 h after which it was allowed to stand at ambient temperature for 24 hours. The precipitate formed was filtered and washed three times with cold ethanol. The precipitate was recrystallized in mixture of methanol and chloroform (1:1). It was filtered off, washed with ether and dried in vacuum. The reaction was monitored with the use of TLC using methanol: chloroform (2:8) mixture.

The aldehyde used in the synthesis is 5-chloro-2-hydroxybenzaldehyde; yield 1.76 g (71.3 %); mp: 229-230 °C; $R_f = 0.82$ ($\text{CHCl}_3/\text{CH}_3\text{OH}$, 4:1, at RT.). $^1\text{H-NMR}$ ($\text{DMSO-}d_6$) δ : 12.36(s, 1H, NH), 11.21 (s, Ar-OH, 1H), 9.13 (d, $J = 1.71$ Hz, 1H, H(3)), 8.83 (dd, $J_1 = 1.2$ Hz, $J_2 = 4.7$ Hz, 1H, H(4)), 8.67 (s, 1H, H-C=N), 8.33 (dt, $J_1 = 1.71$ Hz, $J_2 = 6.15$ Hz, 1H, H(6)), 7.74 (d, $J = 2.61$ Hz, 1H, H(7)), 7.64

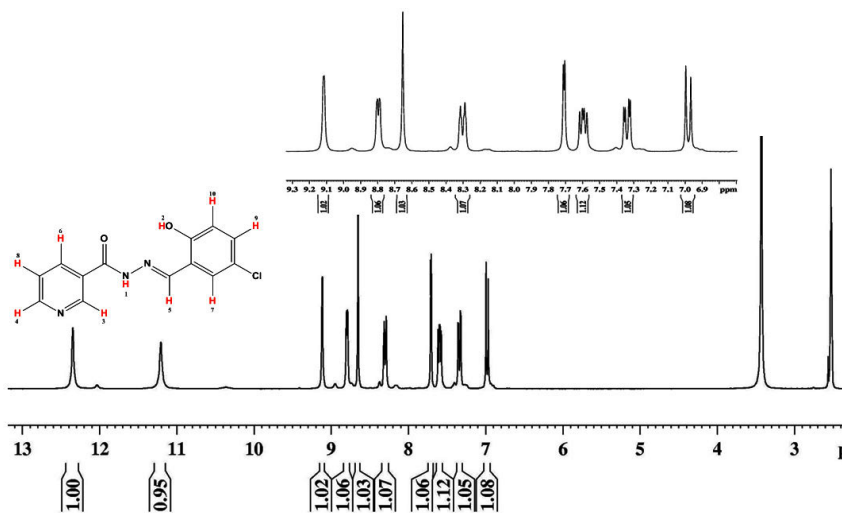


Fig. 1: $^1\text{H-NMR}$ spectrum of H_2L in $\text{DMSO-}d_6$ at 300 MHz

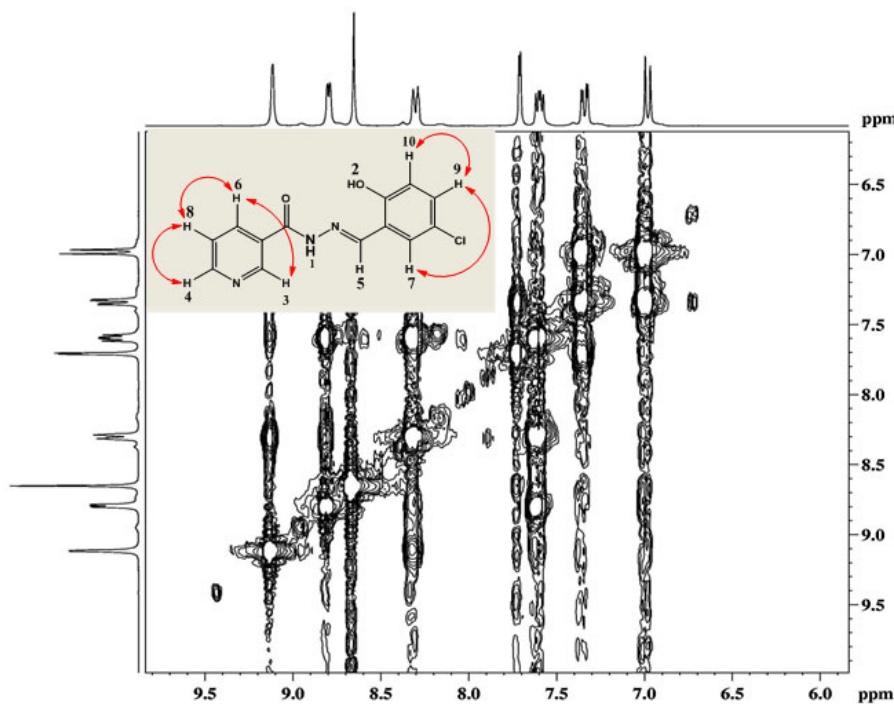


Fig. 2: COSY spectrum of H_2L in $\text{DMSO-}d_6$ at 300 MHz

(m, $J_1 = 4.83$ Hz, $J_2 = 7.86$ Hz, 1H, H(8)), 7.39 (dd, $J_1 = 2.64$ Hz, $J_2 = 8.64$ Hz, 1H, H(9)), 7.02 (d, $J = 8.76$ Hz, 1H, H(10)), ppm. $^{13}\text{C-NMR}$ (DMSO- d_6) δ : 162.12 (CO), 156.56 (C-OH), 152.99 (C(3)), 149.17 (C₍₄₎), 146.76 (HC=N), 136.0 (C(6)), 131.48 (C(7)), 129.13 (C(8)), 127.98 (C(9)), 124.14 (C(10)), 123.58 (C(11)), 121.18 (C(12)) 118.5 C(13), ppm. IR (KBr) cm^{-1} : 3502-3328 (ArOH), 3193 (NH), 1666 (C=O), 1613 (C=N), 1552 (N-N), 1347 (C-O), 1158 (C-N), 707 (C-Cl). MS (ESI+): in m/z : 276.2 [M + H]⁺. Anal. calcd. for C₁₃H₁₀ClN₃O₂ (275.05): C, 57.72; H, 3.64; N, 15.27. Found: C, 57.55; H, 3.83; N, 14.88.

Synthesis of metal complexes

Synthesis of Cu(II) complexes of (E)-N'-(5-chloro-dihydroxybenzylidene) nicotinothiazide [Cu(H₂L)₂] SO₄

