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Synthesis, In vitro Biological Studies of novel Homoleptic Ni(II) and Zn(II) Complexes of Thiosemicarbazide Derivative ligand

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Abstract
Thiosemicarbazide derivatives are currently becoming an important class of S, N –donor ligands to form stable metal complexes showing some biological applications. In this study, thiosemicarbazide-based derivative (4-(4-Chlorophenyl)-1-(4-nitrophenyl) thiosemicarbazide (L) and its homoleptic Ni(II) and Zn(II) complexes were synthesized by modified standard procedures. The structures of the synthesized compounds were characterized by several techniques: Uv-vis, Fourier-transform infrared (FT-IR), proton nuclear magnetic resonance (^{1}H NMR), ^{13}C-NMR spectroscopy, and TG-analysis. To explore the capability of thiosemicarbazide and its metal complexes for growth inhibition of bacteria and as antioxidant potential, evaluation were performed against two Gram-positive bacteria (*Bacillus subtilis ATCC6633* and *Staphylococcus aureus ATCC25923*) and Gram-negative bacteria (*Escherichia coli ATCC 25922* and *Pseudomonas aeruginosa ATCC 27853*) using disc diffusion and DPPH method respectively. For the ligand (L**)**, the antimicrobial tests revealed a higher antibacterial activity against gram-positive *B. subtilis* **(**4.24 ± 0.67**)** whereas the gram-negative *E. coli* bacteria show resistance. The metal complexes $[Ni(L)_2(H_2O)_2]$ and $[Zn(L)_2]$ showed good activity against *S. aureus*, with a mean inhibition zone of 12.86 ± 0.46 and 13.82± 0.46 mm diameter at 500 µg/mL, respectively compared to the standard drug Ciprofloxacin having inhibition zone of 11.0 ± 0.67mm diameter at 500 µg/mL. Both complexes also showed good antiradical potential (48.55% and 56.75% at 100µg/ml). The results indicate that the complexes showed higher antibacterial and antioxidant potential than the free ligand. Thus, this study indicates an insight towards thiosemicarbazide derivative as ligand through metal coordination could enhance bioactivities.
Keywords: Thiosemicarbazide-derivative, Homoleptic-complex, Antibacterial, Antioxidant
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1. Introduction

The challenge of microbial infections has been a growing issue recently, due to the increasing number of multidrug resistant microbial pathogens, newly developed microbes, and the slow rate of new drug development [1, 2]. Thus, bacterial resistance to antibiotics is becoming the greatest challenge in human healthy aspects, due to a continuous increase in the number of infections caused by bacterial resistance to commonly used antibiotics [3]. Even though there are many antibiotics available, the appearance of new antibiotic resistant bacterial species in recent years alarms a continuous need for the discovery of new classes of antibacterial agents.

Recently, interest has been rapidly growing in gaining insight into the properties, applications, and transformations of thiosemicarbazides. They are an important class of sulfur and nitrogen containing compounds acting as important ligands in coordination chemistry and their derivatives show appreciable pharmacological activities as antimicrobial and antioxidant agents [4, 5]. They are potent intermediates for the synthesis of pharmaceutical and bioactive compounds [5]. Many of the thiosemicarbazide derivatives showed good antibacterial activity against *K. pneumoniae* [4, 6] and *S. aureus* [7] in comparison with the standard drug ciprofloxacin.

The synthesis of thiosemicarbazides can be carried out in several ways. The general method involves the preparation of thiosemicarbazides by nucleophilic addition of amines or carbohydrazides to isothiocyanates or carbon disulfide [8]. They behave as chelating ligands and usually react with metallic cations by coordinating with metals through their nitrogen and sulfur donor atoms giving complexes [9]. Recently, metal-based drug development is also a current area of research to develop more bioactive antimicrobial drugs. Thus, thiosemicarbazide and their metal complexes are getting great attention because they are a class of compounds which have gained importance as potential drug candidates such as antibacterial [10], antiviral [11], fungicidal [10] and antioxidant [11] activities. The different thiosemicarbazide ligands and their metal complexes like zinc,

copper, and nickel have shown pharmacological properties [12]. The complexes can exhibit high bioactivities which are not shown by the free ligand [13]. Lipophilicity property of bioactive compounds, which controls the rate of entry into the cell, is adjusted by coordination and the mechanism of action can involve binding to a metal in vivo or the metal complex can serve as a carrier for activation of the ligand as a cytotoxic agent [13].

The mechanism indicates that the decrease in the polarity of the metal ions or after coordination to the NNS donor system of thiosemicarbazide increases the lipophilicity of the molecules, allowing them to more easily pass through the lipid membranes and block essential biological processes in the cell like, enzymatic processes in microorganisms [12]. On the basis of these previous findings, herein, we synthesized a ligand 4-nitrophenyl-4-(phenyl chloride) thiosemicarbazide ligand, its homoleptic zinc and nickel complexes and investigated the in vitro antimicrobial and antioxidant activities.

2. Materials and methods

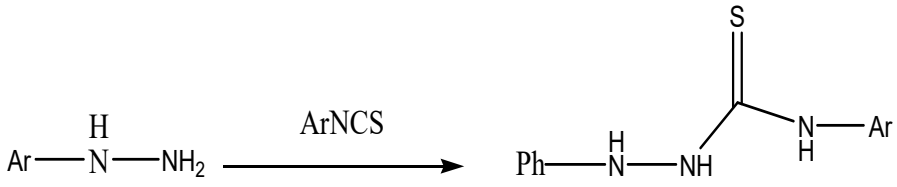
2.1. Materials

The chemicals used were of analytical grade (AR) and of the highest purity available. Metal salts, 4-chlorophenyl isothiocynate, 4-nitrophenylhydrazine, methanol, ethanol, and diethyl ether were procured from Loba Chemie PVT, Ltd and were used as received. The melting points of the synthetic compounds were determined in sealed capillary tubes with an electro-thermal melting point apparatus Stuart SMP3 made in UK. Results were the mean of triplicate readings. Analytical TLC was run on a 0.2 mm thick layer of silica gel 60 with fluorescent indicator UV_{254} (MACHEREY-NAGEL GmbH & Co.KG, Germany). Spots were detected using UV lamp. Column chromatography was performed using silica gel 60 (250–400 mesh) Merck. The absorbance of solutions was measured in UV/Vis range (Scan Wavelength: 200.0 - 800.0, Test Mode: Abs Mode) nm using Chem LAB UV Spectrometer. The IR spectra were recorded as KBr pellets on a Perkin Elmer FT-IR spectrophotometer (400–4000 cm^{-1}), Model No. BX. The NMR spectra were recorded using Bruker Avance 400 spectrometer operating at 400 MHz in deuterated dimethylsulphoxide (DMSO-d_6) as a solvent. Chemical shifts are quoted in δ and were related to thos of the solvents. The thermal analysis was done using a thermal analyzer DTG-60H Simultaneous DTA-TG Apparatus SHIMADZU to scan the weight loss versus temperature. The scanning was performed in the temperature range up to 1500 K at a constant heating rate 10.0 deg/min and the N_2 gas was allowed to pass at a flow rate 20 cc/min.

