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# Synthesis, characterization and cytotoxicity of new 2-isonicotinoyl-N-phenylhydrazine-1-carbothioamide and its metal complexes

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Mohamed H. Abdel-Rhman, Chemistry Department, Faculty of Science, Mansoura University, Mansoura, Egypt. Email: mhassan2371@yahoo.com The reaction of the newly synthesized ligand, 2-isonicotinoyl-Nphenylhydrazine-1-carbothioamide (H<sub>3</sub>L), with acetate salt of Co (II), Cu (II), Ni (II) and Zn (II) led to isolation of four solid complexes. The ligand and complexes structure elucidation were based on elemental analyses, spectral analyses (IR, UV–Visible, <sup>1</sup>H and<sup>13</sup>C-NMR, MS and ESR), TGA, molar conductivity and magnetic moments measurements. The results indicated that the ligand exists in the thioketo form, while on coordination to the metal ions; it behaves as mono-negative bidentate chelate and exists in enol form. The optical band gap measurements of the ligand and its metal complexes are in the range 3.83–4.48 eV indicating their semi-conducting character. The cytotoxicity examination of H<sub>3</sub>L and its Zn (II) complex showed that the ligand have very strong cytotoxicity against both HCT-116 and HEPG-2 cell lines while, Zn (II) complex has moderate activity.

#### KEYWORDS

anticancer activity, isonicotinic hydrazide, optical properties, spectral study, thiosemicarbazides

# **1** | INTRODUCTION

Isonicotinic hydrazide (isoniazid, INH) is one of the most important hydrazides due to its wide biological activity as antitubercular, antimicrobial, anticonvulsant, analgesic, anti-inflammatory, anti-platelet and anti-tumor activities.<sup>[1,2]</sup> Isoniazid (INH) has very high *in vivo* inhibitory activity towards M. tuberculosis H37Rv. Various INH derivatives were synthesized and their activities against various strains of *M. tuberculosis* were studied.<sup>[3]</sup> The copper complexes of INH derivatives have been synthesized and the activities of these complexes have been evaluated as antitumor agents. The antitumor activity of the complexes was attributed to the inhibition of ribonucleotide reductase enzyme.<sup>[1]</sup> Moreover, iproniazid, N'isopropylisonicotinohydrazide, is used as antituberculosis and antidepressant drug.<sup>[4]</sup> Hydrazones derived from INH and pyridinecarboxaldehydes showed activity against

Gram positive and negative bacteria in addition exhibited good cytotoxicity activities.<sup>[5]</sup>

On the other hand, thiosemicarbazides have high biological and antitumor activities. Coordination with metal ions increases the activity of the thiosemicarbazides.<sup>[6]</sup> To obtain efficient chemotherapeutic agents, much more efforts have been directed to modify the structure and to the interpretation of the structure activity relationships (SAR). For example, in the pyridylthiosemicarbazide derivatives, the replacement of the thiocarbonyl sulfur atom by oxygen leads to decrease the activity whereas, selenium retains the activity. Also, the position of the attached substituents to the pyridyl ring is very effective factor in determining the antitumor activity.<sup>[7]</sup> Beside the biological activity of the thiosemicarbazide derivatives, they have wide applications in catalysis, non-linear optics, chemical analysis and various industrial aspects.<sup>[8]</sup>

#### 2 of 11 WILEY Organometallic Chemistry

The wide applications of isoniazid and thiosemicarbazide derivatives prompted us to synthesize and study the cytotoxicity of N-ethyl-2-isonicotinoylhydrazine-1-carbothioamide,<sup>[9]</sup> *N*-(2-isonicotinoyl hydrazine carbonothioyl)benzamide,<sup>[10]</sup>2-isonicotinoyl-N-phenylhydrazine-1-carboxamide<sup>[11]</sup> and N-benzoyl-2-isonicotinoylhydrazine-1-carboxamide<sup>[12]</sup> ligands and some of their metal complexes.

Thus, on continuation to the efforts of looking for new derivatives of isonicotinic thiosemicarbazides with promising anti-cancer activity, this work presents the synthesize and characterization of a new ligand, 2-isonicotinoyl-N-phenylhydrazine-1-carbothioamide and its metal complexes. To shed some light on the possible applications, the cytotoxicity and the optical band gap were tested. The ligand has very strong cytotoxicity against both HCT-116 and HEPG-2 cell lines. Furthermore, the present compounds, are semi-conductors.

# 2 | EXPERIMENTAL

## 2.1 | Reagents

Phenylisothiocyanate (98.0%) and isonicotinic hydrazide (99.0%) were purchased from Sigma-Aldrich. Co (II), Cu (II), Ni (II) and Zn (II) acetate salts were purchased from Merck. Colorectal (HCT-116) and Hepatocellular (HePG-2) carcinoma were obtained from ATCC. The reagents, Fetal Bovine serum purchased from GIBCO, UK. RPMI-1640 medium, MTT and 5-fluorouracil were obtained from Sigma.

# 2.2 | Measurements

The carbon and hydrogen contents were determined by Perkin-Elmer model 2400 CHN analyzer. The metals content were determined volumetrically using suitable indicators.<sup>[13]</sup> The magnetic susceptibilities were measured using a Sherwood Scientific magnetic balance. Molar conductivities were determined on an YSI model 32 conductivity bridge. The FT-IR spectra were recorded, as KBr discs, on a Thermo-Nicolet IS10 spectrometer. Electronic spectra measurements were carried out on Unicam UV/ Vis., spectrometer UV2 using1 cm Silica cells. The NMR <sup>1</sup>H and <sup>13</sup>C spectra of the ligand and its Zn (II) complex were recorded on Bruker Ascend 400 MHz. ESR spectra of the Cu (II) complex were measured at room temperature using Brucker E 500 ESR spectrometer (9.808 GHz, 100 kHz field modulation) from 480 to 6480 Gauss in a 2 mm quartz capillary. The thermal analysis (TG) were carried out using Shimadzu model 50 at heating rate 10 °C/min and the nitrogen flow rate 20 ml/min.