Ethanol solution of H₂L was prepared by dissolving 20 m mol of H₂L in 15 mL of absolute ethanol in a round bottomed flask. The solution was warmed to 50°C on a water bath for 40 min before adding 15 mL of ethanolic solution (10 m mol) of CuSO₄. The mixture was refluxed at 80 °C for 4 h. The green product formed was filtered after cooling the solution in iced water, washed with ethanol and ether, and then dried over P₄O₁₀ *in vacuo*. Yield: 187 mg (84.61%).

Elemental Anal.

found (Calcd.) (%): Cu, 8.86 (8.94); C, 43.85 (43.92); H, 3.07 (2.84); N, 11.54 (11.82). $\mu = 2.1$ BM.

Synthesis of Fe(II) complexes of (E)-N'-(5-chloro-2-dihydroxybenzylidene) nicotinothiazide [Fe(H₂L)Cl₂]

Fe(III) complex of H₂L was prepared by

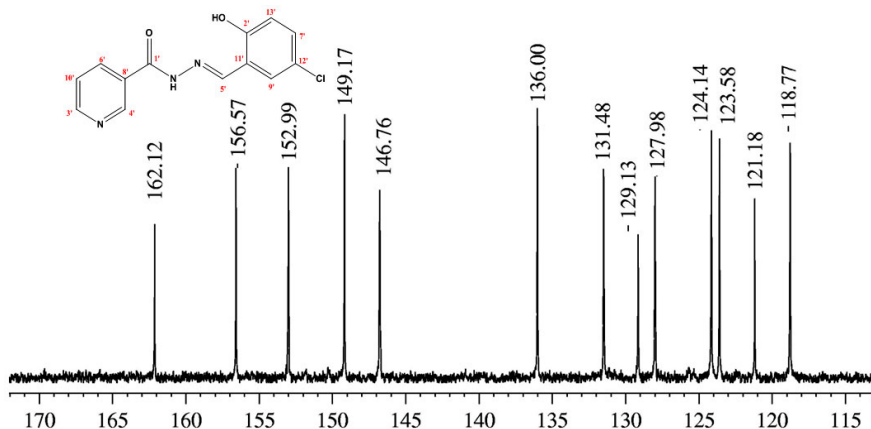


Fig. 3: ^{13}C NMR spectrum of H₂L₄ in DMSO- d_6 at 75 MHz

dissolving 161 mg (10 m mol) of H₂Lin 10 ml of absolute ethanol in a round bottomed flask. The solution was with ethanolic solution of FeCl₂. The mixture was refluxed for 4 h and kept at room temperature for 4 days to obtain a deep brown product which was with cold ether and dried over P₄O₁₀ *in vacuo*. Yield: 128 mg (55.89%).

Elemental Anal.

found (Calcd.) (%): Fe, 15.65 (15.22); C, 43.25 (42.55); H, 3.07 (2.75); N, 11.17 (11.45). $\mu = 5.90$ BM.

Synthesis of Zn(II) complexes of (E)-N'-(5-chloro-2-dihydroxybenzylidene) nicotinothiazide [Zn(H₂L)₂]

10 m mol (229 mg) of Zn(CH₃COO)₂ was dissolved in 10 mL of absolute ethanol, The solution obtained was added gradually to ethanolic solution of H₂L (10 m mol) in a round bottomed flask after which two drops of triethylamine (TEA) was added. The solution was refluxed for 3 h and the yellow precipitate obtained was dried over P₄O₁₀ *in vacuo* after washing with cold ethanol. Yield: 152 mg (78.76%).

Elemental Anal.

found (Calcd.) (%): Zn, 10.52 (10.60); C, 50.78 (50.63); H, 3.49 (3.27); N, 13.87 (13.63). $\mu = 0$ BM.