2.2. Chemistry

2.2.1. Synthesis of 4-(4-chlorophenyl)-1-(4-nitro-phenyl)thiosemicarbazide ligand

The general method for the synthesis of thiosemicarbazides involves the nucleophilic addition of amines or carbohydrazides to isothiocyanates or carbon disulfide [8]. Using isothiocyanate treatment of carbohydrazides with aryl isothiocyantes under different reaction conditions (pyridine, NaOH, KOH, and NaH) gave the general substituted thiosemicarbazide derivatives (Scheme 1).

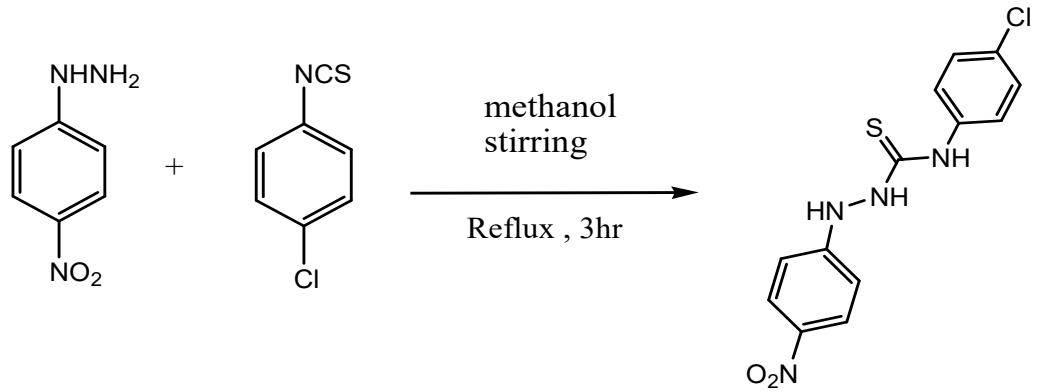


Scheme 1. General synthesis procedure of substituted thiosemicarbazides derivatives

Procedure

The thiosemicarbazide derivative ligand was synthesized by using a modified previously reported procedure(Scheme 2) [14].

A methanol solution (25 ml) of 4-chlorophenyl isothiocynate (1.7g, 0.01mol) was added to a methanol solution (25ml) of 4- nitrophenylhydrazine (1.53gm, 0.01mol). The reaction mixture was left under reflux at 70°C over hotplate with stirring for 3h. After completion of reaction (monitored by TLC), the reaction mass was allowed to cool to room temperature. During which period a precipitate were formed. The resulting brown-reddish precipitates were collected and washed with ethanol, diethyl ether and left to dry. Yield (2.4gm ≈ 78%), yellow-brown crystal; mp. 177–179 °C, *Rf* = 0.64 (EtOAc : methanol = 7 :3), UV-Vis ; λ_{max} (DMSO) = 282 & 315 nm; IR (υ cm^{-1}, KBr): 3271 , 3155 (N-H str.), 2929 (C-H str.), 2046 (aromaticity), 1697(C-C ring str.), 1597(aromatic C=C str.), 1279(C=S str.), 1121(C-Cl str.), 1077(N-N str.) ; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.43 (d, 2H), 8.33 (d, 2H), 7.87 (d, 2H), 7.37 – 7.35 (d, 2H), 6.50 (d, 1H), 5.90 & 1.90(s,2H) . ^{13}C NMR (101 MHz, DMSO-d_6): δ 181.71(C=S), 168.73(C-N), 157.20(C-Cl), 138.86, 132.44, 129.28, 128.25 (aromatic carbons).



Scheme 2. Synthesis of 4-(4-Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide ligand

2.2.2. Preparation of the homoleptic metal complexes

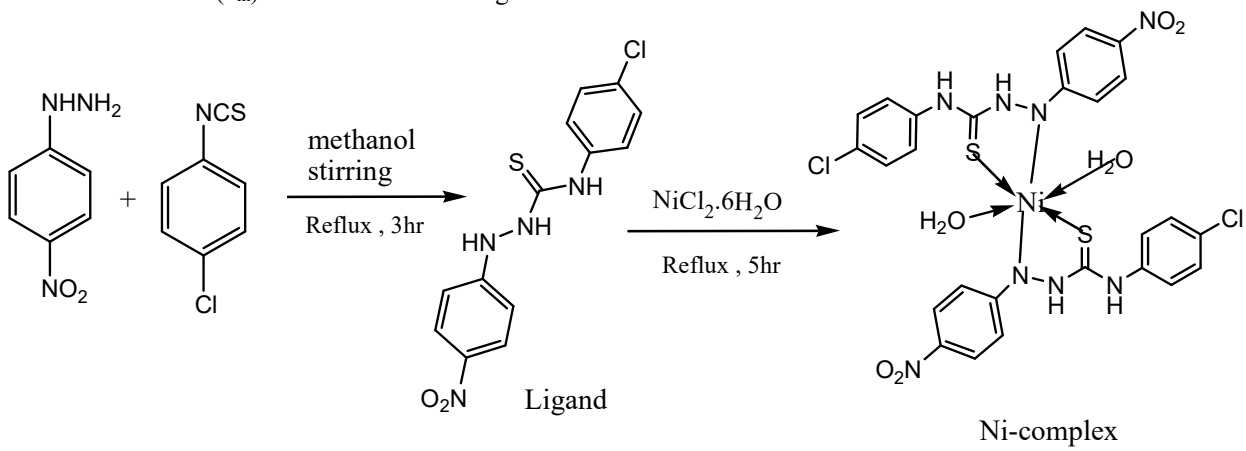
Synthesis of 4-(4-Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide metal complexes

A general method was used for the synthesis of metal complexes(Scheme 3 & 4) [15].

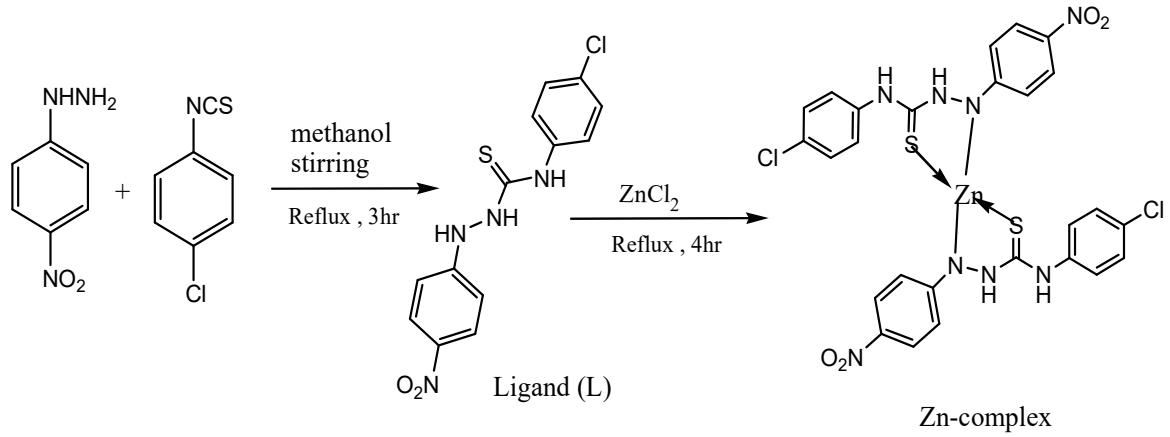
Hot ethanolic solution (25ml) of metal salts, $NiCl_2.6H_2O$ (1mmol, 0.24g) or $ZnCl_2$ (1mmol, 0.136g) were mixed with hot ethanolic solution (25ml) of 4-(4-chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide ligand (2mmol, 0.65 g) drop wise with constant stirring. The mixture was refluxed for 4-5 h at 70 - 80 ºC on a water bath for complete complexation. On cooling the contents, the metal complexes precipitate was separated out in each case. It was filtered, washed with 50% ethanol, dried under vacuum desiccators over silica gel and recrystallized from methanol. Purity of the complexes was checked by thin layer chromatography (TLC).

