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## Synthesis, characterization and biological activity of novel transition metal complexes of *N*-{[2-(pyridin-4-ylmethyl)hydrazinyl]carbonothioyl}thiophene-2-carboxamide

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### ABSTRACT

*N*-{[2-(pyridin-4-ylmethyl)hydrazinyl]carbonothioyl}thiophene-2-carboxamide and its metal complexes have been synthesized and the structure elucidated by elemental analysis, conductivity measurements, UV-Visible, FT-IR, <sup>1</sup>H NMR and thermal analysis. The complexes were soluble in most of the organic solvents and were non-electrolytic in nature. The cobalt and nickel complexes possess tetrahedral geometry while that of copper complex have octahedral geometry. The antimicrobial activities of title compounds have been screened against Gram-positive and Gram-negative bacteria with comparing standard ciprofloxacin as reference. Antifungal activities against two different fungi have been evaluated and compared with Flucanazole as reference. Almost all complexes showed excellent activity against bacteria and fungi strains used. The MIC result showed comparable activity as standard drug. Obtained compounds also subjected to antioxidant activity and they show potent activity when compared with ligand.

**Keywords:** Thiosemicarbazone, thermal analysis, conductivity measurements, antimicrobial and antioxidant activity

### INTRODUCTION

The manipulative of thiosemicarbazone complexes were used for developing wide range of sensitive diagnostic agents and pharmacological applications. The biological properties of thiosemicarbazones were often related to metal coordination [1-3]. The thiosemicarbazone derivatives were of special importance because of their versatile biological and pharmacological activities. The derivatives of thiosemicarbazone found to have applications in drug development for the treatment of central nervous system disorders due to bacterial infection, as well as analgesic and antiallergic agent. Moreover, thiosemicarbazones also found to have wide variety of commercial applications, such as dyes, photographic films, plastic industry and in textile industries. In the recent years, many researchers have demonstrated wide range of biological activity of thiosemicarbazone derivatives such as antimicrobial [4-9], antitumor [10-11], sodium channel blocker [12], anticancer [13-14], antitubercular [15] and antiviral activities [16].

The design and synthesis of thiosemicarbazone metal complexes are of particular interest in pharmacological research to have increased drug activity and to decrease their toxicity which make a tool for variety applications including clinical biology, analytical and industrial [17-23]. In addition, thiosemicarbazone derivatives exhibits

enhanced thermodynamic and kinetic stabilities due to their modified complexation properties relative to the corresponding simple molecular precursor. Keeping all these facts in mind, in this article we reported the synthesis and characterization of *N*-{[2-(pyridin-4-ylmethyl)hydrazinyl]carbonothioyl}thiophene-2-carboxamide and its metal complexes. The title complexes were characterized by elemental analysis, molar conductance measurements, UV-visible spectroscopy, FT-IR, <sup>1</sup>H NMR and thermal gravimetric analysis. The synthesized ligand and its complexes have been screened for antimicrobial activities in order to examine their *in-vitro* microbiological activity against various Gram-positive, Gram-negative bacteria and different fungi in comparison with the standard drugs. The result reveals that all the newly synthesized complexes more potent than the ligand. In addition to that free radical scavenging activity of complexes and ligand was also investigated.

## MATERIALS AND METHODS

The chemicals thiophene-2-carbonyl chloride, Isoniazid, NH<sub>4</sub>SCN were purchased from the Sigma Aldrich. Ni(II)acetate tetrahydrate, Co(II)chloride tetrahydrate, Cu(II)acetate monohydrate were purchased from MERCK and were used as received. The purified solvent was used.

### Physical Measurements

The elemental analyses (C, H, N, S) were performed using Perkin-Elmer 2400 II CHNS/O Elemental analyzer. Melting point of ligand and metal complexes were measured by using melting point apparatus model code NAMPA/045 and are uncorrected. UV-Visible spectra were measured on an ocean optics USB 4000USA, using 1cm path length cuvette at room temperature. Infrared spectra were recorded using FT-IR 8400s Shimadzu spectrometer with KBr pellets in the range of 400-4000 cm<sup>-1</sup>. The molar conductance data were measured using freshly prepared DMSO solutions (10<sup>-3</sup>M) at 25 °C with a systronics model-660A instrument. The <sup>1</sup>H NMR spectra were measured at 400 MHz Varian-AS NMR spectrometer in dimethylsulfoxide-D<sub>6</sub> using tetramethylsilane (TMS) as the internal standard. Thermogravimetric analysis was done by Perkin Elmer-4000 instrument.