Synthesis of Mn(II) complexes of (E)-N'-(5-chloro-2-dihydroxybenzylidene) nicotinothiazide [Mn(H₂L)₂] H₂O

10 m mol (161 mg) of H₂L dissolved in 10

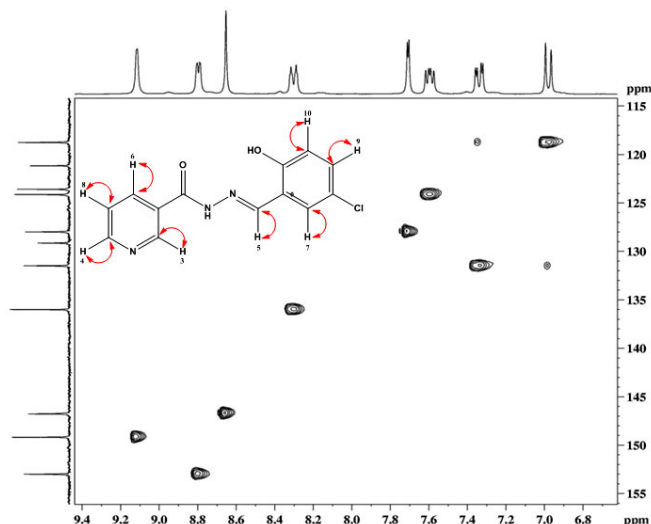


Fig. 4: ^1H - ^{13}C HSQC spectrum of H₂L in DMSO-*d*₆ 75 MHz

ml of absolute ethanol was added to 10 m mol of $\text{Mn}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ in a round bottomed flask. The mixture was refluxed for 4 h after adding two drops of TEA. The brown precipitate obtained was dried over P_4O_{10} *in vacuo* after washing with cold ethanol. Yield: 173 mg (53.2%).

Elemental Anal.

found (Calcd.) (%): Mn, 8.28 (8.80); C, 49.63 (50.02); H, 3.67 (3.55); N, 13.05 (13.46). $\mu = 6.03$ BM.

Synthesis of Ni(II) complexes of (E)-N'-(5-chloro-2-dihydroxybenzylidene)nicotinothiazide [$\text{Ni}(\text{H}_2\text{L})_2$]

10 m mol of $\text{NiCl}_2 \cdot 4\text{H}_2\text{O}$ was dissolved in 10 ml of ethanol and was added to ethanolic solution of H₂L (20 m mol in 10 ml of ethanol) in a round bottomed flask. The solution was refluxed for 4 h and the precipitate formed was allowed to stand at ambient temperature for 12 h after which it was filtered, washed and dried over P_4O_{10} *in vacuo*. Yield: 196 mg (52.10%).

Elemental Anal.

found (Calcd.) (%): Ni, 9.23 (9.34); C, 50.60 (51.19); H, 3.57 (3.30); N, 13.55 (13.78). $\mu = 2.92$ BM.

Anti mycobacterial Activity Study

RESULTS AND DISCUSSION

NMR spectral studies of (E)-N'-(2-hydroxy-5-chlorobenzylidene)nicotinothiazide [H₂L]:

^1H NMR

^1H NMR spectrum of H₂L (Figure 1.0) in DMSO-*d*₆ showed singlet peaks at $\delta = 12.36$ ppm and $\delta = 11.21$ ppm. They were assigned to NH(1) and OH(2) proton respectively. Both peaks were strongly de-shielded due to direct attachment to high electro negative atoms (Cui *et al.*, 2012; Webster and Silver stein, 2006). The doublet signal at $\delta = 9.13$ ppm, and doublet of doublet signal at $\delta = 8.83$ ppm were attributed to H(3) and H(4) respectively. Both peaks appeared down field of TMS as expected because of the de-shielding effect from the adjacent N(1) atom. Also, the singlet peak at $\delta = 8.67$ ppm was assigned to H(5) proton. The lower field δ value observed was attributed to conjugative effect from adjacent N(3)-N(2)-C(1) = O(1) core of the hydrazone. The H(6) and H(8) protons in the pyridyl ring, were assigned to the signal at $\delta = 8.33$ ppm and $\delta = 7.64$ ppm respectively. H(6) appeared as doublet of triplet while H(8) appeared as a multiplet signal. In the phenol ring, the doublet peak that integrated as one proton at $\delta = 7.74$ ppm corresponded to H(7) protons while the H(9) and H(10) protons

were assigned to doublet of doublet peak at $\delta = 7.39$ ppm and to a doublet peak at $\delta = 7.02$ ppm respectively.