Nickel complex; $[Ni(L)_2(H_2O)_2]$. Yield: 78%; Pale Brown crystal, m.p. >300 ˚C; IR (KBr discs) V_{max} cm^{-1}: 3260 (N–H), 2922 (C–H), 1595 (C=C), 1269 (C=S), 1121 (C–Cl), 1023 (N–N), 538 (Ni–N), 442 (Ni–S). UV-Vis: λ_{max} (DMSO) = 333nm. TGA mass loss 4.9 % (53.3-215.5°C, 1st step, calc. 2 × H_2O = 36.24g) and 45.6 % (225.5 – 632.6 °C, 2 step, calc. one ligand formation = 321.54 g). Molar conductance (λm)=7.5 $n^{-1}cm^2mol^{-1}$. Magnetic moment of: 2.01 BM.

Zinc complex; $[Zn(L)_2]$; Yield: 68%; reddish-brown crystal, m.p > 300 ˚C, FTIR (KBr discs) V_{max} cm^{-1}: 3266, 3155 (N-H), 2922 (C-H), 1533 (C=C), 1023 (N-N), 1258 (C=S), 1135 (C-Cl), 525 (M-N), 448 (M-S). UV-Vis: λ_{max} (DMSO) = 351nm. TGA mass loss of 4.6 % (260.6-362 °C) at 1st step indicates loss of part of one ligand. Molar conductance (λ_m)= 2.4 $n^{-1}cm^2mol^{-1}$. Magnetic moment: 1.06 BM.



Scheme 3. Synthesis rout of Ni-complex



Scheme 4. Synthesis rout of Zn-complex

2.2.3. Magnetic Susceptibility

Magnetic moments of the Ni(II) and Zn(II) complexes were recorded at room temperature lying in the range 1.06 -2.01 BM (Table 1).

Table 1. Magnetic moment of Ni(II) and Zn(II) complexes

Complexes	Weight (gm)	Sample length (L) cm	Reading for tube plus sample (R)	Reading of empty tube (R_o)	Mass susceptibility (Xg)	Magnetic moment (BM)
$Zn(L)_2$	0.055	1	149	– 30	$6.789X10^{-6}$	1.06
$Ni(L)_2$	0.055	0.9	85	– 34	$4.062X10^{-6}$	2.01

2.2.4. Thermogravimetric analysis

Thermogravimetric analysis (TGA and DTG) were carried out in a dynamic nitrogen atmosphere (20 ml/min) with a heating rate of 10˚C/min using a Schimadzu TGA-50H thermal analyzer. Thermal analysis provides important information regarding the stability and presence of water molecules in the crystalline network of compounds [12]. The simultaneous TGA/DTG and DSC analysis of the metal complexes were studied where the heating rates were suitably controlled at 10 ^{0}C min^{-1} under nitrogen atmosphere and the weight loss was measured from ambient temperature to 1500 ^{0}C.

2.3. Antibacterial evaluation

Antibacterial activities of the synthesized ligand and its metal complex compounds were tested using the paper disc diffusion method [16,17]. During the process, two Gram-positive bacteria (*Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* ATCC25923) and two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) were used to evaluate the antibacterial activities. The medium was prepared from molten nutrient and Mueller–Hinton agar. Ciprofloxacin was the standard drug used as positive control while DMSO was used as negative control. The four bacterial strains were tested with 300 and 500 μg/mL concentrations using the paper disc diffusion method. The compound was dissolved in DMSO at concentrations of 300 and 500 μg/mL, 6 mm diameter Whatman filter paper discs were soaked with 1mL solution of the above two concentrations for each compound and then these saturated paper discs were inoculated at the center of each Petri dish having bacterial lawn in triplicate. The plates were incubated at 37°C for 48 h and the inhibition zone that appeared around the paper disc in each plate was determined by measuring the diameter of the inhibition zone [18]. The test was done using the disc diffusion technique at Adama Science and Techinology University.

2.4. Radical Scavenging Activity

The radical scavenging activity of the synthesized compounds was evaluated with 1,1-diphenyl-2 picryl hydrazyl (DPPH). In the process, 0.04 mg/mL solution of DPPH in methanol was prepared; 1 mL of this solution was poured into 4 mL of the synthesized samples in methanol to have four different concentrations (12.5, 25, 50, and 100 μg/ mL); and a control was made by adding 1 mL of the DPPH solution to 4 mL of methanol, while 4 mL methanol was used as a blank. The mixtures were shaken and allowed to stand at 37°C for 30 min in a dark oven, and the absorbance was recorded at 517 nm using a double beam UV-Vis spectrophotometer [19]. Ascorbic acid was used for the reference standard in similar concentrations as above. Percentage inhibition of DPPH radical was determined using the following equation: [20].