### Preparation of *N*-{[2-(pyridin-4-ylmethyl)hydrazinyl]carbonothioyl}thiophene-2-carboxamide (S<sub>1</sub>).

A solution of thiophene-2-carbonyl chloride (1.46g, 0.01mol) in 30mL of dry acetone was added to a solution of ammonium thiocyanate (1.44g, 0.02mol) in 10 mL dry acetone. The reaction mixture was refluxed for 6 h with stirring in a round bottom flask equipped with condenser and drying tube. After the reaction, the white solid of NH<sub>4</sub>Cl was removed by filtration. The filtrate containing thiophene-2-carbonyl isothiocyanate was added to a solution of isoniazid (2.05g, 0.015mol) in 10 mL of dry acetone with constant stirring. The mixture was heated under reflux for 3 h. The reaction was monitored with TLC by using silica gel-G coated plates by using ethyl acetate and *n*-hexane (0.1:0.9). After completion of the reaction, the reaction mixture was poured into 300ml of ice cold water. Filtered precipitate was washed with ice cold water and recrystallised from the ethanol. Cream color, Yield: 84%, M.P.171-174 °C. Elemental analysis (%) Calc. for [C<sub>12</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>S<sub>2</sub>]: C, 47.44; H, 3.19; N, 18.34; S, 20.75; O, 10.28. Found: C, 47.04; H, 3.29; N, 18.29; S, 20.93; IR (KBr, cm<sup>-1</sup>): 3250 (-NH), 3160 (-OH), 1672 (C=N), 1069 (N-N), 2786 (S-H), 1256, 848 (C=S), 3090 (Ar C-H), 1527 (Ar C=C), 1502 (C-C). <sup>1</sup>H NMR (DMSO-D<sub>6</sub>, ppm): 11.41 to 11.84 (s, NH), 7.82 to 8.80 (d, Ar-H), 7.27 (t, Ar-H).

### Synthesis of metal complexes.

#### Synthesis of Nickel(II) complex (1).

A solution of nickel(II)acetate tetrahydrate (0.512g, 0.002mol) in ethanol (10 mL) was added to a solution of ligand S<sub>1</sub> (1.22g, 0.004mol) in ethanol 20 mL. The resulting reaction mixture was refluxed for 4h with continuous stirring. The light green solid formed was filtered off, washed successively with 1:1 cold water: ethanol and dried in vacuum. Yield: 78%. M.P. 210-214 °C. Elemental analysis (%) Calc. for Ni[C<sub>24</sub>H<sub>18</sub>N<sub>8</sub>O<sub>4</sub>S<sub>4</sub>]: C, 43.06; H, 2.71; N, 16.74; S, 19.16; O, 9.9; Ni, 8.77. Found: C, 42.43; H, 2.54; N, 16.15; S, 18.88. IR (KBr, cm<sup>-1</sup>): 3250 (-NH), 3394 (-OH), 1612 (C=N), 1068 (N-N), 3155 (Ar, C-H), 1535 (Ar, C=C), 1510 (C-C), 530 (Ni-N), 408 (Ni-S). <sup>1</sup>H NMR (DMSO-D<sub>6</sub>, ppm): 10.48 to 12.11 (s, NH), 7.19 to 8.89 (m, Ar-H), 14.5 (s, OH). Molar conductance 7.68(Ω<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>).