Cosy

The assignments of proton was confirm with the use of ^1H - ^1H correlation spectroscopy (Figure 2.0). The cross peaks along both sides of the diagonal identify the proton that are coupled to each other (Webster and Silver stein, 2006; Benjamin and Dell, 2008). The proton at 12.36, 11.21 and 8.67 ppm assigned to H (1), H (2) and H (5) proton did not show correlation in the COSY spectrum. Therefore, the observation accounted for singlet signals observed in the spectrum. In the pyridyl ring, the signal at 9.13 ppm, assigned to H (3) showed a weak correlation with H (6) at 8.33 ppm and at the same time, H (6) also correlated with H (8), while H(8) showed direct correlation with both H (6) and H(4). Based on this, the signal for H (3), appeared as a doublet, while that of H (6) appeared as a doublet of triplet. Also the signal for H (4) appeared as a doublet of doublet while the signal for H (8) appeared as quartet. In the phenol ring region, the signal at

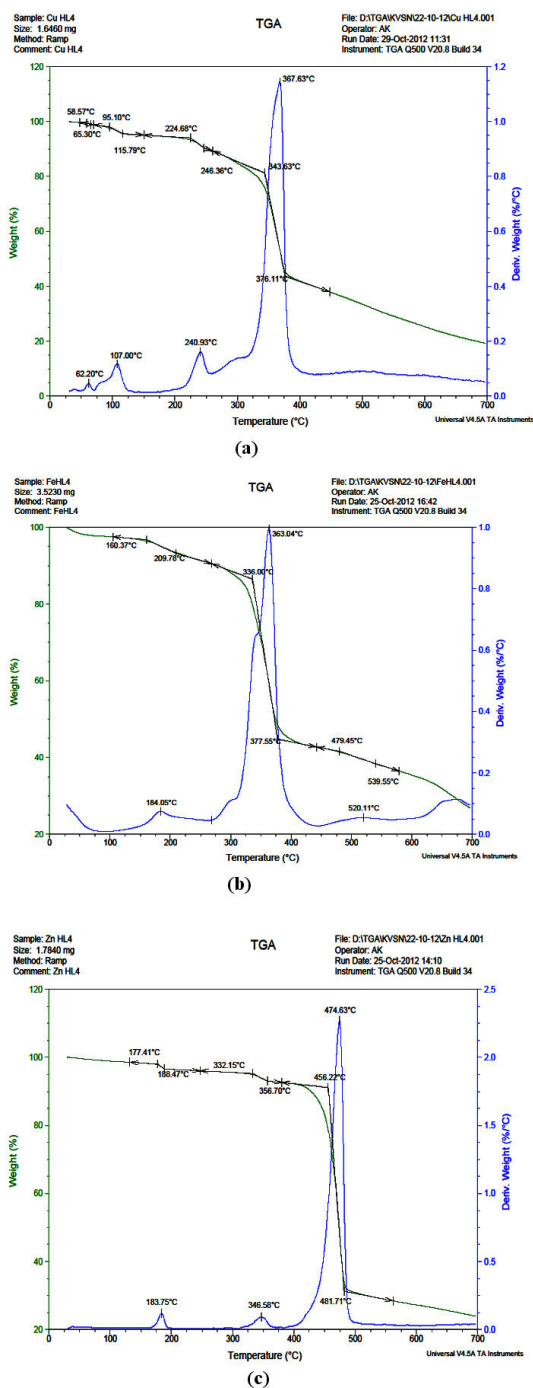


Fig. 5: TGA / DTA spectra of (a); $[\text{Cu}(\text{H}_2\text{L})_2]\text{SO}_4$; (b); $[\text{Fe}(\text{H}_2\text{L})\text{Cl}_3]$ and (c); $[\text{Zn}(\text{H}_2\text{L})_2]$

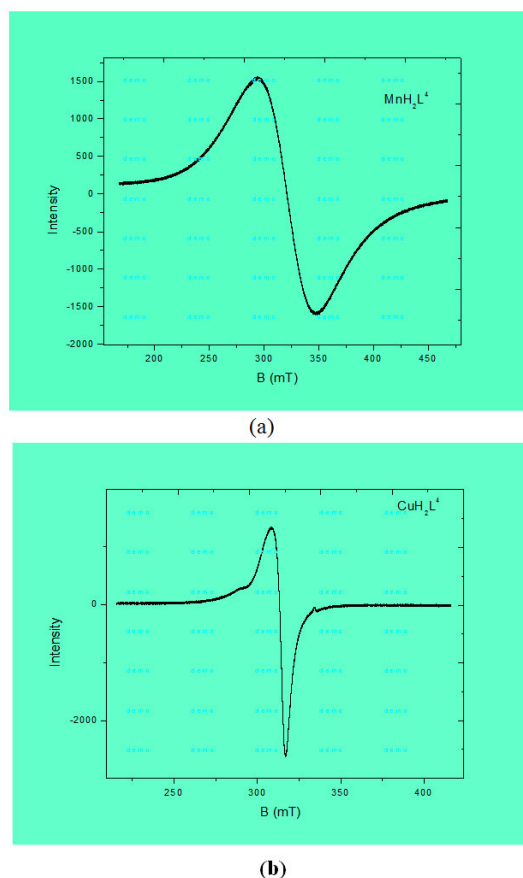


Fig. 6: EPR spectrum of (a); $[\text{Mn}(\text{H}_2\text{L})_2]\text{H}_2\text{O}$ and (b); $[\text{Cu}(\text{H}_2\text{L})_2]\text{SO}_4$ in DMSO at 77 K

7.39 ppm assigned to H (9) proton experienced ortho-coupling from H (10) and metal-coupling from H (7). The correlation accounted for doublet peaks at 7.74 and 7.02 ppm assigned to H (7) and H (10) respectively. The correlations observed in the COSY spectrum are supported by the J values of each of the protons.