# 2.3 | Deconvolution analysis of IR spectra

IR spectra deconvolution analyses were carried out using Peak Fit program.<sup>[14]</sup> The spectra were corrected for the dark current noises and background. The Gaussian peaks exhibited the best-fitted data and the full width at half maximum, the intensity and position were adjusted automatically on the basis of the minimization of the deviations between experimental and simulated spectrum.<sup>[15,16]</sup>

# 2.4 | Synthesis of the ligand

Phenylisothiocyanate (0.01 mol) was added to isonicotinic hydrazide (0.01 mol) in 20 mL absolute ethanol dropwise and the reaction mixture was refluxed for 1 hr. White crystals were isolated after cooling to room temperature; filtered off, washed with ethanol and dried in air. Yield: 85%, m.p.: 184 °C.

# 2.5 | Synthesis of the metal complexes

To 30 ml hot ethanolic solution of the ligand (0.01 mol), equivalent amounts(0.01 mol) of Co (II), Cu (II), Ni (II) or Zn (II) acetates in 25 ml absolute ethanol were added dropwise with constant stirring. The reaction mixtures were refluxed for 2 hr, where colored precipitates were isolated on reflux. The precipitates were filtered off, washed successfully with ethanol, ether and dried at room temperature. Elemental analysis data and colors for the complexes are given in Table 1.

# 2.6 | Cytotoxicity assay

The cell lines, HePG-2 and HCT-116, were used to determine the inhibitory effects of the compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml Penicillin and 100  $\mu$ g/ml Streptomycin at 37 °C in a 5% CO<sub>2</sub> incubator. The cell lines were seeded in a 96-well plate at a density of 1.0x10<sup>4</sup> cells/well at 37 °C for 48 hr under 5% CO<sub>2</sub>. After incubation, different concentration of the compounds under investigation were added to the cells and incubated for 24 hr. After 24 h treatment, 20 µl of MTT solution at 5 mg/ml added and incubated for 4 hr. Dimethyl sulfoxide (DMSO), 100  $\mu$ L, is added into each well to dissolve the purple formazan formed. The colorimetric assay recorded at absorbance of 570 nm using a

TABLE 1 Elemental analyses of the ligand and its metal complexes

				Elemental analyses % Found (Calcd.)		
Compound (Formula)	Color	M.Wt. Yield %	m.p. (°C)	С	Н	М
$H_{3}L(C_{13}H_{12}N_{4}OS)$	White	272.33 95.07	184	57.44 (57.34)	4.32 (4.44)	-
$[Cu(H_2L)Ac]H_2O~(C_{15}H_{16}N_4O_4SCu)$	Dark green	411.92 70.08	>300	43.81 (43.74)	4.01 (3.92)	15.80 (15.43)
$[Co(H_2L)Ac]H_2O(C_{15}H_{16}N_4O_4SCo)$	dark green	407.31 66.18	>300	44.10 (44.23)	4.11 (3.96)	14.55 (14.47)
[Ni(H <sub>2</sub> L)Ac]3H <sub>2</sub> O (C <sub>15</sub> H <sub>20</sub> N <sub>4</sub> O <sub>6</sub> SNi)	dark green	443.10 68.70	>300	40.82 (40.66)	4.37 (4.55)	13.34 (13.25)
$[Zn(H_2L)Ac]H_2O(C_{15}H_{16}N_4O_4SZn)$	yellow	413.76 73.10	>300	43.42 (43.54)	4.00 (3.90)	15.88 (15.80)

plate reader (EXL 800, TM, USA Absorbance Microplate Reader). The relative cell viability in percentage was calculated as ( $A_{570}$  of treated samples/ $A_{570}$  of untreated sample) × 100. Doxorubicin was used as a standard anticancer drug for comparison.<sup>[17,18]</sup>

## **3** | **RESULTS AND DISCUSSION**

The elemental analyses data of the isolated compounds are shown in Table 1. The results indicate 1:1 (M:L) stoichiometric ratio for the isolated complexes. The melting points of the isolated complexes are >300 °C. They are insoluble in polar or nonpolar solvents but soluble in coordinating solvents as DMF and DMSO. The molar conductance of all complexes lies in the range 2.0–  $5.0 \ \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$  indicating their non-electrolytic nature.

#### 3.1 | Infrared spectra

The infrared spectra of the ligand, as KBr disc, showed two strong bands at 3266, 3116  $\text{cm}^{-1}$  with a shoulder at 3218 cm<sup>-1</sup> due to  $\nu$ (N<sup>4</sup>H),  $\nu$ (N<sup>1</sup>H) and  $\nu$ (N<sup>2</sup>H) vibrations, respectively.<sup>[9-11]</sup> Moreover, another two sharp bands were observed at 1673 and 1638 cm<sup>-1</sup> due to  $\nu$ (C=O) and  $\nu$ (C=N)<sub>Pv</sub> vibrations,<sup>[9-11]</sup>, respectively. The spectrum displayed also, four bands in the range 1550-1450 cm<sup>-1</sup> due to amide II, amide III, thioamide I and thioamide  $\text{II.}^{[10,11,19]}$  Furthermore, the band at 844 cm<sup>-1</sup> was assigned to  $\nu$ (C=S), while those at 773 and 746 cm<sup>-1</sup> were attributed to  $\rho$  (NH) vibration.<sup>[19]</sup> Thus, the above observations clarify the existence of the ligand in the ketoform, where NH's, carbonyl and thiocarbonyl vibrations exist (Figure 1). On comparison with the isonicotinic hydrazide spectrum, it was found that the  $\nu$ (C=O) vibration was shifted to a higher position in addition to the appearance of  $\nu(N^2H)$  vibrations as a shoulder may be attributed to the involvement in intramolecular hydrogen bond (Figure 2). The appearance of a new band at 1965 cm<sup>-1</sup> is an additional evidence for intramolecular hydrogen bond.<sup>[20]</sup>