#### Synthesis of Cobalt(II) complex (2).

A solution of cobalt(II)acetate tetrahydrate (0.498g, 0.002mol) in ethanol (10 mL) was added to a solution of ligand S<sub>1</sub> (1.22g, 0.004mol) in ethanol 20mL. The resulting reaction mixture was refluxed for 5 h with continuous stirring. The parrot-green solid formed was filtered off, washed successively with 1:1 cold water: ethanol and dried in vacuum. Yield: 68%. M.P. 192-193 °C. Elemental analysis (%) Calc. for Co[C<sub>24</sub>H<sub>18</sub>N<sub>8</sub>O<sub>4</sub>S<sub>4</sub>]: C, 43.05; H, 2.71; N, 16.73; S, 19.15; O, 9.56; Co, 8.80. Found: C, 44.19; H, 2.46; N, 16.60; S, 20.80. IR (KBr, cm<sup>-1</sup>): 3250 (-NH), 3490

(-OH), 1611 (C=N), 1070 (N-N), 3150 (Ar, C-H), 1525 (Ar, C=C), 1512 (C-C), 482 (Co-N), 410 (Co-S). Molar conductance  $8.60(\Omega^{-1}\text{cm}^2\text{mol}^{-1})$ .

#### Synthesis of Copper(II) complex (3).

A solution of copper(II)acetate monohydrate (0.399g, 0.002mol) in ethanol (10mL) was added to a solution of ligand  $S_1$  (1.22g, 0.004mol) in ethanol 20mL. The resulting reactions mixture was refluxed for 3 h with continuous stirring. The dark green solid formed was filtered off, washed successively with 1:1 cold water: ethanol and dried in vacuum. Yield: 81% .M.P. 220-223 °C. Elemental analysis (%) Calc. for  $\text{Cu}[\text{C}_{24}\text{H}_{18}\text{N}_3\text{O}_4\text{S}_4]$ : C, 42.75; H, 2.69; N, 16.62; S, 19.02; O, 9.50; Cu, 9.42. Found: C, 42.78; H, 2.71; N, 16.69; S, 19.10. IR (KBr,  $\text{cm}^{-1}$ ): 3250 (-NH), 3410 (-OH), 1628 (C=N), 1048 (N-N), 3012 (Ar, C-H), 1539 (Ar, C=C), 1511(C-C), 537 (Cu-N), 417 (Cu-S). Molar conductance  $8.41(\Omega^{-1}\text{cm}^2\text{mol}^{-1})$ .

#### Antimicrobial activity.

The synthesized ligand ( $S_1$ ) and its complexes (**1-3**) were screened *in-vitro* for their biological activity, *Gram positive Staphylococcus aureus*, *Gram negative, Klebsiella pneumonia*, *Pseudomonas aures*, *Escherichia coli*, two fungal strains were *aspergillus niger* and *candida albicans* with ligand ( $S_1$ ) and its complexes (**1-3**) was performed by the Agar well diffusion method [24]. The bacterial strains were collected from different infectious status of patients who had not administered any antibacterial drugs for atleast two weeks with the suggestions of an authorized physician, in Kiran diagnostic health centre of Chitradurga, Karnataka state, India. Fungal strains were procured from the culture maintained at National College of Pharmacy Shimoga. The compounds were tested at 40  $\mu\text{g}/\text{mL}$  concentration against both bacterial and fungal strains. DMSO was used as a vehicle. Ciprofloxacin (40  $\mu\text{g}$  in100 $\mu\text{l}$ ) and Fluconazole (40  $\mu\text{g}$  in100 $\mu\text{l}$ ) were used as standard drugs for comparison of antibacterial and antifungal activities respectively. The zone of inhibition was compared with standard drug after 24 h of incubation at 37 °C for antibacterial activity and 72h at 25 °C for antifungal activity.