¹³C NMR

The carbon skeleton of the hydrazone was identified by recording its ¹³C NMR spectrum (Figure 3.0). From the spectrum, it was observed that the carbonyl carbon C (1) resonated at 162.12 ppm while the aromatic carbon C (2) resonated at 156.57 ppm. The two carbons are the most deshielded carbon due to delocalization of π -electron in the carbonyl and the presence of hydrogen

bonding in the hydroxyl group, which reduced the electron density around the carbon atoms (Benjamin and Dell, 2008; Kate *et al.*, 2016). The signals at 152.99 and 149.17 ppm were assigned to C (3) and C (4) respectively. The two carbons resonated down field due to the high electro negative effect of the adjacent N (1) atom. The resonances at 136.00, 129.13 and 124.14 ppm were assigned to the other pyridyl carbons i.e. C(6), C (8) and C (10) respectively. Azomethine carbon C(5) resonated at 146.76 ppm while the δ values assigned to the other carbons in the phenol ring are C (7), 131.48; C (9), 127.98; C (11), 123.58; C (12), 121.18 and C (13), 118.77 ppm. The two carbons, C (7) and C (9), adjacent to C (12)-Cl resonated at lower field than the other phenol carbons due to conjugative effect.

Table 1: Analytical data of H₂L⁴ and its metal complexes.

Complex	Colour	M. pt. oC	Found (Calc.), %				LM <i>S cm² mol⁻¹</i>	μ_{eff} μB
			M	C	H	N		
[Cu(H ₂ L) ₂]SO ₄	Green	>300	8.86 -8.96	43.85 -44.05	3.07 -2.56	11.54 -11.85	43	2.1 -1.73
[Fe(H ₂ L)Cl ₃]	Deep brown	>300	15.65 -15.26	43.25 -42.66	3.07 -2.48	11.17 -11.48	52	5.9 -4.92
[Zn(H ₂ L) ₂]	Yellow	>300	10.52 -10.64	50.78 -50.8	3.49 -2.95	13.87 -13.67	45	-
[Mn(H ₂ L) ₂]H ₂ O	Brown	>300	8.28 -8.77	49.63 -49.86	3.67 -3.86	13.05 -13.42	48	6.03 -5.92
[Ni(H ₂ L) ₂]	Yellow	>300	9.23 -9.59	50.2 -51.02	3.57 -3.62	13.55 -13.73	46	2.92 -2.83

H₂L = C₁₃H₁₀ClN₃O₂

Table (2.0): Infrared data of H₂L and its metal complexes.

Ligand/ Complex	$\nu(OH)$ cm ⁻¹	$\nu(C=O)$ cm ⁻¹	$\nu(C=N)$ cm ⁻¹	$\nu(N-N)$ cm ⁻¹	$\delta(C-O)$ cm ⁻¹	$\delta(C-N)$ cm ⁻¹	$\nu(M-O)$ cm ⁻¹	$\nu(M-N)$ cm ⁻¹
H ₂ L	3328- 3193 m	1666 s	1613 m	1552 s	1345 m	1158 m	-	-
[Cu(H ₂ L) ₂]SO ₄	3413 s,b	1616m	1523 m	1456 m	1371 w	1179 m	597w	515 w
[Fe(H ₂ L)Cl ₃]	3425 s,b	1605 m	1531 w	1454 w	1379 w	1183 m	559 w	427 m
[Zn(H ₂ L) ₂]	3418 s,b	1614 m	1528 m	1470 m	1377w	1188 m	559 w	510 w
[Mn(H ₂ L) ₂]H ₂ O	3424 s,b	1610m	1560 w	1455 m	1377 m	1177 m	612 m	427 w
[Ni(H ₂ L) ₂]	3420 s,b	1667 w	1608 m	1553 m	1374 w	1182 m	518 w	497 w