FIGURE 1 Infrared spectra of H<sub>3</sub>L



FIGURE 2 Structure of the ligand

On the other hand, the spectral data of H<sub>3</sub>L metal complexes (Table 2) indicate that, all the complexes showed a band in 3428–3390  $\text{cm}^{-1}$  range attributed to  $\nu$ (OH)<sub>solvent</sub>.<sup>[21]</sup> Moreover, the spectra of the metal complexes displayed strong bands in the range 3272-3259 cm<sup>-1</sup> due to  $\nu$ (N<sup>4</sup>H) vibration.<sup>[10,11]</sup> Comparison with the ligand spectral data revealed that the position of the azomethine of the pyridyl ring remains at the same position indicating that it did not take part in coordination to the metal ions. On contrary, the  $\nu$ (N<sup>2</sup>H) vibration band was observed at lower wavenumber  $(3142 \text{ cm}^{-1})$  in case of Co (II) and Zn (II) complexes which is taken as an evidence for its involvement in coordination to the metal ions. While, in the case of Cu (II) and Ni (II) complexes, it was observed at 3210 which indicates that, this group is free.<sup>[9]</sup> The region 1750–1400  $\text{cm}^{-1}$  has overlapped bands, to overcome this overlapping deconvolution analysis was employed to resolve these bands (Table 3). The data

**TABLE 2**Infrared spectral data of the ligand and its metalcomplexes

		Complexes			
	$H_3L$	Cu (II)	Co (II)	Ni (II)	Zn (II)
ν (OH) <sub>solvent</sub>	-	3428	3390	3398	3419
$\nu(N^4H)$	3266	3259	3270	3272	3265
$\nu(N^2H)$	3218	3210	3141	3209	3142
$\nu(C=N^1)$	-	1612	1629	1614	1605
$\nu$ (C=N) <sub>Py</sub>	1638	1635	1636	1650	1628
Amide II	1550	1544	1562	1575	1560
Thioamide I	1515	1521	1524	1516	1522
Thioamide II	1448	1456	1457	1460	1455
Thioamide III	1255	1224	1224	1224	1224
Amide III	1228	1224	1224	1224	1224
ν(N-N)	1149	1168	1166	1164	1164
ν(C-O)	1066	1066	1062	1064	1066
ν(C=S)	844	836	838	836	838
ρ (NH)	773, 746	754	754	752	752
ν(M-O)	-	574	566	572	568
ν(M-N)	-	495	489	491	485

showed two new bands in 1709-1697 cm<sup>-1</sup> and 1591-1557 cm<sup>-1</sup> assigned to  $v_{as}$  (OAc) and  $v_{s}$  (OAc) vibrations, respectively of coordinated acetate ion in a bidentate fashion (the difference between  $v_{as}$  and  $v_s$  is ~ 150 cm<sup>-1</sup>).<sup>[19]</sup> In addition, the spectra of the metal complexes displayed new bands in the region 1613-1629 cm<sup>-1</sup> attributed to the vibrations of the newly formed azomethine group v(C=N<sup>1</sup>).<sup>[9-11]</sup> Furthermore, two new bands observed in the regions 574–566 and 495–485  $\text{cm}^{-1}$  may be due to  $\nu$ (M-O) and  $\nu$ (M-N) vibrations, respectively.<sup>[19]</sup> Thus, it could be deduced that the ligand coordinates to the metal ions in a mononegative bidentate fashion through the deprotonated enolic carbonyl oxygen and the amine nitrogen as supported by the following: The obscure of  $\nu(N^{1}H)$ and  $\nu(C=O)_{Pv}$  bands with appearance of new  $\nu(C=N^1)$ band (Figure 3). The shift to lower wavenumber of  $\nu$ (N<sup>2</sup>H) band was taken as an evidence for the involvement of these groups in coordination to metal ions. The position of the band due to  $\nu$ (C=S) vibration, approximately did not suffer from any change which indicates the inertness of sulfur atom towards coordination to the metal ions.

#### 3.2 | Nuclear magnetic resonance spectra

<sup>1</sup>H-NMR spectral data of the ligand, in DMSO-d<sub>6</sub>, exhibits three singlet signals corresponding to the protons of  $N^4H$ ,  $N^2H$  and  $N^1H$  at 10.48, 9.86 and 9.79 ppm,<sup>[22]</sup>

**TABLE 3** Deconvolution analyses data of complexes infraredspectra in 1710–1400  $\text{cm}^{-1}$  range

	Co (II) complex (R <sup>2</sup> = 0.9996)		Ni (II) complex $(R^2 = 0.9999)$			
Peak	Center	FWHM	% Area	Center	FWHM	% Area
1	1483	8.14	1.02	1284	24.51	0.78
2	1490	13.49	2.78	1308	43.50	4.49
3	1502	22.19	6.10	1346	66.61	10.18
4	1524	37.39	16.85	1422	98.77	21.90
5	1562	46.53	31.47	1460	26.01	1.22
6	1591	16.10	2.60	1485	43.90	4.01
7	1605	24.82	13.81	1516	59.91	10.17
8	1629	12.41	0.65	1574	85.93	27.73
9	1636	53.15	15.49	1613	39.02	4.95
10				1650	50.23	9.13
11				1697	49.13	5.38
	$Zn (II) = (R^2 = 0.$	complex 9999)		Cu (II) complex (R <sup>2</sup> = 0.9995)		
Peak	Center	FWHM	% Area	Center	FWHM	% Area
1	1487	13.88	1.59	1456	13.94	0.91
2	1500	23.10	4.77	1490	29.43	5.44
3	1522	36.73	14.73	1521	55.88	31.24
4	1557	53.06	32.22	1570	56.71	40.07
5	1601	41.14	10.19	1600	20.81	1.75
6	1627	90.27	32.94	1612	31.21	11.16
7	1702	52.64	3.53	1635	35.38	7.25
8				1709	28.73	2.14

respectively. The appearance of these signals confirms the existence of the ligand in keto-thion form. Multiplet signals observed in the region 7.44–7.17 ppm due to the phenyl group protons (Table 4). The pyridyl ring showed two doublet signals at 8.78 and 7.86 ppm due to the protons at (2 and 6) and (3 and 5) positions,<sup>[23]</sup> respectively.