#### Minimum Inhibitory Concentrations (MIC).

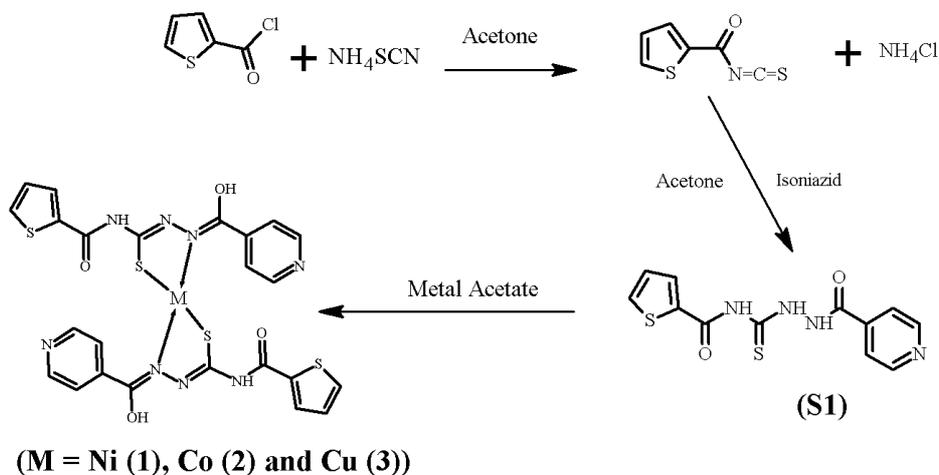
The Minimum Inhibitory Concentrations (MIC) of ligand ( $S_1$ ) and its complexes (**1-3**) was determined by a micro dilution method [24]. The respective clinical strain was spread separately on the medium. The wells were created using a stainless steel sterilized cork borer under aseptic conditions. The synthesized ligand ( $S_1$ ) and its complexes (**1-3**) at different concentrations and later loaded into corresponding wells. The drugs Ciprofloxacin (40  $\mu\text{g}$  in100 $\mu\text{l}$ ) and Fluconazole (40  $\mu\text{g}$  in100 $\mu\text{l}$ ) were used as standard drugs for comparison of antibacterial and antifungal activities respectively. The zone of inhibition was compared with standard drug after 24 h of incubation at 37 °C for antibacterial activity and 72h at 25 °C for antifungal activity.

#### Antioxidant activity.

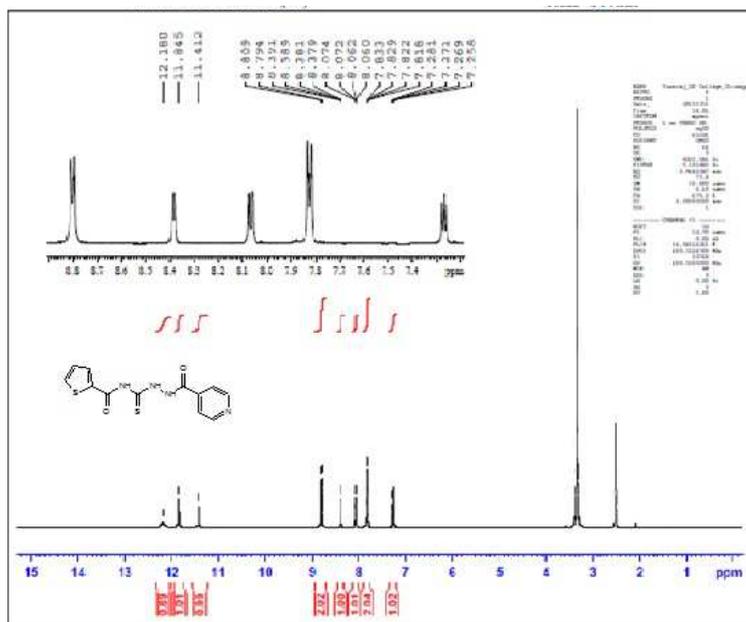
The DPPH free radical scavenging activity of the ligand ( $S_1$ ) and its complexes (**1-3**) were measured according to the standard method and compared with ascorbic acid (standard) [24]. The DPPH radical is a stable free radical having  $\lambda_{\text{max}}$  at 517 nm. Different concentration (5, 10, 25, 50, 100 and 200  $\mu\text{g}/\text{mL}$ ) of compounds and standard were prepared in methanol. In clean and labeled test tubes 2ml of DPPH solution (0.002% in methanol) was measured at 517 nm using UV-visible spectrophotometer. The absorbance of the DPPH control was also noted. The scavenging activity was calculated using the formula: scavenging activity (%) =  $(A-B)/A \times 100$ , where A is the absorbance of control (methanol) and B is the absorbance of sample.

## RESULTS AND DISCUSSION

The scheme 1 shows the template condensation reaction between thiophene-2-carbonyl chloride, ammonium thiocyanate and ionized to produce new ligand and also the synthesized complexes. The condensation reaction of thiophene-2-carbonyl chloride and ammonium thiocyanate were taken in dry acetone, to give thiophene-2-carbonyl isocyanate, which when react with isoniazid to produce the ligand  $S_1$ . The complexes were synthesized by refluxing separately 0.002mol of nickel(II)acetate tetrahydrate, cobalt(II)acetate tetrahydrate and copper(II)acetate monohydrate with 0.004mol of ligand ( $S_1$ ) in ethanol. The  $^1\text{H-NMR}$  spectrum of the ligand ( $S_1$ ) is shown in Figure 1. It showed sharp signals in the range 11.41-12.18 ppm for different -NH protons (3H) and a multiple observed in the range 7.27-8.80 ppm are assigned to the aromatic ring protons (7H).