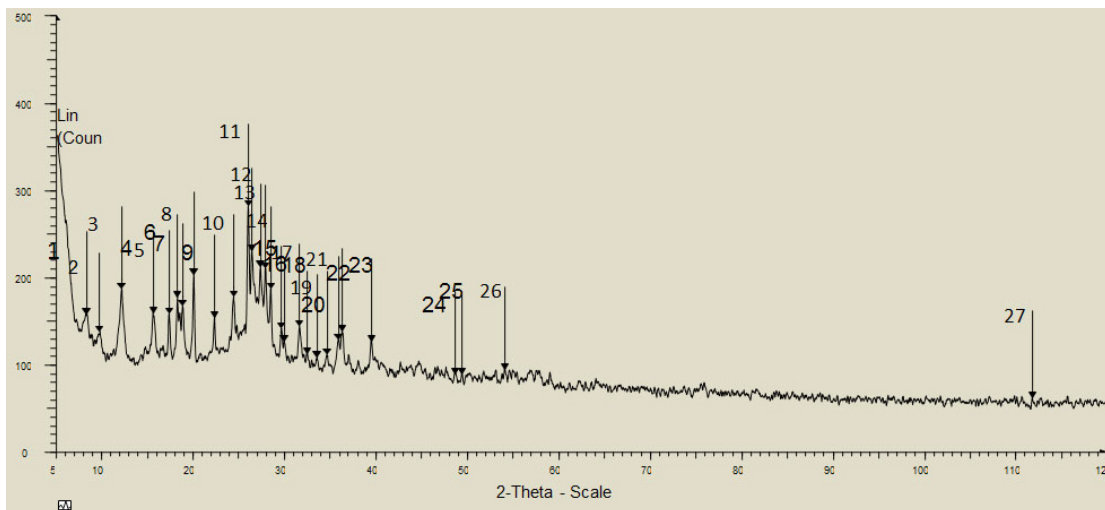
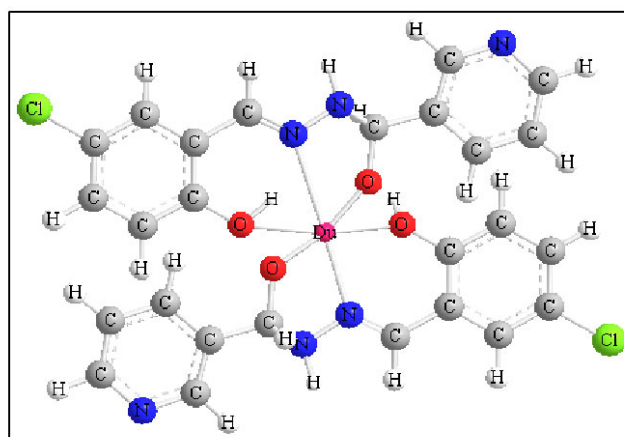
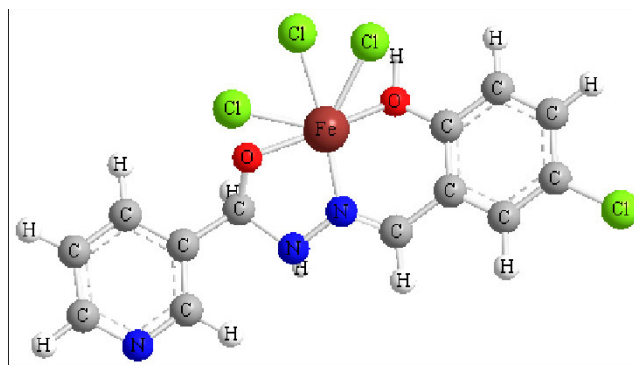


Fig. 7: X-ray diffraction pattern of $[\text{Cu}(\text{H}_2\text{L})_2]\text{SO}_4$



When Du: Cu:- $[\text{Cu}(\text{H}_2\text{L})_2]$; Zn:- $[\text{Zn}(\text{H}_2\text{L})_2]$
 Mn:- $[\text{Mn}(\text{H}_2\text{L})_2]$; Ni:- $[\text{Ni}(\text{H}_2\text{L})_2]$



$[\text{Fe}(\text{H}_2\text{L})\text{Cl}_3]$

$[\text{Fe}(\text{H}_2\text{L})\text{Cl}_3]$

Figure 8.0: Proposed structures for the complexes

^1H - ^{13}C HSQC

The ^1H - ^{13}C HSQC correlation spectrum for the hydrazine (Figure 4.0) was recorded to ascertain the ^1H NMR and ^{13}C NMR assignments. HSQC contour was not observed for carbon atoms at 162.12, 156.57, 129.13, 123.58 and 121.18 ppm. These signals were assigned to C (1), C (2), C (8), C (11) and C (12) respectively. Both C (8) and C (11) are quaternary carbons (Webster and Silver stein, 2006; Kate *et al.*, 2016). HSQC spectrum shows eight contours for protonated carbon as expected. Each contour confirmed ^1H NMR assignments in correlation with the ^{13}C NMR assignments.

Physical properties of Cu(II), Fe(II), Zn(II), Mn(II) and Ni(II) complexes of (E)-N'-(2-hydroxy-5-chlorobenzylidene)nicotinohydrazide [H_2L]

The metal complexes of H_2L are of various colours. All the metal complexes precipitated as amorphous powder. The metal complexes were found to possess high melting point (>300 °C). They are sparingly soluble in methanol, chloroform and DMF but are soluble in DMSO and pyridine. The elemental analysis data obtained are in agreement with the theoretical values obtained. The results obtained, combined with other analytical data were used to arrive at the molecular formulae presented in Table (1.0). The magnetic susceptibility data recorded at ambient temperature for Cu(II), Fe(II), Mn(II) and Ni(II) complexes demonstrated full agreement with the calculated spin only magnetic moments for the complexes. As expected, Zn(II) complex was found

to be diamagnetic. Low conductance values obtained using conductivity meter for all the complexes in DMSO demonstrated non - electrolytic character of the complexes.