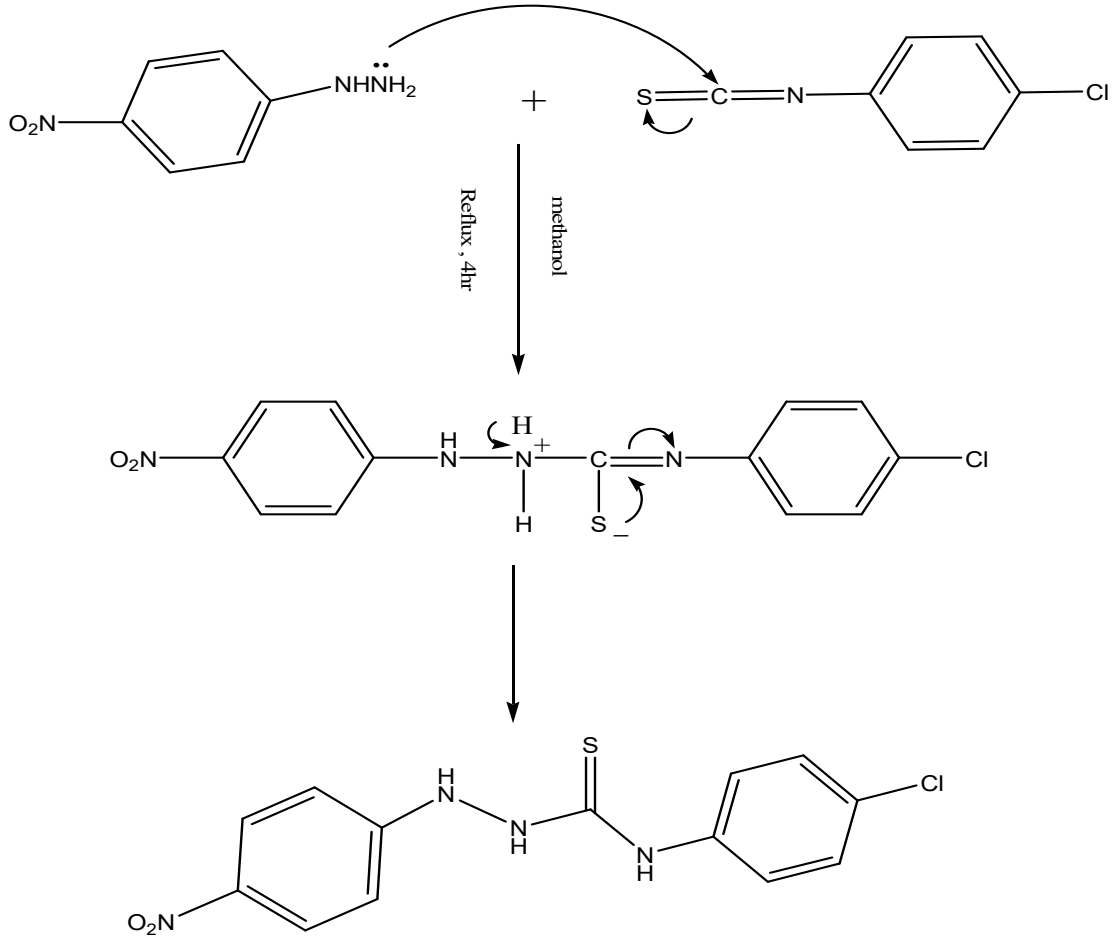
$$\textbf{DPPH scavenged (\%)} = \frac{\textbf{A control} - \textbf{A test}}{\textbf{A control}} \textbf{ x 100}$$

Where: -**A control** is the absorbance of the control reaction (without compound)
A test is the absorbance in the presence of the test compounds

3. Results and Discussion

3.1. Chemistry. In order to synthesize new thiosemicarbazide derivative ligand and its transition metal complexes which have enhanced biological activities as antibacterial and antioxidant activities, a previous reported modified procedure was used with necessary reaction conditions.

During the process, 4-(4-Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide ligand was prepared by refluxing 4-chlorophenyl isothiocynate and 4-nitrophenylhydrazine mixtures using modified previously reported procedure [8]. The nucleophilic additions of 4- nitrophenylhydrazine to 4-chlorophenyl isothiocynate under the reaction conditions gave the new substituted thiosemicarbazide derivative ligand as shown in scheme 5 below.



Scheme 5. Proposed reaction mechanism for synthesis of 4-(4-Chlorophenyl)-1-(4-nitrophenyl) thiosemicarbazide ligand

The analytical data of the ligand with the stoichiometry proposed is summarized in Table 2 below.

Table 2. Analytical results for the ligand compound

Compound	Empirical formula	Formula weight	Colour	mpt. (°C)	yield %	% Calculated/found			
						C	H	N	S
Ligand (L)	$C_{13}H_{11}N_4O_2SCl$	322.75	Yellow brown	178	78	48.37 (48.24)	3.44 (3.42)	17.36 (17.32)	9.93 (9.78)

The isolated solid ligand is stable in air and yellow-brownish in color. It melts at 178 °C. Calculated/fund elemental analysis data (C, H, N and S) was in a good agreement with those required for the suggested formula. Also, the synthesis of Ni and Zn-complexes of the synthesized ligand 4-(4-Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide following the metal complexation protocol adopted by *Sanjay Goel et al., 2016* approach [21] the data in Table -3 below were obtained.

Table 3. Analytical results for the prepared Ni, Zn-complexes of 4-(4-Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide

Compound	Empirical formula	Mwt. g/mol	Colour	mpt. (°C)	% Calc/found			λ_m ($n^{-1}cm^2$ mol^{-1})
					C	H	N	
$[Ni(L)_2(H_2O)_2]$	$C_{26}H_{24}NiN_8O_6S_2Cl_2$	738.01	Pale Brown	>300	42.3/ 41.8	3.3/ 3.2	15.2/ 15.4	7.5
$[Zn(L)_2]$	$C_{26}H_{20}ZnN_8O_4S_2Cl_2$	708.62	Reddish Brown	>300	40.1/ 39.9	2.6/ 2.5	14.4 / 14.5	2.4

From table - 3 above in case of 4-(4-Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide, the ligand react with metals ions as shown in scheme 3 & 4. It was found that the stoichiometry for the complexes is 1:2 $[ML_2]$, for the M/L system (where M = divalent, Ni or Zn)

The structures of the new compounds were confirmed by spectroscopic data and TG analysis.

3.2. FT- IR spectra analysis

The IR spectral data is one of the evidence to determine the structural confirmation of the synthesized compounds by identifying the bands corresponding to the main functional groups. The significant vibrational frequency bands corresponding to the 4-(4-Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide and its Ni(II) and Zn(II)-complexes are assigned and compared carefully. The important vibrational bands are presented in table 4.

Table 4. FT-IR V(cm^{-1}) of the ligand and its Ni(II) and Zn(II) complexes.

Compounds	V(N-H)	V(C-H)	V(C=C)	V(N-N)	V(C=S)	V(C-Cl)	V(M-N)	V(M-S)
L	3288, 3163	2937	1597	1077	1279	1121	-	-
$[Ni(L)_2(H_2O)_2]$	3260, 3140	2922	1595	1023	1269	1118	538	442
$[Zn(L)_2]$	3266, 3155	2922	1533	1023	1258	1135	525	448

In the IR spectra of the ligand (L), the N-H stretching bands were observed in the region 3288 - 3163 cm^{-1}. The aromatic C-H stretching vibrations gave rise to a band at 2937 cm^{-1}. The band at 2054 cm^{-1} confirms the existence of aromaticity and the bands at 1705, 1597,1279, 1121, 1077cm^{-1} corresponds to C-C ring, aromatic C=C, C=S, C-Cl, N-N stretching frequencies respectively [22]. A study and comparison of the infrared spectra of the free ligand and its metal complexes (Table 4) infers that the ligand behaves as a neutral bidentate and its metal complexes are coordinated through N and S of the thio-keto group as confirmed by Figures 1,3 and 5 (see Supplementary Material).

