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A new series of Cu(II) and Ni(II) complexes of NO bidentate 4-NO_2-benzoylhydrazones: synthesis, characterization, and biological studies

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Abstract: A series of new nickel(II) and copper(II) hydrazone complexes **1**–**14**, containing a bidentate NO-donor hydrazone ligand, derived from 4-nitrobenzoylhydrazide and several aliphatic and aromatic aldehydes were synthesized, and their chemical structures were confirmed by means of FT-IR, UV-Vis, ^{1}H and ^{13}C NMR, mass spectral data, conductance measurements, and elemental analyses. The spectral data of the newly synthesized complexes show the formation of a 1:2 [metal:ligand] ratio. The ligands and their complexes were also investigated for their possible in vitro antimicrobial activities against *S. aureus*, *S. epidermidis*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *P. mirabilis*, *E. faecalis*, and *C. albicans*. Among the fourteen new complexes synthesized, complex $Cu(L_4)_2$ (**7**) containing a direct aromatic moiety in the ligand (HL_7) was found to be most active against selected test microorganisms.

Key words: Hydrazone derivatives, bidentate Schiff base ligand, copper complex, nickel complex, antimicrobial activity

1. Introduction

Schiff bases are an important class of nitrogen-donor ligands, pioneered by Hugo Schiff (1834–1915) with the discovery of them in 1864.[1] From the coordination chemistry perspective, hydrazone-type Schiff bases are multidentate ligands, i.e. they have multiple coordinating sites. For example, acylhydrazone Schiff base complexes are extensively investigated in the form of coordination polymers.[2] Analytical chemistry also uses these kinds of compounds as metal-chelating agents.[3] The keto-enol tautomerism is important for forming complexes with usual and unusual properties due to different donation properties and adopting unusual coordination numbers.[4] Using various hydrazides and carbonyl compounds, the resulting ligands and complexes thereby formed by these ligands have suitable structural and functional variations.[5]

Schiff base complexes with transition metals are useful model compounds of the active parts of biologically important complexes. Reported biological and pharmaceutical activities of hydrazone-type Schiff base complexes include antimicrobial, antituberculostatic, anticancer, and antioxidant behaviors.[6,7] Aroylhydrazone complexes of transition metals are also an interesting class with amebicidal, antibacterial, and antileukemic activities with potential activities for antineoplastic, antiviral, and antiinflammatory effects.[8–10] It is reported that the long list of biological activities of Schiff base complexes can also include antifungal activity.[11] Insecticidal and herbicidal activities are other interesting outcomes of Schiff base complexes.[12] Inhibition of lipid peroxidation and enzymes was also reported for this versatile class of compounds.[13–15]

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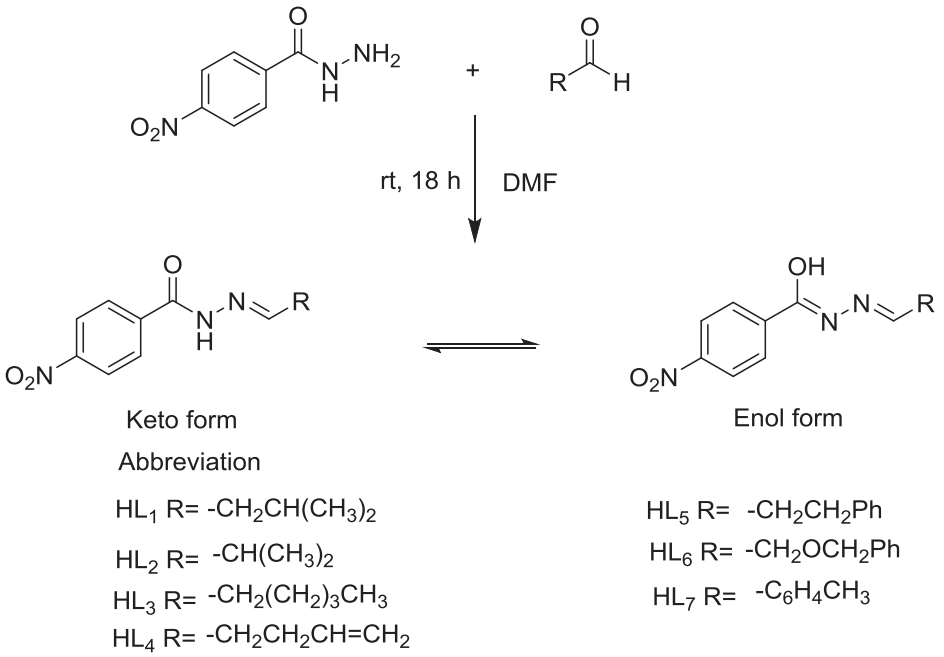
Technological applications of hydrazone-based Schiff base complexes include, but are not limited to, light emission diodes (LEDs),[16] corrosion inhibitors,[17] potentiometric sensors,[18] synthetic intermediates to heterocyclic compounds, and conjugate azomethine-based polymers.[19] With some postsynthetic modification, hydrazone-based Schiff base metal complexes can be varied for further applications such as energy transfer cassettes,[20] fluorogenic or chromogenic probes, and metal complex dyes.[21]