Infrared spectra

The infrared spectral data of the ligands H_2L and its metal complexes are presented in Table 2.0. The broad peak between 3328 and 3193 cm^{-1} attributed to the $\nu(\text{C-OH})$ vibrational stretching mode (Stuart, 2004; Webster and Silver stein, 2006) in the hydrazone spectrum was observed as strong and broad peak at higher wave numbers between of 3413 and 3425 cm^{-1} in the metal complexes. The observation supported the bonding of the OH group to metal ions in the complexes. The conjugation effect also resulted to the shifting of $\nu(\text{C-O})$ deformation mode to higher wave number in the complexes.

The strong peak observed at 1666 cm^{-1} in the spectrum of the free ligand was attributed to the characteristics of $\nu(\text{C=O})$ vibrational stretching mode (Stuart, 2004). Similar peak was observed at lower wavelength in the spectral of the complexes except in Ni(II) complex due to effect of ring restrain that prevent free vibration of the chromophoric group after coordination to the metal ion. However, in Ni(II) complex, the band appeared at higher wave number with reduction in intensity which indicates formation of covalent bond between the oxygen atom of the carbonyl and Ni(II) ion in the complex. This is further supported by the significant change in

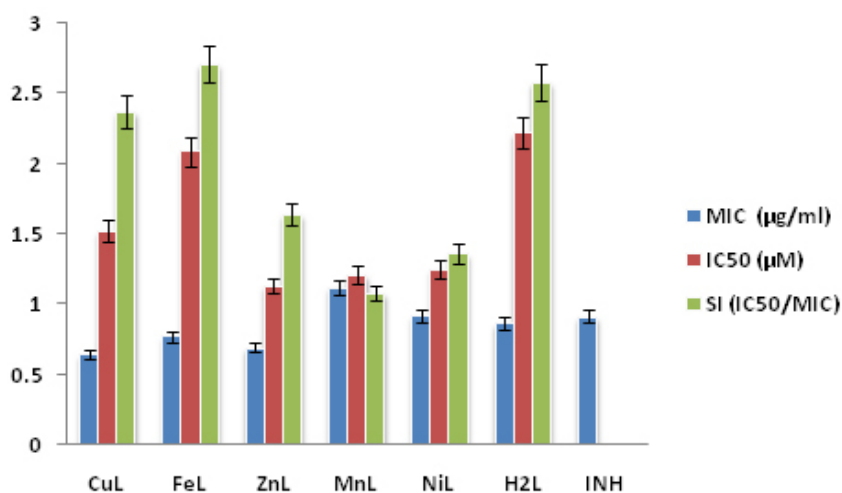


Fig. 9: Comparing of MIC ($\mu\text{g}/\text{mL}$) values of the metal complexes of H_2L with the ligand (H_2L) and isoniazid (INH) drug.

the wavelength of $\nu(\text{C-O})$ deformation bands which shifted to higher wave number in the spectral of the complexes due to the coordination of CO to the metal ion.

The azomethine $\nu(\text{C=N})$ vibrational stretching mode at 1613 cm^{-1} in the spectrum have undergone hypsochromic shift in the metal complexes due to restrain in its vibrational frequency after coordination to metal ions (Shi *et al.*, 2009; Dhanaraj and Nair). The observation is further supported by the higher wavenumber shift for the $\delta(\text{C-N})$ deformation bands in the metal complexes. However, the medium peak at 3502 cm^{-1} , which is characteristic of secondary amine of the amide group in the hydrazone, disappeared in the spectra of the metal complexes due to the conjugate effect of the coordination to the azomethine N atom. Other bands observed in the complexes in the regions $518 - 597\text{ cm}^{-1}$ and $515 - 427\text{ cm}^{-1}$ are due to $\nu(\text{M-O})$ and $\nu(\text{M-N})$ respectively.

TGA / DTA Analyses

Thermal analysis curves (TGA / DTA) for Cu(II), Fe(II) and Zn(II) complexes obtained under inert atmosphere within the temperature range of ambient temperature to $700\text{ }^\circ\text{C}$ are as shown in Figure 5.0. The thermal decomposition of Cu(II) complex proceeded in four degradation steps. The first three degradation steps ranging from $58 - 65$, $95 - 115$ and $224 - 246\text{ }^\circ\text{C}$ corresponded to the loss of the SO_4^{2-} ion from the complex (Gabbott, 2008). The degradation accounted for 14% (Calcd. 13%) loss in the total weight of the complex. The coordinated ligand decomposed to residue within the temperature range of $246 - 450\text{ }^\circ\text{C}$. This

Table 3.0: MIC ($\mu\text{g/mL}$), IC_{50} (μM) and SI ($\text{IC}_{50}/\text{MIC}$) values of the metal complexes of H_2L .

Compound Code	MIC ($\mu\text{g/ml}$)	IC_{50} (μM)	SI ($\text{IC}_{50}/\text{MIC}$)
CuL	0.64 ± 0.073	1.52	2.37
FeL	0.77 ± 0.056	2.09	2.71
ZnL	0.69 ± 0.022	1.13	1.64
MnL	1.12 ± 0.159	1.21	1.08
NiL	0.92 ± 0.271	1.25	1.36
H_2L	0.86 ± 0.201	2.22	2.58

INH (Control) = 0.91 ± 0.133 ($\mu\text{g/mL}$)

decomposition corresponded to the loss of 62% (Calcd. 61%) of the complex. The residue, which is about 24% (Calcd 23%) of the complex, was considered to be metal oxide and some carbon residue.

