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Synthesis, Characterization and Antibacterial Studies of Nickel(II) and Palladium(II) Complexes of Thiosemicarbazone

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A series of four benzaldehyde derivatives have been synthesized and underwent complexation with Ni(II) and Pd(II) ions using a microwave-assisted method. All compounds were characterized structurally using CHNS, FT-IR, and ^{1}H and ^{13}C-NMR spectroscopies. The results from FT-IR and NMR spectroscopies showed that the thiosemicarbazone ligands bind to the thionic sulfur and azomethine nitrogen. The antibacterial activities of the compounds were first tested by the broth microdilution method against two Gram-positive and three Gram-negative bacteria. Then, the minimum inhibitory concentration (MIC) was determined using gentamicin as a reference drug. The tested compounds showed significant activity to both types of tested bacteria compared to gentamicin.

Key words: Microwave-assisted; thiosemicarbazone; metal complexes; antibacterial activity

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The use of microwave irradiation as an energy source for a reaction has become an attractive alternative and earned researchers' attention against the conventional reflux method. This method provides a clean, cost-effective, and highly accelerated rate of reaction that helps to perform the reaction in much less time and good yields [1].

Thiosemicarbazone and its metal complexes play a key role in our understanding of the coordination chemistry of transition metal ions due to the presence of the thionic sulfur and azomethine nitrogen [2]. Thiosemicarbazone complexes have gained significant attention owing to the their biological activities such as antibacterial, antifungal, and anti-inflammatory behaviors [3] [4]. With the growing interest of thiosemicarbazones, the present work has been undertaken to investigate the ligation behavior of thiosemicarbazone towards Ni(II) and Pd(II) ions, which can be used as an antibacterial drug against Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

All chemicals and reagents used in the synthesis were of analytical grade and procured from Sigma-Aldrich and Merck. The solvents for the synthesis of ligands and complexes were used without further purification.

1. Synthesis of Ligands

All ligands were synthesized by the condensation of benzaldehyde derivatives (5 mmol) with 4-phenylthiosemicarbazide (5 mmol) as shown in **Figure 1** for 3 hours in a 1:1 molar ratio using absolute ethanol (25 mL) as the reaction medium. The method was adopted from Abdalla, Farina, & Ibrahim (2015)

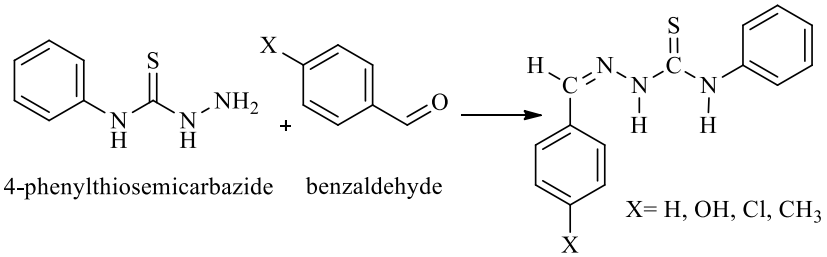

Figure 1. Synthesis of ligands

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with some mo
difications [5]. The resulting precipitates were filtered, washed with cold ethanol, and dried over silica gel.

L1: Color: White. Yield: 72.10%. Melting Point: 191℃. Calc. for $C_{14}H_{13}N_3S$: C, 65.85; H, 5.13; N, 16.46; S, 12.56. Found: C, 64.74; H, 5.13; N, 17.85; S, 12.09%. M/w: 255.34. IR (KBr, cm^{-1}): *ν*(NH) 3304, 3160; *ν*(C=N) 1570; *ν*(C=S) 858. ^{1}H-NMR ($CDCl_3$, ppm): 10.35 (s, 1H, NH); 9.21 (s, 1H, NH), 7.97 (s, 1H, C=N); 7.26-7.76 (m, 9H, Ar-H); ^{13}C-NMR ($CDCl_3$, ppm): 176.2 (C=S); 143.6 (C=N); 126.4-138.0 (Ar-H).