<sup>1</sup>H-NMR spectrum of  $[Zn(H_2L)OAc]H_2O$  shows two singlet signals at 9.59 and 9.42 ppm assigned to the protons of N<sup>4</sup>H and N<sup>2</sup>H group, respectively. On comparison with the ligand spectrum, it is clear that N<sup>1</sup>H signal has disappeared which is taken as an evidence for the enolization of the ligand with liberation of the hydrogen ion after chelation.<sup>[24]</sup> Furthermore, a new singlet signal was observed at 1.88 ppm attributed to the methyl protons of the acetate group.<sup>[10,11]</sup> Moreover, the phenyl and pyridyl protons appeared at more or less the same positions.

 $^{13}$ C-NMR spectrum of H<sub>3</sub>L, in DMSO-d<sub>6</sub> (Figure 4), shows number of signals represent the number of chemically non-equivalent carbon atoms of the organic moiety. The signal due to the carbonyl carbon appears at





FIGURE 3 Suggested structures of the metal complexes

M = Co(II), Cu(II) and Zn(II); n = 1

TABLE 4 NMR data of ligand and its Zn (II) complex

<sup>1</sup> H-NMR			<sup>13</sup> C-NMR	
Protons	$H_3L$	Zn (II) complex	Carbons	$H_3L$
$N^{4}H$	10.84	9.59	2,6-Py	150.15
$N^{2}H$	9.86	9.42	3-Py	121.67
$N^{1}H$	9.79	-	5-Py	120.63
2,6-Py	8.78	8.66	4-Py	139.61
3,5-Py	7.86	7.81	2,6-Ph	125.15
2,6-Ph	7.44	7.58	3,5-Ph	128.06
3,5-Ph	7.34	7.34	4-Ph	139.61
4-Ph	7.17	7.15	1-Ph	139.11
(CH <sub>3</sub> ) <sub>OAc</sub>	-	1.88	C=0	164.49
			C=S	181.11

164.49 ppm,<sup>[22]</sup> while the thiocarbonyl carbon were observed at  $\approx 181$  ppm.<sup>[22]</sup> The pyridyl ring carbons at 3- and 5-positions were observed at 121.67 ppm, while those at 2- and 6-position were at 150.15 ppm. The phenyl group carbons displayed signals in the range 125.15–139.11 ppm.<sup>[22]</sup> The data confirm the existence of the ligand in the keto-form.

# 3.3 | Electronic spectra and magnetic moments

The electronic spectrum of the ligand, in DMSO, showed two bands at 34965 and 33335 cm<sup>-1</sup> attributed to  $(\pi \rightarrow \pi^*)$  transition of pyridyl and phenyl rings,<sup>[9]</sup> respectively. Moreover, two bands at 25910 and 25000 cm<sup>-1</sup> with a shoulder at 27625 cm<sup>-1</sup> due to  $n \rightarrow \pi^*$  transitions of

thiocarbonyl, carbonyl groups and  $(n \rightarrow \pi^*)$  of pyridyl ring,<sup>[9]</sup> respectively.

The spectrum of Co (II) complex, in DMSO, showed only one band at 17065 cm<sup>-1</sup> attributed to  ${}^{4}A_{2} \rightarrow {}^{4}T_{1}(P)$ transition, which suggests tetrahedral geometry around the Co (II) ion.<sup>[25]</sup> Another band observed at 23700 cm<sup>-1</sup> and assigned to the ligand to metal charge transfer (LMCT). Moreover, the spectral data in Nujol mull showed the d-d transition and the LMCT bands at 16610 and 22025 cm<sup>-1</sup>, respectively, confirming the tetrahedral structure of the solid complex and indicated that DMF did not alter the geometrical structure.<sup>[9,25]</sup> The magnetic moment of Co (II) complex was found to be 4.59 B.M. (Table 5). Utilizing the magnetic moment value and d-d transition, the 10Dg can be calculated using the following equation, where  $\lambda = -170 \text{ cm}^{-1}$ and  $\mu_{spin-only} = 3.87$  B.M. The obtained 10Dq was found to be  $3579 \text{ cm}^{-1}$  and confirms the tetrahedral geometry of the Co (II) complex.<sup>[25]</sup>

$$\mu_{\text{eff}} = \mu_{\text{spin}} - \text{only} \left( 1 - \frac{4\lambda}{10\text{Dq}} \right)$$

Moreover, the spectrum of Cu (II) complex recorded in DMSO, exhibited a broad band which the deconvolution analysis resolved it two overlapped bands at 16130 and 13335 cm<sup>-1</sup> (Figure 5A). In addition, the Nujol mull spectrum showed a broad band at 16805 cm<sup>-1</sup> with a shoulder at 14870 cm<sup>-1</sup> attributed to  ${}^{2}B_{1g} \rightarrow {}^{2}E_{1g}$  and  ${}^{2}B_{1g} \rightarrow {}^{2}A_{1g}$  transitions, respectively, of square planar geometry.<sup>[25,26]</sup> Several bands were observed also in the range 21000–22600 cm<sup>-1</sup> due to LMCT. The magnetic moment value of the complex is 1.68 B.M., which is lower than the normal value (1.73–2.0 B.M.) of Cu (II) complexes due



FIGURE 4 <sup>13</sup>C-NMR spectra of H<sub>3</sub>L

TABLE 5 Electronic spectral and magnetic moment data of ligands and its complexes