The assignments of the significant IR spectra bands of the $[Ni(4\text{-nitro-phClTS})_2(H_2O)_2]$ complex is shown in figure 3 which clearly show the shifting of the bands corresponding to the v(N-H) lowered by 20cm^{-1} and v(C=S) is lowered by 10cm^{-1} of the ligand towards the lower side on complexation. This negligible effect on these frequencies after complexation precludes the possibility of complexation at these functional groups [23].

Also for the significant IR spectra bands of the $[Zn(4\text{-nitro-phClTS})_2]$ complex shown in figure 5 shows the shifting of v(C=S) and v(N-N) towards the lower side (around 20-30cm^{-1}) on complexation. The IR spectral bands in the complexes also show some new bands with medium to weak intensity in the 525–447 cm^{-1} region which were assigned to (M–N) and (M–S) formation. This suggests that the ligand is bidentate chelating agent coordinating through nitrogen of –HN-N-H group and sulfur of C=S group [23].

3.3. Electronic Spectral Analysis

The UV-Vis spectra of the ligand with its complexes of Ni(II) and Zn(II) were recorded in, Ethanolic solution. It display a group of bands at 282 nm corresponds to the π-π* transitions in benzene and 315 nm is due to n–π* transition of the thiol (C=S) group [24]. Both complexes display similar bands corresponding to the intra-ligand transitions below 360 nm and an expected blue shift was observed compared to the ligand as shown in figures 2, 4, 6 (see Supplementary Material). The electronic transition found in thiosemicarbazide due to thiol(C=S) functional group was shifted on complexation. The Zn(II) and Ni(II) complex of the ligand exhibit a band with tolerable intensity near 400 nm corresponding to the ligand to metal charge transfer transitions [24]. This observation transparently indicates the stabilization of exited state in complexes. The magnetic moment of the complexes of Ni (II) and Zn(II) are 2.01 and 1.06 B.M. respectively, consistent with a tetrahedral geometry [25].

3.4. NMR spectra analysis

Additional evidence for the formation of thiosemicarbazide was obtained from the ^1H NMR and ^{13}C NMR spectrum data, which provides diagnostic tools for the positional elucidation of the protons and carbons respectively as shown in Figures 7 and 8 (see Supplementary Material).

In the ^{1}H NMR spectra of the ligand, the signal due to the N-H protons; H_A and H_B appeared at 1.91 and 5.90 ppm, respectively. Other aromatic protons were observed at expected regions (H_C = 6.6 pm, H_D =7.36 ppm, H_E = 7.89 ppm, H_F = 10.43 ppm). Thus, the recorded signals obtained in the ^{1}H NMR spectra are in agreement with the proposed structures of thiosemicarbazide.

The ^{13}C NMR spectrum of the thiosemicarbazide ligand was recorded in DMSO-d_6. The spectral signals of thiosemicarbazide showed signal at 181.71 ppm assigned due to thioamide (C=S) and 168.73 (C-N), 157.20 (C-Cl), 138.86, 132.44, 129.28, 128.25 (aromatic carbons) and are in good agreement with the probable structures [26].

3.5. Conductivity Measurements of metal complexes

The molar conductance measurements recorded for $1x10^{-3}$M DMSO solutions of the metal complexes are listed above (Table 3). The data show negligible molar conductance values (2–8 λm ($n^{-1}cm^2mol^{-1}$), indicating that the complexes are non-electrolytes nature, because a value below 50 $n^{-1}cm^2mol^{-1}$ is for non-electrolyte [27] and the presence of chloride ions within the coordination sphere of Zn(II) complexes [28]. This suggested that there were no anions present outside the coordination sphere of the complexes [29]. Higher values of molar conductivity observed in the Ni (II) complexes compared to the Zn (II) complexes may be due to the presence and absence of solvolysis in the complexes rather than ionic dissociation. Solvolysis is a special type of nucleophilic substitution or elimination where the nucleophile is a solvent molecule. DMSO, which was used for conductivity measurement, is a coordinating solvent and capable of causing solvolysis [30].

3.6. Thermogravimetric analysis

The thermal stability and water content of the complexes was studied by using thermo gravimetric analysis. The TGA/DTG and DSC data of the obtained complexes are summarized in table 5 and the representative thermogram curves are given in Figures 1-4 as shown below.

Table 5: Thermo analytical results of 4-(4-Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide metal complexes

Complexes	**Temprature of TGA (^{0}C)**	**Temprature of DTG**	**Weight loss (%)**		**Assignments**	**DSC Temp (^{0}C) Endothermic peaks**
			Calc	found		
Ni(II) complex	(1) 53.3-215.5	85	4.9	4.8	loss of coordinated two water molecules	261.1,319.7,424,546.3 ^{0}C
	(2) 225 – 632.6	242	45.5	45.6	loss of one ligand molecule	
Zn(II) complex	(1) 260.6-362	314	4.5	4.6	loss of coordinated part of one ligand	333.3, 369.3, 675.5 ^{0}C

Nickel and zinc complexes are thermally stable above 70 ^{0}C and 260 ^{0}C, respectively, which indicate the presence of water molecules in nickel complex and absence of water in zinc complexes but indicate the loss of some part of one ligand in the zinc complex [31]. The results in Table 5 also show that the zinc complex exhibits higher thermal stability, as it decomposes at higher temperatures than the nickel complex, which agrees with the experimental melting temperatures. The complexes were found to decompose in a two or more-step process. The first decomposition of nickel complex occurs between 70 – 215 ^{0}C with an endothermic peak at 84 ^{0}C and the second decomposition from 225.5 – 632.6 ^{0}C with an endothermic peak at 242 ^{0}C as shown in Figure 1 and 2.

The TG of Ni(II) complex indicates a weight loss of 4.8 % (calc. 4.9 %, 36.16 g), which is observed in the temperature range of 70 – 215 ^{0}C associated with the loss of two coordinated water molecules. The DTG curve of this complex shows a short peak around 102 ^{0}C which is assigned to the loss of water, another peak at around 186 ^{0}C, with some decomposition followed by a very sharp and high intense peak at 242 ^{0}C for complete decomposition of the complex. An endothermic peak at 261 ^{0}C on the DSC curve of the Ni(II) complex may be associated with some reduction or phase transitions followed by another peak at 319 ^{0}C which can be considered as its melting point and undergoes further decomposition at around 546 ^{0}C. The complex left as metal residue above 736 ^{0}C.