L2: Color: Yellow. Yield: 79.93%. Melting Point: 215℃. Calc. for $C_{14}H_{13}N_3OS$: C, 61.97; H, 4.83; N, 15.49; S, 11.82. Found: C, 61.99; H, 4.81; N, 15.47; S, 11.87%. M/w: 271.34. IR (KBr, cm^{-1}): *ν*(NH) 3394, 3277; *ν*(OH) 3166; *ν*(C=N) 1605; *ν*(C=S) 854m. ^{1}H-NMR ($CDCl_3$, ppm): 11.62 (s, 1H, OH); 9.94 (s, 1H, NH); 9.90 (s, 1H, NH), 8.03 (s, 1H, C=N); 6.77-7.68 (m, 9H, Ar-H); ^{13}C-NMR ($CDCl_3$, ppm): 175.9 (C=S); 159.9 (OH); 143.8 (C=N), 115.8-139.3 (Ar-H).

L3: Color: Yellow. Yield: 95.76%. Melting Point: 204℃. Calc. for $C_{14}H_{12}ClN_3S$: C, 58.03; H, 4.17; N, 14.50; S, 11.07. Found: C, 58.14; H, 4.01; N, 14.30; S, 11.41%. M/w: 289.78. IR (KBr, cm^{-1}): *ν*(NH) 3310, 3138; *ν*(C=N) 1596; *ν*(C=S) 815. ^{1}H-NMR ($CDCl_3$, ppm): 10.26 (s, 1H, NH); 9.18 (s, 1H, NH), 7.95 (s, 1H, C=N); 7.65-7.28 (m, 9H, Ar-H); ^{13}C-NMR ($CDCl_3$, ppm): 175.9 (C=S); 141.7 (C=N) 124.7-136.7 (Ar-H).

L4: Color: Brown. Yield: 86.78%. Melting Point: 189℃. Calc. for $C_{15}H_{15}N_3OS$: C, 66.88; H, 5.61; N, 15.60; S, 11.90. Found: C, 65.01; H, 5.34; N, 15.10; S, 12.05%. M/w: 269.36. IR (KBr, cm^{-1}): *ν*(NH) 3350, 3300; *ν*(C=N) 1595, *ν*(C=S) 813. ^{1}H-NMR ($CDCl_3$, ppm): 10.58 (s, 1H, NH); 9.81 (s, 1H, NH), 8.08 (s, 1H, C=N); 7.16 7.67 (m, 9H, Ar-H); ^{13}C-NMR ($CDCl_3$, ppm): 175.7 (C=S); 143.5 (C=N); 126.33 141.3 (Ar-H), 21.7 (CH_3).

2. Synthesis of Metal Complexes

Eight metal complexes (**Figure 2**) were successfully synthesized by the reaction of respective ligands with $Ni(OAc)_2$ and $Pd(OAc)_2$ via microwave-assisted synthesis method. The mixtures were heated in the microwave reactor for 5 minutes at 90℃ using absolute ethanol (15 mL) as the reaction medium with a 2:1 molar ratio. The resulting precipitates were filtered, washed with cold ethanol, and dried over silica gel.

NiL1: Color: Dark Brown. Yield: 79%. Melting Point: >300℃. Cal. for $C_{28}H_{28}N_6NiS_2$: C, 60.21; H, 4.38; N, 14.24; S, 10.50. Found: C, 58.86; H, 4.94; N, 14.71; S, 11.22%. M/w: 571.39. IR (KBr, cm^{-1}): *ν*(NH) 3312, 3182; *ν*(C=N) 1573; *ν*(C=S) 876. ^{1}H-NMR (DMSO, ppm): 9.80 (s, 1H, NH); 8.18 (s, 1H, NH), 8.16 (s, 1H, C=N); 7.04-7.78 (m, 9H, Ar-H); ^{13}C-NMR (DMSO, ppm): 171.9 (C=S); 155.99(C=N) 119.9-140.6 (Ar-H).

NiL2: Color: Light Brown. Yield: 83.59%. Melting Point: >300℃. Calc. for $C_{28}H_{28}N_6NiO_2S_2$: C, 57.03; H, 4.17; N, 13.55; S, 10.79. Found: C, 55.74; H, 4.68; N, 13.93; S, 10.63%. M/w: 603.38. IR (KBr, cm^{-1}): *ν*(NH) 3374, 3254; *ν*(OH) 3178; *ν*(C=N) 1609; *ν*(C=S) 847. ^{1}H-NMR (DMSO, ppm): 10.31 (s, 1H, OH); 9.60 (s, 1H, NH); 8.05 (s, 1H, NH), 8.04 (s, 1H, C=N); 6.74-7.57 (m, 9H, Ar-H); ^{13}C-NMR (DMSO, ppm): 173.0 (C=S); 160.5 (OH); 140.8 (C=N), 115.1-134.3 (Ar-H).