Compound	Band position (cm <sup>-1</sup> )	Assignment	$\mu_{\rm eff}$ (BM)
H <sub>3</sub> L	34965, 33335, 27625, 25910, 25000	$\begin{split} (\pi \to \pi^*)_{\rm Py},  (\pi \to \pi^*)_{\rm Ph},  (n \to \pi^*)_{\rm Py},  (n \to \pi^*)_{\rm C=S}, \\ (n \to \pi^*)_{\rm C=O}, \end{split}$	-
Cu (II) complex	33115 (34015), 31450, 28410 (28410), 27475, 25125 (25640), 23925, 22625 (20000) (21645), 16130 (16805), 13335 (14870)	$\begin{array}{l} (\pi \to \pi^*)_{Ph}, (\pi \to \pi^*)_{C=S}, (\pi \to \pi^*)_{C=N}^{-1}, (n \to \pi^*)_{Py}, \\ (n \to \pi^*)_{C=S}, (n \to \pi^*)_{C=N}^{-1}, \text{ LMCT}, \ ^2B_{1g} \to \ ^2E_{1g}, \\ \ ^2B_{1g} \to \ ^2A_{1g} \end{array}$	1.77
Co (II) complex	32680 (33115), 31445, 28250 (28570), 27475, 25380, 24275 (24390), 23700 (22026), 17065 (16610)	$\begin{aligned} (\pi \to \pi^*)_{Ph}, (\pi \to \pi^*)_{C=S}, (\pi \to \pi^*)_{C=N}^{-1}, (n \to \pi^*)_{Py}, \\ (n \to \pi^*)_{C=S}, (n \to \pi^*)_{C=N}^{-1}, LMCT, {}^4A_2 \to {}^4T_1(P) \end{aligned}$	4.59
Ni (II) Complex	33115, (31450), 28735, 27780 (27030), 25640, 24630, 22935 (22940), 20835 (20920), 18655 (17730), 16610 (14795)	$\begin{split} &(\pi \to \pi^*)_{Ph}, (\pi \to \pi^*)_{C=S}, (\pi \to \pi^*)_{C=N}{}^1, (n \to \pi^*)_{Py}, \\ &(n \to \pi^*)_{C=S}, (n \to \pi^*)_{C=N}{}^1, \\ &LMCT, {}^3A_{2g}(F) \to {}^3T_{1g}(P), {}^3A_{2g}(F) \to {}^3T_{1g}(F) \end{split}$	2.95
Zn (II) complex	34015 (34015), 31450, 28090, 27475 (27030), 25910, 23255 (22625)	$\begin{array}{l} (\pi \to \pi^{*})_{Py}, (\pi \to \pi^{*})_{C=S}, (\pi \to \pi^{*})_{C=N}^{-1}, (n \to \pi^{*})_{Py}, \\ (n \to \pi^{*})_{C=S}, (n \to \pi^{*})_{C=N}^{-1}, LMCT \end{array}$	-

Values between brackets for Nujol mul



FIGURE 5 Electronic spectra of  $[Cu(H_2L)\ Ac]H_2O(A)$  and  $[Ni(H_2L)Ac]3H_2O\ (B)$ 

to the presence of some Cu-Cu interactions between the neighboring molecules.

The spectrum of Ni (II) complex, in DMSO, displayed two bands at 22935 and 20835 cm<sup>-1</sup> attributed to the ligand to metal charge transfer transition (LMCT). In addition, a broad band at 16610 cm<sup>-1</sup> assigned to  ${}^{3}T_{1} \rightarrow {}^{3}T_{1}(P)$  transition of tetrahedral geometry around Ni (II) ion,<sup>[9,25]</sup> respectively (Figure 5B). Moreover, the Nujol mull spectrum displayed the LMCT transitions at 20920 and 19160 cm<sup>-1</sup>, while transitions were observed at 17730 and 14795 cm<sup>-1</sup>, respectively, were assigned to  ${}^{3}T_{1} \rightarrow {}^{3}T_{1}(P)$  and  ${}^{3}T_{1} \rightarrow {}^{1}E$  transitions, respectively. Furthermore, the magnetic moment values of the complex confirmed the tetrahedral geometry where it was found to be 3.4 B.M., which is in the range of tetrahedral structure (3.4–4.3 BM).

Finally, the electronic spectral data of Zn (II) complex displayed intra-ligand transitions, shown in Table 5. The spectrum displayed three bands at 34015, 31450 and 28090 cm<sup>-1</sup> attributed to  $(\pi \rightarrow \pi^*)_{Ph}$ ,  $(\pi \rightarrow \pi^*)_{C=S}$  and  $(\pi \rightarrow \pi^*)_{C=N^*}$ , respectively, in addition to LMCT band at 21740 cm<sup>-1</sup>. The obscure of bands due to  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transitions of carbonyl group was taken as an additional evidence for enolization of both groups.

#### 3.4 | Electron spin resonance

For Cu (II) complexes, in which the Cu (II) ion has electronic configuration  $3d^9$ , ESR spectroscopy can distinguish the ground states based on the principal values of the g-tensor. Thus, when  ${}^2B_{1g}$  is the ground state the  $g_{||} > g_{\perp} > 2.0023$ , while in case of  ${}^2A_{1g}$  ground state the  $g_{\perp} > g_{\parallel} > 2.0023$ .<sup>[27]</sup>



FIGURE 6 ESR spectrum of [Cu(H<sub>2</sub>L) Ac]H<sub>2</sub>O

The ESR spectrum of Cu (II) complex (Figure 6) showed g-tensor values  $g_{II} = 2.15$  and  $g_{\perp} = 2.06$ . The observed values of  $g_{II}$  is less than 2.3 suggesting a significant covalent character of metal-ligand bonds.<sup>[28]</sup> Also, The g-values were  $g_{II} > g_{\perp} > 2.0023$  which indicates that the Cu (II) has a  $d_{x^2-y^2}$  ground state characteristic for the