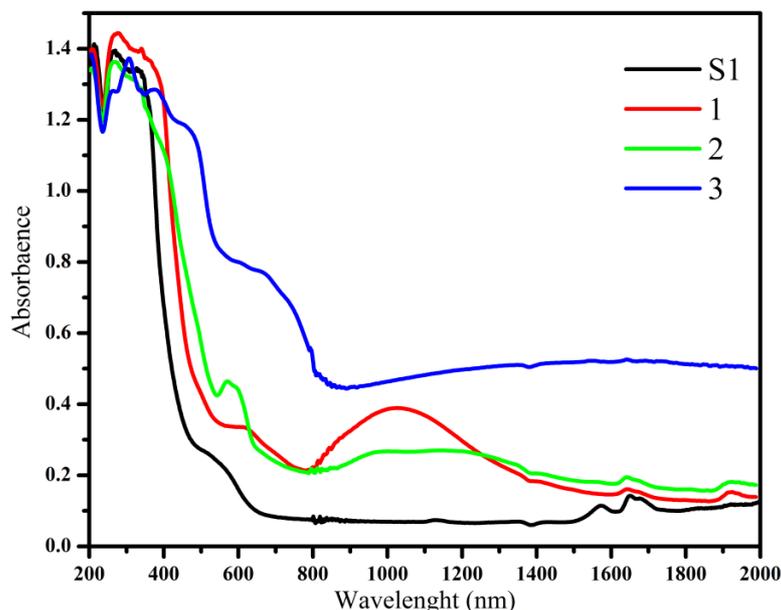


Scheme-1: synthesis of N-[[2-(pyridin-4-ylmethyl)hydrazinyl]carbonothioyl]thiophene-2-carboxamide ( $S_1$ ) and its complexes (1-3)