NiL3: Color: Dark Brown. Yield: 89.09%. Melting Point: >300℃. Calc. for $C_{28}H_{26}Cl_2N_6NiS_2$: C, 51.63; H, 3.43; N, 12.27; S, 10.58. Found: C, 52.52; H, 4.09; N, 13.13; S, 10.02%. M/w: 640.28. IR (KBr, cm^{-1}): *ν*(NH) 3315, 3126; *ν*(C=N) 1606; *ν*(C=S) 825. ^{1}H-NMR (DMSO, ppm): 9.85 (s, 1H, NH); 8.22 (s, 1H, NH), 8.21 (s, 1H, C=N); 7.05-7.80 (m, 9H, Ar-H); ^{13}C-NMR (DMSO, ppm): 172.5 (C=S); 154.5 (C=N); 120.2-140.5 (Ar-H).

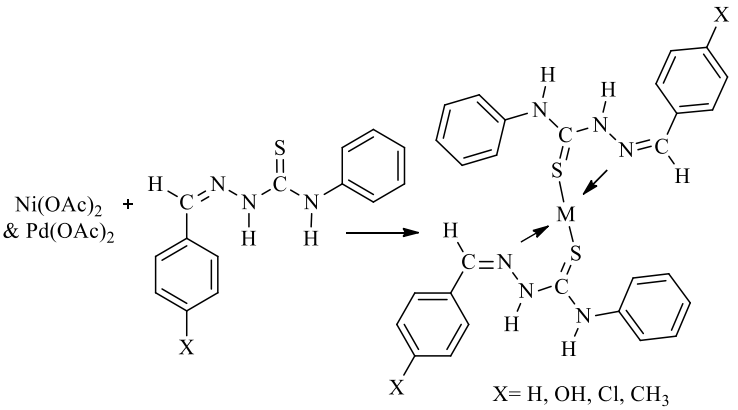

Figure 2. Synthesis of complexes where M= Ni(II) and Pd(II)

NiL4: Color: Light Brown. Yield: 79.17%. Melting Point: >300℃. Calc. for $C_{30}H_{32}N_6NiS_2$: C, 61.01; H, 4.84; N, 13.51; S, 10.11. Found: C, 60.11; H, 5.38; N, 14.02; S, 10.70%. M/w: 599.44. IR (KBr, cm^{-1}): *ν*(NH) 3351, 3310; *ν*(C=N) 1601, *ν*(C=S) 826. ^{1}H-NMR (DMSO, ppm): 9.75 (s, 1H, NH); 8.08 (s, 1H, NH), 8.06 (s, 1H, C=N); 7.04-7.66 (m, 9H, Ar-H); ^{13}C-NMR (DMSO, ppm): 171.7 (C=S); 156.0 (C=N); 119.9-141.8 (Ar-H), 20.9 (CH_3).

PdL1: Color: Brown. Yield: 83.38%. Melting Point: >300℃. Cal. for $C_{28}H_{28}N_6PdS_2$: C, 53.88; H, 3.99; N, 12.61; S, 11.23. Found: C, 54.32; H, 4.56; N, 13.57; S, 10.36%. M/w: 619.11. IR (KBr, cm^{-1}): ν(NH) 3314, 3157; ν(C=N) 1597; *ν*(C=S) 860. ^{1}H-NMR (DMSO, ppm): 9.79 (s, 1H, NH); 8.94 (s, 1H, NH), 8.25 (s, 1H, C=N); 7.08-8.24 (m, 9H, Ar-H); ^{13}C-NMR (DMSO, ppm): 170.5 (C=S); 153.3 (C=N) 120.3-140.7 (Ar-H).