-WILEY-Organometallic 7 of 11 Chemistry

octahedral or square planar geometry.<sup>[29]</sup> The exchange interaction between Cu (II) centers in solid state may be expressed by the axial symmetry parameter, G, where  $G = (g_{II} - 2)/(g_{\perp} - 2)$ . If G > 4, the exchange interaction is negligible, whereas when G < 4, a an exchange interaction is indicated. The calculated G value was 2.54 suggesting the presence of copper-copper exchange interactions in the solid state between neighboring molecules.<sup>[30]</sup> This G value is close to the value reported for previously synthesized Schiff base complex (MC)(CuCl)<sub>2</sub>.2H<sub>2</sub>O.<sup>[31]</sup>

The orbital reduction factor, K, was used as a measure of covalency, where for ionic environment K = 1, and for covalent K < 1. The orbital reduction factor is calculated using the following simplified expressions where  $K_{\parallel}$  and  $K_{\perp}$  are the parallel and perpendicular components of orbital reduction factor, respectively,  $\lambda_{\circ}$  is the spin-orbit coupling constant(-828 cm<sup>-1</sup>).<sup>[31-33]</sup>

$$\begin{split} K_{\parallel}^{2} &= \frac{\left(g_{\parallel} - 2.0023\right)}{8 \times \lambda_{\circ}} \times d\text{-}d \text{ transition} \\ K_{\perp}^{2} &= \frac{\left(g_{\perp} - 2.0023\right)}{2 \times \lambda_{\circ}} \times d\text{-}d \text{ transition} \\ K^{2} &= \frac{\left(K_{\parallel}^{2} + 2K_{\perp}^{2}\right)}{3} \end{split}$$



**FIGURE 7** Mass spectra of  $H_3L$ 



**SCHEME 1** Fragmentation pattern of the ligand

The obtained data showed that K = 0.51, implies total covalent character.<sup>[34]</sup> For pure  $\sigma$ -bonding,- $K_{\parallel} \approx K_{\perp} \approx 0.77$ while if  $K_{\parallel} < K_{\perp}$ , reveal the in-plane  $\pi$ bonding, whereas out-of-plane  $\pi$ -bonding showed  $K_{\parallel} > K_{\perp}$ . The calculated values of  $K_{\parallel}$  and  $K_{\perp}$  were 0.60 and 0.77, respectively. These values lie in the same range (0.48–0.72) as that of a series of large covalent Schiff base complexes reported by Shauib *et al.*<sup>[31]</sup> This comparison indicates that the covalency in-plane is greater than of the out-of-plane  $\pi$ -bonding.<sup>[35,36]</sup>

Finally, the magnetic moment for the Cu (II) complex,  $\mu_{eff}$ , can be calculated utilizing the ESR spectral data, g-values, by the following expression.<sup>[37]</sup> The data indicated that it is close to the obtained value from magnetic susceptibility measurements (1.70 B.M.) and found to be1.81 B.M., respectively.

$$\mu_{e\!f\!f}=\!\frac{1}{2}\sqrt{g_{\parallel}^2+2g_{\perp}^2}$$

#### 3.5 | Mass spectra

The mass spectrum of the H<sub>3</sub>L ligand shows the molecular ion peak at m/z = 272 (27.5%), which matches with the suggested formula (Figure 7). The ligand has two possible fragmentation pathways where in the first it loses H<sub>2</sub>O giving a peak at m/z = 254 (1.12%) and in the second it loses (PhNH<sub>2</sub>) leading to a peak at m/z = 179 (1.16%).

**TABLE 6** Thermal gravimetry analysis data of metal complexes

Both the pathways undergo further fragmentation steps to give a base peak at m/z = 78 (100.0%) corresponding to the pyridyl ring (Scheme 1).

#### 3.6 | Thermal analyses

Thermal gravimetric analyses (TGA) were carried for the isolated solid complexes to have some idea on their thermal stabilities and to help in supporting the suggested structures. The decomposition stages of the synthesized metal complexes were listed in Table 6.

The TG diagram of  $[Cu(H_2L)Ac]H_2O$  showed a decomposition step (36–125 °C) corresponds to loss of  $H_2O$  molecule (Calcd. 4.37; Found 4.10%). The second stage (165 to 250 °C) due to loss of the  $C_2H_3O_2$  (Calcd. 14.33; Found 14.51%). The next steps were due to decomposition of the ligand leaving a residue of CuO (Calcd. 19.33; Found 21.23%).

TG curve of  $[Co(H_2L)Ac]H_2O$  shows four stages of decomposition, the first starts from 18 to 100 °C and corresponds to the loss of water molecule outside the coordination sphere (Calcd. 4.42; Found 4.81%). The second step (100–500 °C) was attributed to the loss of  $C_2H_3O_2$  and  $C_7H_6NS$  fragments (Calcd. 47.93; Found 47.49%). The other two steps occur in the range 500–1000 °C, due to complete decomposition of the ligand leading to a residue of  $Co_2O_3$  (Calcd. 19.18; Found 20.37%).

The TGA of  $[Ni(H_2L)Ac]3H_2O$  decomposes in three steps, the first in 38–125 °C range, corresponding to loss

complex	Temp. range °C	Wt. loss %	Fragment loss (Fragment %)
[Cu(H <sub>2</sub> L)Ac]H <sub>2</sub> O	36–165	4.10	H <sub>2</sub> O (4.37)
	165–250	14.51	$C_2H_3O_2(14.33)$
	250–300	22.02	$C_6H_6N$ (22.36)
	300–360	15.02	CHNS (14.35)
	360–1000	23.19	$C_6H_5N$ (22.14)
	residue	21.23	CuO (19.33)
[Co(H <sub>2</sub> L)Ac]H <sub>2</sub> O	18–100 100–500 500–625 625–1000 residue	4.81 47.49 7.74 21.01 19.18	$\begin{array}{l} H_2O~(4.42)\\ C_2H_3O_2+~C_7H_6NS(47.93)\\ N_2H~(7.12)\\ C_5H_5N~(22.3)\\ CO_2O_3(20.37) \end{array}$
[Ni(H <sub>2</sub> L)Ac]3H <sub>2</sub> O	38–115	3.98	$H_2O$ (4.07)
	155–505	45.51	$2H_2O + C_2H_3O_2 + C_7H_6N$ (44.96)
	505–1000	33.21	$C_5H_5N + CHN_2OS$ (34.28)
	residue	17.31	NiO (17.25)
[Zn(H <sub>2</sub> L)Ac]H <sub>2</sub> O	40–210	4.09	H <sub>2</sub> O (4.35)
	210–485	48.14	$C_2H_3O_2 + C_7H_6NS(47.19)$
	485–850	19.06	$C_5H_5N$ (18.87)
	850–1000	9.71	NH (9.97)
	residue	19.00	ZnO (19.96)