The TGA / DTA curve for Fe(III) complex showed one main degradation step between 209 and $539\text{ }^\circ\text{C}$ which correlated with the decomposition of the ligand in the complex and it was found experimentally to be 65% (Calcd. 66%) weight loss. However, the decomposition observed between 160 and $209\text{ }^\circ\text{C}$, equivalent of 18% weight loss, was attributed to loss of coordinated chlorine atoms in the complex. The residue, which is mainly metal oxide, was found to be equivalent to 16% (Calcd. 16.5%) of the complex.

The thermogram for Zn(II) complex showed one main decomposition step which corresponded to 75% (Calcd. 77%) weight loss. This was attributed to the decomposition of the coordinated ligand, H_2L . However, the slight decomposition at $183\text{ }^\circ\text{C}$ and $346\text{ }^\circ\text{C}$ were considered to be part of the ligand decomposition. The residue, which was mainly metallic oxide, accounted for 25% (Calcd. 23%) of the complex.

Electron paramagnetic resonance spectra

The EPR spectra for representative [Mn(II) and Cu(II)] complexes recorded in DMSO at 77 K are as shown in Figures 6.0. The EPR spectrum of Mn(II) complex appeared as a broad peak with no hyper fine splitting. However, the g_{iso} observed (2.0549) was found to be greater than free electron value of 2.0023. Also, the observed $g_{\parallel} = 3.6193$ was found to be greater than g_{\perp} of 1.9597. The observation suggests that Mn(II) is in octahedral environment (Pilbrow, 1990).

The spectrum of the Cu(II) complex at 77 K showed one intense absorption band at the high field and was isotropic due to the tumbling motion of the molecules. The Cu(II) complex exhibited the g_{\parallel} value of 2.5921 and g_{\perp} value of 2.0771. These values suggests a distorted octahedral structure, thus ruling out the possibility of a trigonalbipyramidal structure which would be expected to have $g_{\perp} > g_{\parallel}$.

Powder X-ray diffraction studies

The crystal lattice parameters for Cu(II)

complex were measured by using a Bruker AXS D8 Advance diffractometer. An X-ray diffractogram was recorded (Figure 7.0) in the range of 5° to 120° on 2θ scale. The major peaks of relative intensity greater than 10% were indexed to yield the Miller indices (hkl), the unit cell parameters and the unit cell volume. The unit cell parameters of Cu(II) complex yielded values of lattice constants: $a = 21.315 \text{ \AA}$, $b = 25.031 \text{ \AA}$ and $c = 10.045 \text{ \AA}$ and a unit cell volume V of 5359.36 \AA^3 . The parameters were found to be in agreement with orthorhombic lattice system in which the conditions such as $a \neq b \neq c$ and $\alpha = \beta = \gamma = 90^\circ$ were satisfied. Therefore, $[\text{Cu}(\text{H}_2\text{L})_2]\text{SO}_4$ complexes was considered to have a distorted octahedral geometry (Shoemaker and Garland, 1989).

Based on the analytical data and spectroscopic data obtained, the computer model structures of the complexes are shown below:

Anti-mycobacterial Study

The anti-mycobacterial properties of the H_2L ligand and its corresponding metal complexes were established by determining their minimum inhibitory concentration (MIC) represented in Table and Figure 8.0. The results indicated that Cu(II), Fe(III) and Zn(II) complexes are more potent than the standard antibiotic (isoniazid) with MIC values of 0.64, 0.77 and 0.69 $\mu\text{g/mL}$ respectively. This indicates that the metal ions play significant role in inhibitory activities of the complexes (Anna *et al.*, 2009; Petra, *et al.*, 2003; Koen, *et al.*, 2006). However, Mn(II) and

Ni(II) complexes were found to possess moderate inhibitory activity as compare to isoniazid. The two complexes show lesser activity than the standard antibiotic. Generally, the complexes demonstrated higher activity against drug-resistant strains of *Mycobacterial Tuberculosis*. However, they were found to be toxic to Vero cell as indicated by their IC_{50} values (Table 3.0).

CONCLUSION

(*E*)-*N'*-(5-chloro-2-hydroxybenzylidene) nicotino-hydrazone ligand and five of its transition metal complexes were synthesized with good yields under simple reflux conditions. The structures of the desired complexes were determined by spectral, thermal and powder xrd studies. The ligand coordinated to the transition metal in an octahedral mode forming M-ONO. The powder X-ray diffraction data for $[\text{Cu}(\text{H}_2\text{L})_2]\text{SO}_4$ complex further supported the geometry of the complex. The Complexes, except MnL and NiL, revealed higher activity against drug-resistant strains of *Mycobacterial Tuberculosis* is indicating that the compounds are potential antitubercular agents.

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