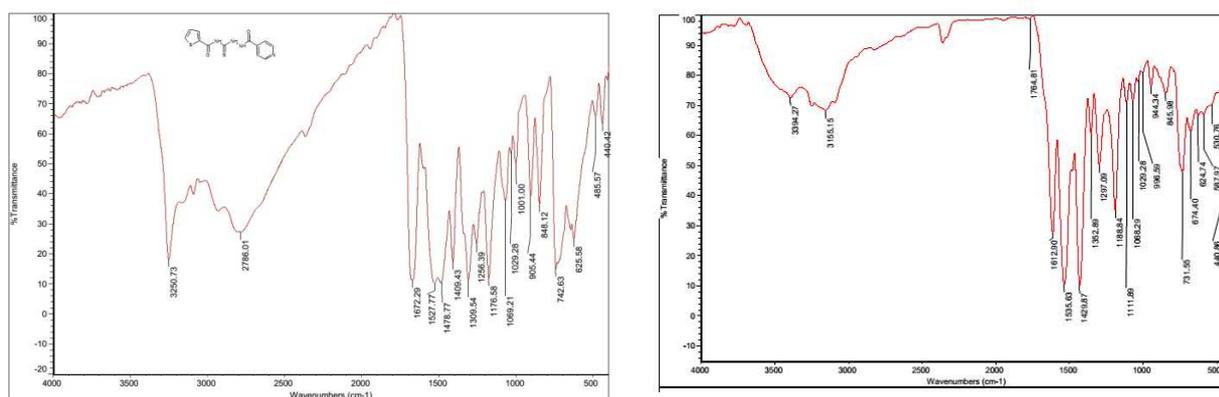
### FT-IR Spectral Studies

The IR spectra of the ligand showed a strong absorption band at  $1672\text{ cm}^{-1}$  which was assigned to the azomethene group (C=N) [25]. the strong band observed at  $1256\text{ cm}^{-1}$  and  $848\text{ cm}^{-1}$  in the spectrum was due to the (C=N) and (C=S) [26]. The bands observed at  $3160\text{ cm}^{-1}$  and  $3250\text{ cm}^{-1}$  were assigned to (O-H) and (N-H) vibrations respectively. This indicates that the ligand present in thione form. The diagnostic IR spectral bands of the complexes were presented in figure 2. The spectra of all complexes with the azomethene moiety (C=N) was shifted to lower frequency compared to the uncoordinated ligand, indicating its involvement in coordination with metal ion. The (C=S) stretching frequency observed at  $1256\text{ cm}^{-1}$  in ligand was shifted in the spectra of the complexes, indicating the involvement of the sulphur in the coordination. These findings were further supported by the appearance of new bands at  $530\text{ cm}^{-1}$  and  $408\text{ cm}^{-1}$ , which were assignable to (M-N) and (M-S) vibrations, respectively in nickel, copper and cobalt complexes.

Fig-2. IR spectrum of Ligand ligand ( $S_1$ ) and their metal complex (1)

### Electronic Absorption Spectra.

The electronic absorption spectra of the ligand ( $S_1$ ) and their metal complexes (**1-3**) were carried using DMSO solvent is as shown in figure 3. The electronic spectrum of  $[\text{Ni}(\text{II})\text{L}_2]$  complex showed a weak band at  $6070 \text{ cm}^{-1}$  due to the transition  ${}^3\text{T}_1(\text{F}) \rightarrow {}^3\text{T}_2(\text{F})$  ( $\nu_1$ ). A broad band at  $9690 \text{ cm}^{-1}$  is assigned to the  ${}^3\text{T}_1(\text{F}) \rightarrow {}^3\text{A}_2(\text{F})$  ( $\nu_2$ ) transition and a weak shoulder like band occur at  $15380 \text{ cm}^{-1}$  is due to the transition  ${}^3\text{T}_1(\text{F}) \rightarrow {}^3\text{T}_1(\text{P})$  ( $\nu_3$ ). The spectral data indicate that the complex have tetrahedral geometry. The  $[\text{Co}(\text{II})\text{L}_2]$  complex spectrum showed a weak band at  $5150 \text{ cm}^{-1}$  which is assigned to the transition  ${}^4\text{A}_2 \rightarrow {}^4\text{T}_2(\text{F})$  and a band at  $8770 \text{ cm}^{-1}$  is assigned to the transition  ${}^4\text{A}_2 \rightarrow {}^4\text{T}_1(\text{F})$  and another transition occur at  $16670 \text{ cm}^{-1}$  is due  ${}^4\text{A}_2 \rightarrow {}^4\text{T}_2(\text{P})$  transition. Based on the spectral results, it can be suggested that the Co(II) complex possess tetrahedral geometry. The Cu(II) complex results a broad absorption but split band at  $12500$  and  $14260 \text{ cm}^{-1}$  were assigned to the transitions from  ${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$  respectively. The split in band may due to John-Teller distortion normally occur in copper(II) complexes, thus  $[\text{Cu}(\text{CH}_3\text{COO})_2\text{L}_2]$  complex is proposed to have distorted octahedral geometry [27].

Figure 3. UV spectra of the ligand ( $S_1$ ) and its complexes (**1-3**)

### Thermal and Molar Conductivity Measurements

Thermogravimetric analysis was carried out at the rate of  $10^\circ\text{C}$  per min. from  $40$  to  $740^\circ\text{C}$  in nitrogen atmosphere  $80.0\text{mL}/\text{min}$ . The weight loss curves and the corresponding differential thermogravimetric curves for the complex

are shown in figure 4. The nickel complex showed two well defined steps for weight loss, the first at 195 °C where, the weight loss was 89% (89.8%) and the second step, where about 10.5% (10.5%) weight loss occur at the temperature above 330 °C can be explained by considering the formation of metal oxide residue (NiO). In the similar manner, the thermal decomposition of Co(II) complexes were also occur, the first step of decomposition at 150 °C, the weight loss was 89% (89.5%) and the second step of drop of the curve from 340 °C 11% (11.5%) was due the formation of cobalt oxide [28].