**FIGURE 8** Energy gap plots of (A) H<sub>3</sub>L,(B) Zn (II)and(C) Ni (II) complexes

of water molecules outside the coordination sphere (Calcd. 11.98; Found 12.2%). The second stage from 155 to 505 °C due to the loss of  $C_2H_3O_2$  in addition to  $C_7H_6N$  fragments (Calcd. 36.38; Found 36.51%). Finally, the third stage starts at 505 °C leading to a residue of NiO (Calcd. 17.25; Found 17.31%).

 $[Zn(H_2L)Ac]H_2O$  displayed three thermal decomposition steps, the first corresponds to loss of  $H_2O$  (Calcd.

WILEY Organometallic 9 of 11 Chemistry

**TABLE 7** Cytotoxic activity of some compounds against human tumor cells

	In vitro Cytotoxicity IC <sub>50</sub> (µg/ml)*			
Compounds	HCT116	HePG2		
DOX**	$5.23 \pm 0.3$	$4.50 \pm 0.2$		
$H_{3}L$	$7.68 \pm 0.8$	$8.44 \pm 0.9$		
Zn (II) complex	$53.00 \pm 3.1$	$38.30 \pm 2.4$		

\*IC<sub>50</sub> (μg/ml): 1-10 (very strong), 11-20 (strong), 21-50 (moderate), 51-100 (weak) and above 100 (non-cytotoxic).
\*\*DOX: Doxorubicin.

4.35; Found 4.09%) was observed at 40–110 °C. The second decomposition step occurs from 210 to 485 °C, due to the loss of  $C_2H_3O_2$  and  $C_7H_6NS$  fragments (Calcd. 47.19; Found 48.14%). The last steps were observed in the ranges 485–850 and 850–1000 °C due to the decomposition of the organic chelator leaving a residue matches with ZnO (Calcd. 19.96; Found 19.00%).

#### 3.7 | Optical band gap

Molecular materials have recent applications in molecular lar electronics because of their semi-conductivities.<sup>[38–40]</sup> To have some idea about the conductivity of the isolated complexes, the optical band gaps (Eg) have been determined from UV-visible spectra. The relation:  $\alpha h\nu = A$  ( $h\nu - E_g$ )<sup>m</sup>, (m = 2 for direct transition and 1/2 for indirect transition; A = energy constant)<sup>[41,42]</sup> was applied. The graphical representation of  $(\alpha h\nu)^2$  versus  $h\nu$  gives the band gap of the ligand and its metal complexes (Figure 8). The band gap (Eg) value for H<sub>3</sub>L was 3.88 and for its complexes were Cu (II) = 3.83; Co (II) = 3.82; Ni (II) = 4.48 and Zn (II) = 3.83 eV. It is clear that, these compounds are semi-conductors and are suitable to be used in solar cells applications.<sup>[43,44]</sup>

#### 3.8 | Cytotoxic effect

The *in vitro* cytotoxicity activities of the ligand ( $H_3L$ ) and Zn (II) complex were studied as representative examples of isolated compounds. Their activities were compared with the standard doxorubicin (Tables 7, 8). The minimum inhibitory concentration of  $H_3L$ was found to be 8.20 and 8.0 µg/ml against HCT-116 and HEPG-2 cell lines, respectively. On the other hand, the minimum inhibitory concentration of Zn (II) complex was found to be 38.30 and 32.1 µg/ml against HCT-116 and HEPG-2 cell lines, respectively (Figure 9). The colorimetric cytotoxic tests exhibited that the ligand  $H_3L$  has very strong cytotoxicity against the two investigated cell lines. While,

#### **TABLE 8**Relative viability of cells (%)

	DOX		H <sub>3</sub> L		Zn (II) complex	
Conc. (µg/ml)	HCT116	HePG2	HCT116	HePG2	HCT116	HePG2
100	7.1	6.3	8.2	8.0	38.3	32.1
50	13.9	11.2	16.5	15.6	51.2	43.2
25	18.7	14.1	25.3	23.7	64.1	57.4
12.5	31.4	28.3	31.4	36.8	75.4	68.6
6.25	47.9	45.8	54.7	56.2	92.6	90.7
3.125	60.5	57.6	71.5	73.9	100	100
1.56	73.8	71.2	89.1	93.1	100	100



FIGURE 9 In vitro cytotoxic activities of H<sub>3</sub>Land Zn (II) complex

Zn (II) complex shows weak cytotoxicity against HCT-116 and moderate cytotoxicity against HEPG-2 cell lines.

#### 4 | CONCLUSION

The ligand, 2-isonicotinoyl-N-phenylhydrazine-1carbothioamide (H<sub>3</sub>L) forms coordinated species with Co (II), Cu (II), Ni (II) and Zn (II) ions. The free ligand exists in the keto-thion form, while it chelates the metal ions in enolized form. The optical band gap measurements indicated that the ligand and its metal complexes are semi-conductors and suitable for solar cells applications. The ligand showed very strong cytotoxicity against both HCT-116 and HEPG-2 cell lines.