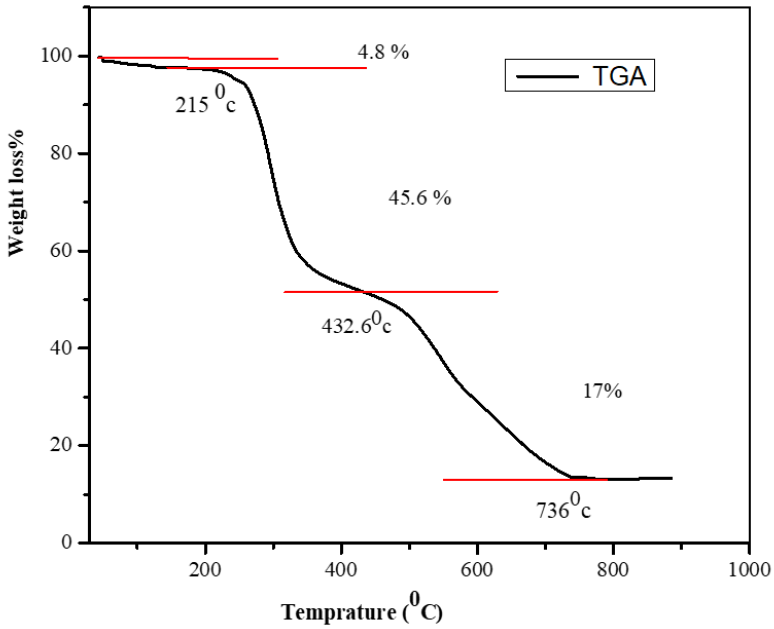


Figure 1. TGA curve of $[Ni(L)_2(H_2O)_2]$ complex

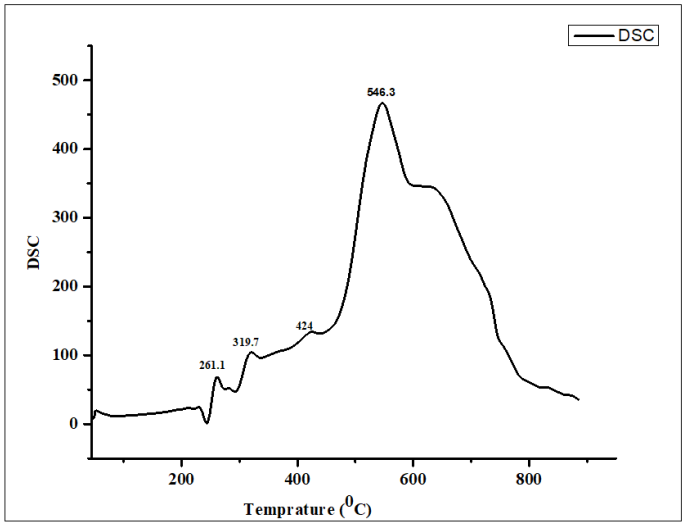


Figure 2. DSC curve of $[Ni(L)_2(H_2O)_2]$ complex

The thermal decomposition of zinc complex starts from 260 ^{0}C with an endothermic peak at 314 ^{0}C and the second decomposition occurs from 516 –760 0C with an endothermic peak at 674 0C.

TGA of Zn(II) complex indicated a total weight loss of 4.6 % (calcd. 4.56 %, 35.44 g), which is observed in the temperature range of 260 - 362 0C, due to the loss of coordinated chlorine molecules and its DTG curve showed large peaks at 314 0C representing decomposition (Figure 3). DSC analysis of the complex shows a very small endothermic peak at 333.3 and 369.3 0C which may be associated with reduction or phase transition (Figure 4). A very sharp endothermic peak at 675.5 0C shows the melting point of the complex after which it may suffer endothermic decomposition. The percentage weight loss of the complex is in agreement with the calculated values. The complex left as a metal residue above 760 ^{0}C [32, 33]. The TG and DSC curves of zinc complex are shown (figure 3 and 4) below.

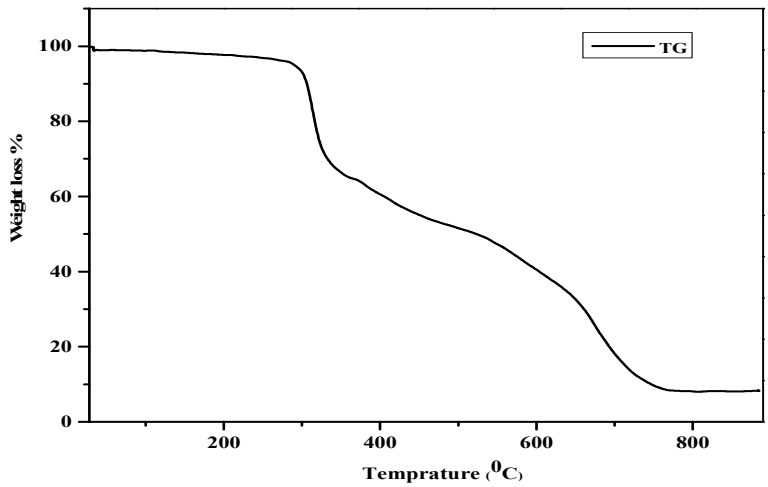


Figure 3. TGA curve of $[Zn(L)_2]$ complex

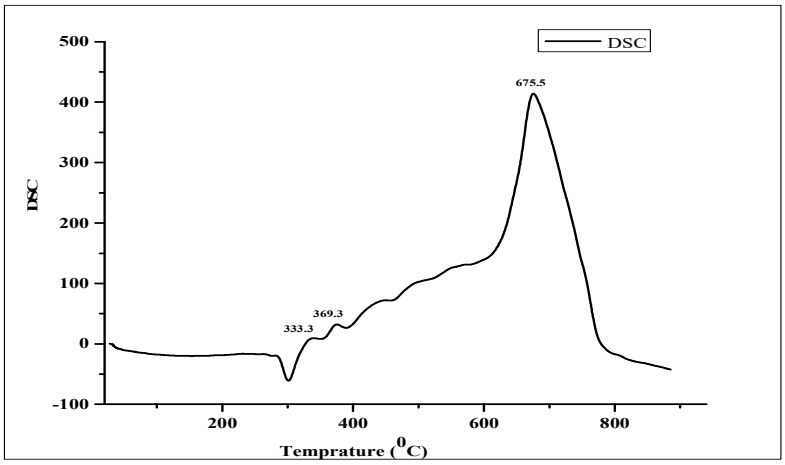
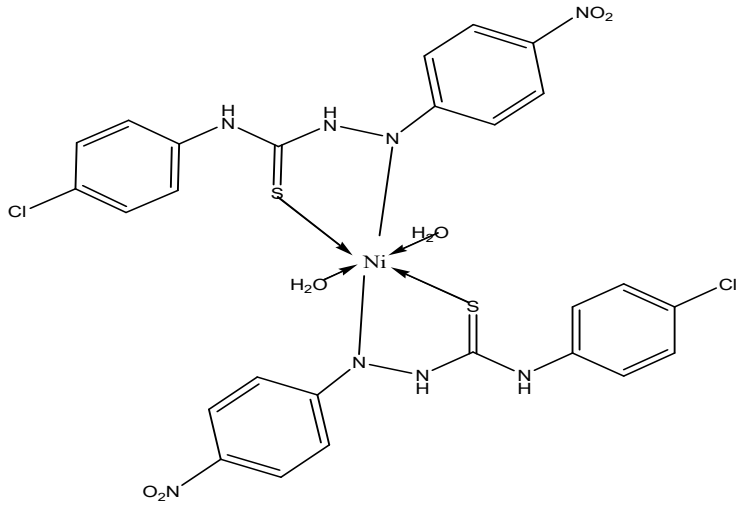
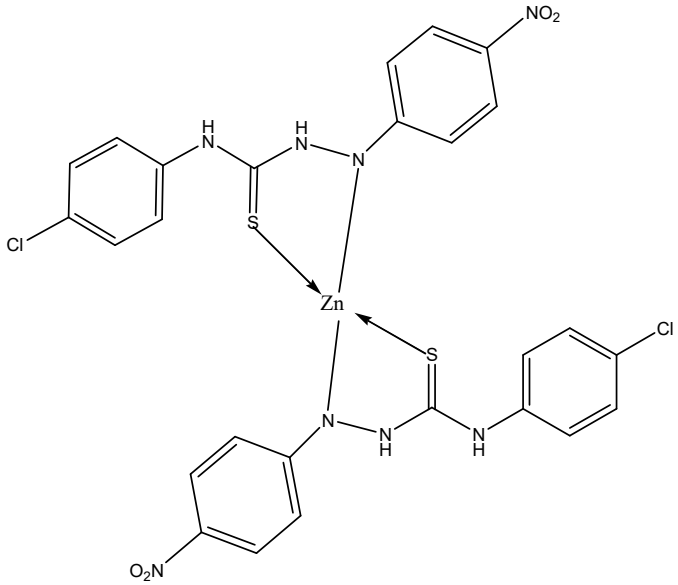