PdL2: Color: Orange. Yield: 78.20%. Melting Point: >300 ℃ . Calc. for $C_{28}H_{28}N_6PdO_2S_2$: C, 51.60; H, 3.81; N, 12.17; S, 10.43. Found: C, 51.65; H, 4.33; N, 12.91; S, 9.85%. M/w: 651.11. IR (KBr, cm^{-1}): *ν*(NH) 3371, 3234; *ν*(OH) 3172; *ν*(C=N) 1611; *ν*(C=S) 821. ^{1}H-NMR (DMSO, ppm): 10.23 (s, 1H, OH); 9.59 (s, 1H, NH); 8.73 (s, 1H, NH), 8.14 (s, 1H, C=N); 6.76-8.13 (m, 9H, Ar-H); ^{13}C-NMR (DMSO, ppm): 169.3 (C=S); 157.2 (OH); 140.9 (C=N), 114.7-134.9 (Ar-H).

PdL3: Color: Brown. Yield: 71.98%. Melting Point: >300 ℃ . Calc. for $C_{28}H_{26}Cl_2N_6PdS_2$: C, 47.74; H, 3.17; N, 11.28; S, 9.87. Found: C, 48.88; H, 3.81; N, 12.22; S, 9.32%. M/w: 688.00. IR (KBr, cm^{-1}): *ν*(NH) 3309, 3118; *ν*(C=N) 1606; *ν*(C=S) 837. ^{1}H-NMR ($CDCl_3$, ppm): 9.87 (s, 1H, NH); 9.61 (s, 1H, NH), 7.59 (s, 1H, C=N); 7.16-7.56 (m, 9H, Ar-H); ^{13}C-NMR ($CDCl_3$, ppm): 170.2 (C=S); 142.5 (C=N); 125.3-139.1 (Ar-H).

PdL4: Color: Light Brown. Yield: 76.58%. Melting Point: >300 ℃ . Calc. for $C_{30}H_{32}N_6PdS_2$: C, 53.88; H, 3.99; N, 12.61; S, 10.90. Found: C, 55.68; H, 4.56; N, 13.57; S, 10.36%. M/w: 647.17. IR (KBr, cm^{-1}): *ν*(NH) 3355, 3307; *ν*(C=N) 1599, *ν*(C=S) 835. ^{1}H-NMR ($CDCl_3$, ppm): 9.99 (s, 1H, NH); 8.55 (s, 1H, NH), 7.93 (s, 1H, C=N); 6.76-7.92 (m, 9H, Ar-H); 2.42 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, ppm): 170.3 (C=S); 159.2 (C=N); 121.2-132.6 (Ar-H), 21.7 (CH_3).

3. Preparation of Antibacterial Studies

The synthesized compounds were screened for their antibacterial activity by the broth microdilution method [6]. The bacterial strains Gram-positive (*Staphylococcus aureus* and *Staphylococcus haemolyticus*) and Gram-negative (*Escherichia coli*, *Staphylococcus typhimurium*, and *Klebsiella pneumoniae*) bacteria were inoculated in MBH medium for 18 hrs at 37℃. The pure compounds were loaded accordingly into wells. The cultures were adjusted to a standardized final OD_{625nm} of 0.10. Then, these adjusted bacterial inoculums were dispensed into each well, except the blank wells. The final volume in each well was 100 μL. The microtiter plates were incubated at 37℃ for 18 hrs and the reading of absorbance at OD_{625nm} was recorded [7]. A standard drug, gentamycin, acted as the positive control while DMSO was used as the negative control. The percentage of inhibition for each sample was calculated [8].

RESULTS AND DISCUSSION

The reaction of thiosemicarbazone with $Ni(OAc)_2$ and $Pd(OAc)_2$ was produced with good yields. The metal complexes were synthesized by reacting the ligands with the metal ions in a 2:1 molar ratio in an ethanolic medium. This was according to the percentage composition of C, H, N, and S obtained from the elemental analysis, which showed good agreement with the experimental data. The chemical structures of all the compounds were confirmed through FT-IR and ^{1}H and ^{13}C-NMR spectroscopies.