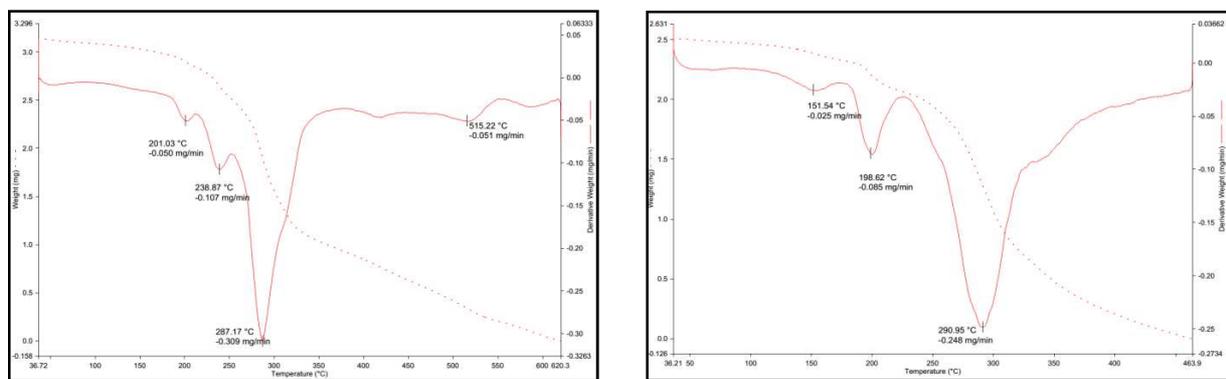


Figure 4. TGA and DTA curves for 1 and 2 complexes

The molar conductance was carried out in DMF at the concentration of 10<sup>-3</sup>M, show the very low conductance value (7-9 ohm<sup>-1</sup> cm<sup>2</sup> mol<sup>-1</sup>), which is due to non-electrolytic nature of complexes. The low molar conductance may be due to large size of anionic coordination sphere. The molar conductance value of complexes (1-3) is listed in Table 1.

Table 1: physical and analytical data of the ligand (S<sub>1</sub>) and its metal complexes (1-3)

SL. No	Molecular formula & molecular weight	Color	Yield (%)	M.P (°C)	Molar conductance (Ω <sup>-1</sup> m <sup>2</sup> mol <sup>-1</sup> )	%N (Found & Cal.)	C% (Found & Cal.)	%H (Found & Cal.)	%S (Found & Cal.)	%M (Found & Cal.)
S <sub>1</sub>	C <sub>12</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> 306.36	Cream	84	171-174	-	18.29 (18.34)	47.04 (47.44)	3.29 (3.19)	20.93 (20.75)	-
1	Ni(C <sub>24</sub> H <sub>18</sub> N <sub>8</sub> O <sub>4</sub> S <sub>4</sub> ) 669.40	Light-green	78	210-214	7.68	16.15 (16.74)	42.43 (43.06)	2.54 (2.71)	18.88 (19.16)	(8.77)
2	Co(C <sub>24</sub> H <sub>18</sub> N <sub>8</sub> O <sub>4</sub> S <sub>4</sub> ) 669.64	Parrot-green	68	190-193	8,60	16.60 (16.73)	44.19 (43.05)	2.46 (2.71)	20.08 (19.15)	(8.80)
3	Cu(C <sub>24</sub> H <sub>18</sub> N <sub>8</sub> O <sub>4</sub> S <sub>4</sub> ) 674.25	Dark-green	81	220-223	8.41	16.69 (16.62)	42.78 (42.75)	2.71 (2.69)	19.10 (19.02)	(9.42)

**Antimicrobial activity**

The results of antimicrobial activity of ligand (S<sub>1</sub>) and their metal complexes (1-3) are shown in Table 2. The results of antibacterial study indicate that the compounds were inhibition to bacteria. Among the synthesized compounds, marked inhibition was observed for compounds 2 and 3, while the least activity was observed for complex 1 and S<sub>1</sub>. The minimum inhibitory concentrations (MIC) for synthesized compounds were evaluated against test bacteria for the concentration ranging from 10µg/mL to 50µg/mL. The complexes 3 and 4 showed high inhibition at low concentration. The MIC results of antimicrobial activity are reported in Table 3.