Figure 4. DSC curve of $[Zn(L)_2]$ complex

All the data suggest that the nickel and zinc complexes have been formulated as: $[Ni(L)_2(H_2O)_2]$ and $[Zn(L)_2]$ and on the basis of spectral studies, the following structure may be suggested for the complexes as shown Scheme 6 & 7.



Scheme 6: Ni-complexes of bis(4-(4-Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide ligand



Scheme 7: Zn-complexes of bis(4-(4-Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide ligand

3.7. Antibacterial Activity

Thiosemicarbazides and their metal complexes are pharmacologically active compounds used to treat various life-threatening diseases. In an attempt to determine lead compounds against bacteria, thiosemicarbazide derivatives and their metal complexes have been synthesized and subsequently tested for their antibacterial activities against selected types of two Gram positive and two Gram negative bacteria pathogens such as; *S. aureus, B. subtilis*, *E. coli* and *P. aeruginosa* as shown in table 6 below.

Table 6. The inhibition zone of the synthetic compound in mm (mean ± SD)

Compound	**Conc. (µ g/ml)**	***E. Coli***	***P. aeruginosa***	***S. Aureus***	***B. Subtilis***
$C_{13}H_{11}N_4O_2SCl$ (L)	300	NA	1.00 ± 0.00	2.34 ± 0.44	4.24 ± 0.67
	500	1.33 ± 0.68	2.34 ± 0.44	4.65 ± 0.67	6.84 ± 0.96
$[Ni(L)_2)\ (H_2O)_2]$	300	6.34 ± 0.44	8.40 ± 0.32	10.24 ± 0.89	9.00 ± 0.00
	500	6.67± 0.44	10.24±0.64	12.86 ± 0.46	9.68 ± 0.33
$[Zn(L)_2]$	300	5.00 ± 0.64	5.64 ± 0.54	13.67 ± 0.86	16.00 ± 0.84
	500	6.12 ± 0.68	5.89 ± 0.44	13.82± 0.46	17.67 ± 0.44
Ciprofloxacin (as standard drug)	300	18.89 ±0.67	7.64 ± 0.64	9.0 ± 0.68	28.0± 0.82
	500	20.46±0.56	8.0 ± 0.66	11.0 ± 0.67	30.33 ± 0.68

NA: no inhibition zone, results are expressed as M ± SD of triplicates.

The above data (Table 6) shows all synthesized compounds except the ligand on *E. coli* bacteria which shows no activity, the entire compounds shows medium to good activity against two or more bacterial strains. The results show that the complexes have higher activity than the free ligand at similar experimental condition. The mean inhibition zones ranged from the lowest (1.00 mm at 300μg/mL) to the highest (17.67 mm at 500 μg/mL). The metal complex compounds $[Ni(L)_2(H_2O)_2]$ and $[Zn(L)_2]$ showed good activities against *S. aureus,* with mean inhibition zone of 12.86 ± 0.46 and 13.82 ± 0.46 mm diameter at 500 μg/mL respectively compared to the standard drug Ciprofloxacin inhibition zone of 11.0 ± 0.67 mm diameter at 500 μg/mL. In addition the complex $[Ni(L)_2(H_2O)_2]$ showed higher antibacterial activity against *P. aeruginosa* (10.24 ± 0.64 mm at 500 μg/mL) than Ciprofloxacin (8.0 ± 0.66 μg/mL). Overall, the inhibition zones of the ligand and its complexes at different concentrations showed that the complexes had enhanced bactericidal activity than the ligand and the antibacterial activities of the metal complexes are directly proportional to their concentration as shown in Figure 15.

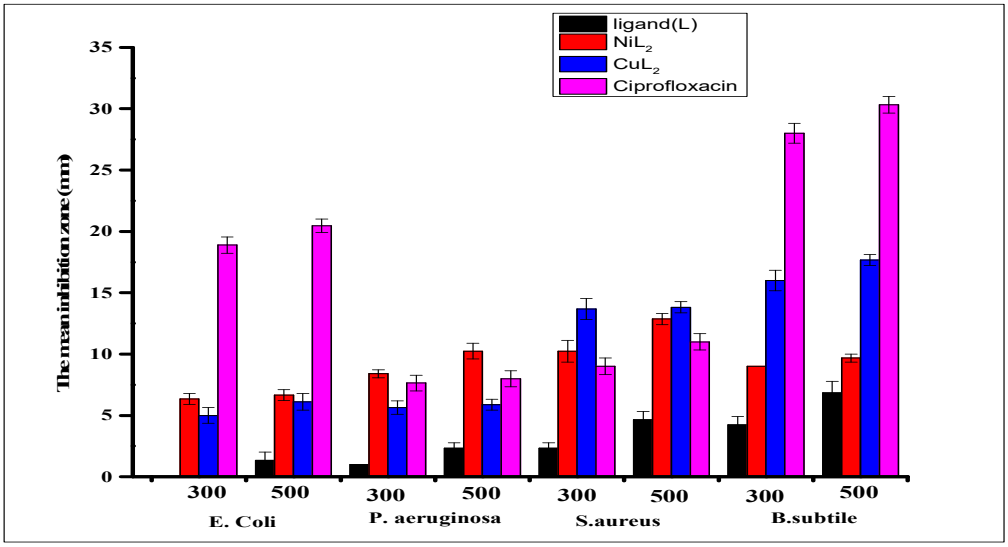
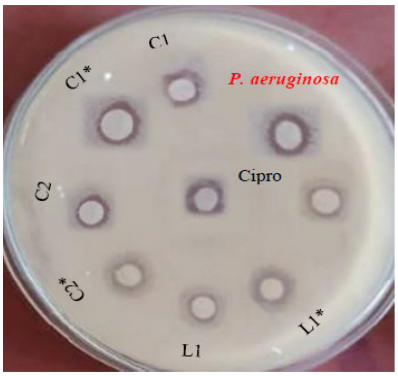


Figure 5. The inhibition zone of the synthetic compounds in mm (mean ± SD) at 300 μg/mL &500 μg/mL.