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#### REFERENCES

- K. Tummalapalli, P. Munusami, M. Pathak, Appl. Organomet. Chem. 2017, 31, 3680.
- [2] S. Rollas, S. G. Küçükgüzel, Molecules 2007, 12, 1910.
- [3] B. K. Kaymakçıoğlu, S. Rollas, Il Farmaco 2002, 57, 595.
- [4] E. Bavin, D. Drain, M. Seiler, D. Seymour, J. Pharm. Pharmacol. 1952, 4, 844.
- [5] M. T. Cocco, C. Congiu, V. Onnis, M. C. Pusceddu, M. L. Schivo, A. De Logu, *Eur. J. Med. Chem.* **1999**, *34*, 1071.
- [6] N. Farrell, Coord. Chem. Rev. 2002, 232, 1.
- [7] D. L. Klayman, J. P. Scovill, J. F. Bartosevich, J. Bruce, J. Med. Chem. 1983, 26, 35.
- [8] A. Prakash, D. Adhikari, Int. J. Chem. Tech. Res 2011, 3, 1891.
- [9] M. I. Orif, M. H. Abdel-Rhman, Polyhedron 2015, 98, 162.
- [10] N. M. Hosny, H. M. Mahmoud, M. H. Abdel-Rhman, *Heteroat. Chem.* 2018, 29, 21415.
- [11] N. M. Hosny, N. Y. Hassan, H. M. Mahmoud, M. H. Abdel-Rhman, J. Mol. Struct. 2018, 1156, 602.
- [12] M. I. Orief, M. H. Abdel-Rhman, J. Mol. Struct. 2018, 1173, 323.
- [13] G. Jeffery, J. Bassett, J. Mendham, R. Denney, Vogel's quantitative chemical analysis, in *Vogel's Quantitative Chemical Analysis*, 5th ed., Longman Scientific & Technical Longman Group UK Limited, Essex, UK 1989.
- [14] J. Scientific, PeakFit-Peak separation and analysis software for Windows, San Rafael CA, USA 1995.
- [15] A. M. Abdelghany, Spectrochim. Acta, Part a 2013, 100, 120.
- [16] S. J. Azahari, M. H. Abdel Rhman, M. M. Mostafa, Spectrochim. Acta, Part A 2014, 132, 165.
- [17] T. Mosmann, J. Immunol. Methods 1983, 65, 55.
- [18] F. Denizot, R. Lang, J. Immunol. Methods 1986, 89, 271.
- [19] K. Nakamoto, Infrared and Raman spectra of Inorganic and Coordination Compounds, 6th ed., John Wiley & Sons Inc, Hoboken, NJ 2009.
- [20] M. M. Mostafa, Spectrochim. Acta, Part a 2007, 66, 480.
- [21] N. M. Hosny, Y. E. Sherif, Monatshefte für Chemie-Chemical Monthly 2016, 147, 1893.
- [22] R. Silverstein, F. Webster, D. Kiemle, Spectrometric identification of organic compounds, 7th ed., John Wiley & Sons, Hoboken, NJ 2006.

- [23] M. H. Abdel-Rhman, M. M. Hassanian, A. A. El-Asmy, J. Mol. Struct. 2012, 1019, 110.
- [24] N. M. Hosny, E. S. A. El Morsy, Y. E. Sherif, *J. Rare Earths* **2015**, *33*, 758.
- [25] A. B. P. Lever, *Inorganic electronic spectroscopy*, 1st ed., Elsevier Publishing Company, Amsterdam, Netherlands 1968.
- [26] M. Hassanien, I. Gabr, M. Abdel-Rhman, A. El-Asmy, Spectrochim. Acta, Part a 2008, 71, 73.
- [27] B. J. Hathaway, Struct. Bonding 1984, 57, 55.
- [28] D. Kivelson, R. Neiman, J Chem Phys 1961, 35, 149.
- [29] I. M. G. M. M. Hassanien, M. H. Abdel-Rhman, A. A. El-Asmy, Spectrochim. Acta, Part a 2008, 71, 73.
- [30] G. Speier, J. Csihony, A. M. Whalen, C. G. Pierpont, *Inorg. Chem.* 1996, 35, 3519.
- [31] N. M. Shauib, A. Z. A. Elassar, A. El-Dissouky, Spectrochim. Acta, Part a 2006, 63, 714.
- [32] B. Hathaway, A new look at the stereochemistry and electronic properties of complexes of the copper (II) ion, in: *Complex Chemistry*, Springer, **1984**, pp. 55–118.
- [33] M. M. Shakdofa, F. A. El-Saied, A. J. Rasras, A. N. Al-Hakimi, *Appl. Organomet. Chem.* 2018, 32, e4376.
- [34] S. Abdou, M. M. Shakdofa, M. A. Whaba, *Spectrochim. Acta, Part a* **2015**, *136*, 1941.
- [35] R. Ray, G. B. Kauffmann, Inorg. Chim. Acta 1990, 174, 237.
- [36] N. A. Mangalam, M. P. Kurup, Spectrochim. Acta, Part a 2011, 78, 926.

- [37] V. T. Kasumov, F. Köksal, R. Köseoğlu, J. Coord. Chem. 2004, 57, 591.
- [38] F. Karipcin, B. Dede, Y. Caglar, D. Hür, S. Ilican, M. Caglar, Y. Sahin, *Opt. Commun.* **2007**, *272*, 131.
- [39] N. M. Hosny, Chem. 2010, 40, 391.
- [40] N. M. Hosny, J. Mol. Struct. 2009, 923, 98.
- [41] J. Tauc, R. Grigorovici, A. Vancu, Phys. Status Solidi B 1966, 15, 627.
- [42] M.-L. Fu, G.-C. Guo, X. Liu, B. Liu, L.-Z. Cai, J.-S. Huang, *Inorg. Chem. Commun.* 2005, *8*, 18.
- [43] N. M. Hosny, Mater. Chem. Phys. 2014, 144, 247.
- [44] N. M. Hosny, A. Dahshan, J. Mol. Struct. 2015, 1085, 78.

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