Table 2: Anti-microbial activity of ligand (S<sub>1</sub>) and their metal complexes (1-3)

Compound	Zone of inhibition in (mm)					
	<i>S.aureus</i>	<i>B.subtilis</i>	<i>S.typhi</i>	<i>E.coli</i>	<i>A.niger</i>	<i>C.albicans</i>
	Antibacterial strains			Antifungal Strains		
S1	19	20	19	20	18	20
1	21	20	19	20	19	20
2	22	21	20	21	20	21
3	22	22	20	21	21	22
Ciprofloxacin	23	23	21	22	-	-
Flucanazole	-	-	-	-	22	23
Control	0	0	0	0	0	0

Standard - Ciprofloxacin (antibacterial); Standard - Flucanazole (antifungal)

Table 3: MIC of ligand (S<sub>1</sub>) and their metal complexes (1-3)

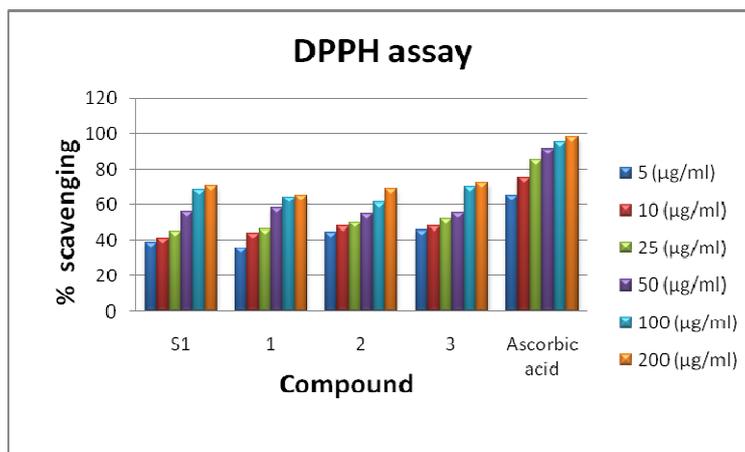
Compound	MIC (µg/µL)					
	<i>S.aureus</i>	<i>B.subtilis</i>	<i>S.typhi</i>	<i>E.coli</i>	<i>A.niger</i>	<i>C.albicans</i>
10-50 (µg)	Antibacterial strains			Antifungal Strains		
S <sub>1</sub>	30	30	30	30	40	40
1	20	20	30	30	30	20
2	30	20	20	30	30	30
3	20	20	30	20	20	30
Control	0	0	0	0	0	0

### Antioxidant activity

Antioxidant activity of ligand (S<sub>1</sub>) and their metal complexes (1-3) at different concentrations in methanol and ascorbic acid was determined in terms of free radical scavenging ability which was evaluated using DPPH free radical assay is as shown in figure 5. The compounds exhibited marked antioxidant activity by scavenging DPPH, and the activity was found to be dose dependent [29]. The compounds 2 and 3 was shown to be more potent than 1 and S<sub>1</sub> and the results were tabulated in Table 4

Table 4: DPPH radical scavenging activity of ligand (S<sub>1</sub>) and their metal complexes (1-3)

Conc. (µg/ml)	Radical scavenging activity (%)				
	S <sub>1</sub>	1	2	3	Ascorbic acid
200	70.61	65.14	68.99	72.48	98.06
100	68.69	64.14	61.56	70.45	95.09
50	56.16	58.56	55.36	55.77	91.36
25	45.14	45.99	50.16	52.36	85.46
10	41.20	44.14	48.14	48.14	75.08
5	38.69	35.69	44.36	45.59	64.96

Figure 5. Plot of the radical scavenging effects (%) of ligand (S<sub>1</sub>) and its metal complexes (1-3) at different concentrations

### CONCLUSION

In the present work, we successfully designed and developed a N-{{[2-(pyridin-4-ylmethyl)hydrazinyl]carbonothioyl} thiophene-2-carboxamide (S<sub>1</sub>) and its metal complexes. The ligand and their complexes have been characterized by various physicochemical techniques. Obtained results were good agreement with the proposed structure. The IR spectra indicate that the ligand acts in bidentate fashion by bonding to the central metal ion through the nitrogen and sulphur atoms. The antimicrobial and antioxidant activity results reveal that the complexes 2 and 3 exhibited good activity compared to the uncoordinated ligand and the complex 1.

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