1. Fourier-Transform Infrared Spectroscopy

Assignments of chosen signature IR bands gave significant evidence for the formation of thiosemicarbazone and its complexes. A strong band at 1570-1605 cm^{-1} was assigned to *ν*(C=N) stretch of azomethine linkage in the spectra of free thiosemicarbazone. Upon complexation, this band shifted to a higher frequency by 5–13 cm^{-1}. The shifting was attributed to the coordination of azomethine nitrogen with metal and formation of M–N [9]. In the solid state, all synthesized compounds remained in the thione form as indicated by the absence of any bands in the region of 2528-2675 cm^{-1} due to *ν*(C-SH) stretch [10]. This was further corroborated by the presence of *ν*(-NH) stretching in the range of 3291-3370 cm^{-1}, indicating that the thiosemicarbazones remained in their thione form in a solid state. The downward shift of *ν*(C=S) to 11-15 cm^{-1} in the spectra of the complexes suggested that the coordination of sulfur was in thionic form. The sharp bands in the range of 745-785 and 1520-1535 cm^{-1} were due to the aromatic *ν*(C-H) and *ν*(C=C), respectively. In addition, *ν*(N-N) stretching band was observed with medium intensity in the region of 1062-1089 cm^{-1}.

Table 1. The antibacterial activities of the synthesized ligands and their complexes against the tested bacteria strains

Compound	Percentage Inhibition (%)				
	Gram-Positive		Gram-Negative		
	S.A	S.H	E.C	S.T	K.P
L1	9.54	3.15	-	0.80	9.76
NiL1	39.02	2.30	46.70	2.55	40.01
PdL1	41.44	71.91	27.27	20.74	56.54
L2	11.49	0.21	-	2.18	15.56
NiL2	15.31	3.41	7.18	9.83	17.88
PdL2	72.34	82.29	39.84	63.81	86.42
L3	4.17	3.06	-	-	7.72
NiL3	68.38	13.68	35.38	10.03	64.99
PdL3	38.09	40.27	28.16	8.17	45.90
L4	9.40	5.61	-	2.52	9.32
NiL4	52.70	18.43	40.46	8.95	59.02
PdL4	43.28	43.72	18.23	12.37	55.85
+ve control	101.25	100.84	103.06	102.48	100.37
-ve control	5.43	7.23	-	-	4.43

2. ^{1}H and ^{13}C-NMR Spectroscopies

There was no peak observed at 4.0 ppm related to –SH proton resonance. The presence of two single peaks at 11.58-9.18 ppm indicated the presence of –NH protons, signifying that even in a polar solvent such as $CDCl_3$ and DMSO-d_6 they remained in the thione form [11]. Within their respective complexes, the thioamide (–NH) protons signal typically shifted by 0.27–0.99 ppm upfield. The ^{13}C-NMR revealed the presence of two signals at 176.25 and 143.69 ppm assignable to thioamide (C=S) and azomethine carbon (C=N), respectively, in thiosemicarbazone. Therefore, 0.52–3.72 ppm of C=S in thiosemicarbazone observed in complexes suggested thione sulfur coordination. The azomethine carbon signal was shifted downfield in the complexes as a result of the various electron densities, indicating coordination of the nitrogen lone pair to the metal. Other carbons in these compounds resonated almost at the same frequency as those in the experimental thiosemicarbazone.

3. Antibacterial Studies

The antimicrobial screening data (**Table 1**) showed that the ligands exhibited antibacterial properties. The metal chelates had higher inhibitory effects than their parent ligands [12]. The antibacterial activities could be explained by the Overton and Tweedy's chelation theory [13]. The lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials. The Overton's concept of cell permeability suggests that liposolubility is an important factor that controls antibacterial activity. On chelation, due to the overlap of the ligand orbital and partial sharing of the metal ion's positive charge, the polarity of the metal ion will be reduced to a greater extent [14]. It also increases the delocalization of π-electrons across the entire chelate ring and increases the lipophilicity of the complexes. Hence, increased lipophilicity enhances the penetration of the complexes into the lipid membrane and blocks the metal-binding sites in the enzymes of microorganisms [15]. The chelation tends to make the complexes act as potent bactericidal agents, thus killing more of the bacteria than the ligands.

CONCLUSION

The thiosemicarbazone ligands and their metal complexes were characterized by elemental analysis and spectral studies. Based on the above data, the thiosemicarbazone appeared to behave as a bidentate ligand coordinating through the azomethine nitrogen and the thione sulfur atom. The antibacterial studies showed that the complexes exhibited higher antibacterial activities than the free ligands.

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