Where: L & L = ligand conc. at 300 and 500 µg/ml ; C & C* = complex conc. at 300 and 500 µg/ml*
Figure 6. Distance of inhibition by samples on *S. aureus* bacteria species

3.8. Antioxidant Activity of the Synthetic Compounds

DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay was widely used to assess the ability of compounds as scavengers of free radicals and hence evaluate the antioxidant activity of synthetic compounds [34]. It is a stable free radical accepting hydrogen from the corresponding donor, which causes it to lose the characteristic deep purple color at λ_{max} = 517 nm. Compounds exhibiting antioxidant activity reduced the absorbance at 517 nm, which is due to DPPH radicals in addition to their ability to turn the purple-colored DPPH to yellow [34]. In this work, the radical scavenging activity of the synthesized compounds was evaluated using DPPH, and the findings are shown in Table 7.

Table 7. Percent free radical scavenging activities of synthesized compounds

Componds	**Concentrations in µg/ml**			
	12.5	**25**	**50**	**100**
$C_{13}H_{11}N_4O_2SCl$ (L)	18.64 ± 0.44	20.04 ± 0.68	24.06 ± 0.45	30.06 ± 0.57
$[Ni(L)_2(H_2O)_2]$	28.56 ± 0.71	31.08 ± 0.33	44.68 ± 0.46	48.55 ± 0.48
$[Zn(L)_2]$	36.46 ± 0.67	37.50 ± 0.68	46.09 ± 0.47	56.75 ± 0.67
Ascorbic acid	84.8 ± 0.68	85.0 ± 0.45	85.67 ± 0.48	86.48 ± 0.24

The DPPH radical scavenging activity of the synthesized ligand and its Ni(II) and Z(II) complexes were compared with ascorbic acid which is used as positive control. The metal complexes displayed better radical scavenging activity in this study, which has been elaborated in comparison with ascorbic acid as shown in figure 16 bellow. The data in table 7 reveal that the new synthetic ligand and its metal complexes showed moderate antioxidant activities, while the metal complexes exhibited better antioxidant activity (56.75 % radical scavenging activity at 100 μg/mL concentration). In $[Zn(L)_2]$ complex, there is no easily transferable hydrogen; however, sulfur of thiocyanate functional group may donate an electron to lone pair electron on the nitrogen

atom to form bond with sulfur.

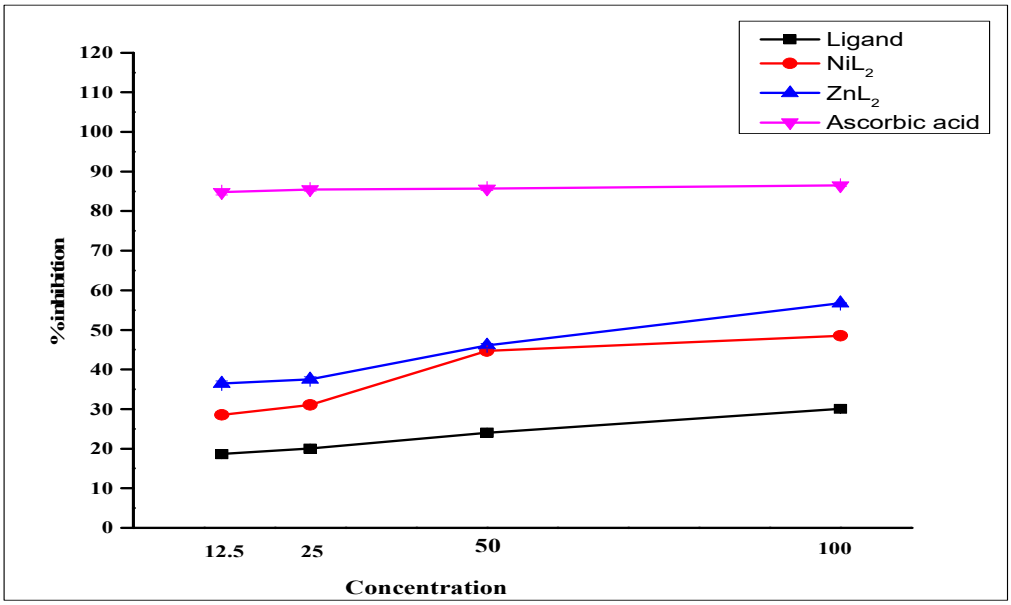


Figure 16. % inhibition of DPPH radical by the synthetic compounds

4. Conclusions

Thiosemicarbazide-derivatives are a useful scaffold to develop bioactive metal complexes by coordinating through their heteroatoms nitrogen and sulfur. In this study, ligand 4-(4 Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide and its Ni(II), Zn(II) complex were synthesized successfully by a modified previously reported complexation protocol procedure and characterized. The complexes formed are neutral with no free anions outside the coordination sphere. The antibacterial activities of the synthesized compounds were screened by the disc-diffusion method against two Gram-negative and two Gram-positive bacteria, and most of them were found to have moderate activity against the bacterial strains used for the screening. Among them, the metal-complexes $[Ni(L)_2(H_2O)_2]$ and $[Zn(L)_2]$ exhibited maximum activity against *Pseudomonas aeruginosa and Staphylococcus aureus* compared to the standard drug ciprofloxacin. The Ni (II) and Zn(II) complexes exhibited better antibacterial activities than the ligand under the same experimental situation. The antioxidant activity data of the new synthesized compounds also shows that the metal complexes have better antioxidant activity than the free ligands. Generally, the synthesized compounds have potential of antibacterial and antioxidant activity and can be further modified to serve as lead compounds.

Data Availability

The data used to support the findings of this study are included within the manuscript and submitted as Supplementary Materials.

Conflict of Interest

This work is our own original work that has no any conflicts of interest.

Acknowledgments

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Supplementary Materials

The FT-IR, UV-Vis, ^{1}H NMR and ^{13}C NMR spectra of the synthesized compounds

Figure 1. FTIR (υ cm^{-1}, KBr) spectrum of 4-(4-Chlorophenyl)-1-(4-nitrophenyl) thiosemicarbazide ligand
Figure 2. UV-Vis spectrum of 4-(4-Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide ligand Figure 3. FTIR (υ cm^{-1}, KBr) of $[Ni(L)_2(H_2O)_2]$ complex
Figure 4. UV-Vis spectrum of $[Ni(L)_2(H_2O)_2]$ complex
Figure 5. FTIR (υ cm^{-1}, KBr) of $[Zn(L)_2]$ complex
Figure 6. UV-Vis spectrum $[Zn(L)_2]$ complex

Figure 7. ^{1}H NMR spectrum of 4-(4-Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide ligand
Figure 8. ^{13}C NMR spectrum of 4-(4-Chlorophenyl)-1-(4-nitrophenyl)thiosemicarbazide ligand

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