According to the literature, numerous metal complexes were synthesized employing several hydrazone ligands. In these hydrazone ligands, the pyridine nitrogen or hydroxyl moiety bound to the aromatic ring are extra donor groups, as expected.[8,22–26] Singh et al. synthesized metal complexes in which the ligand was 2-acetylthiophene benzoyl hydrazone.[27] The originality of our study is that we have used, for the first time, 4-nitrobenzoylhydrazone as the precursor to the ligand to have a tetradentate fashion without using the aforementioned pyridine or hydroxy moieties. To investigate the relationship between the structure of the metal complex and the biological activity it possesses, we have used aliphatic and aromatic substituted 4-nitrobenzoylhydrazone ligands. The present work intends to describe new candidates of this class, and this work reports the synthesis and spectral characterization (including ^{1}H and ^{13}C NMR, FT-IR, UV-Vis and ESI-MS analyses) of seven hydrazone-based Schiff base ligands (HL_n; n = 1–7) and their Cu(II) and Ni(II) metal complexes **1**–**14** [in the form of $Cu(L_n)_2$ and $Ni(L_n)_2$; n = 1–7], along with an overview of their biological activities against eight well-known microorganisms in the presence of several antibiotics.

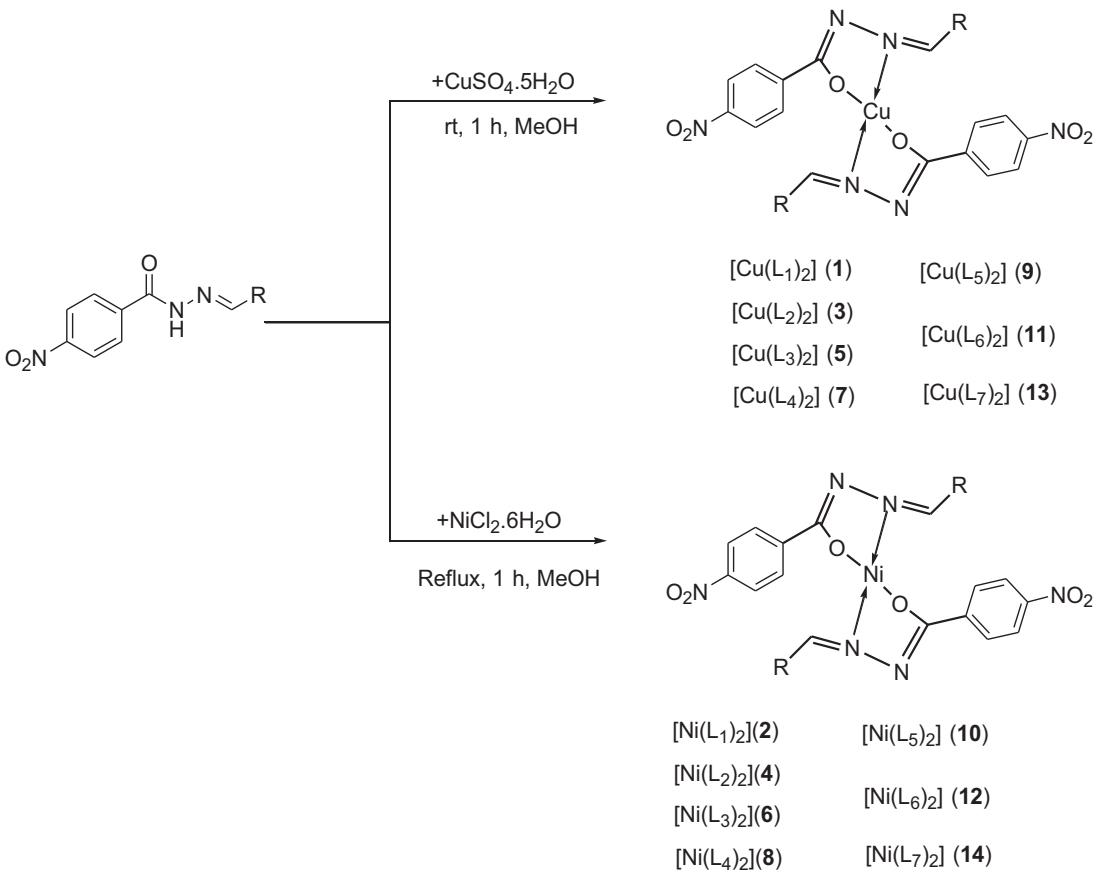
2. Results and discussion

The ligands were synthesized by condensation of 4-nitrobenzoylhydrazide with several aliphatic and aromatic aldehydes (Scheme 1). The reaction of these ligands with metal salts in a 1:2 metal:ligand molar ratio in methanol yielded four coordinate complexes $M(L_n)_2$ (M = Cu(II), Ni(II); n = 1–7) (Scheme 2) and in the complexes, the ligands are enolized and deprotonated during complexation (Scheme 1).[28,29] The presence of the nitro group in the hydrazone is crucial; instead of 4-NO_2-benzoylhydrazones, we have also attempted to synthesize 4-H-benzoylhydrazones. A quick comparison yields the observation that if there is an electron-withdrawing NO_2 group in the aromatic ring, the Cu complexes are formed instantly whereas the Ni complexes require 1 h to complete. The analytical data and physical properties of the ligands and coordination compounds are listed in Table 1. All of the synthesized compounds are quite stable in air at room temperature without decomposing for a long time. The metal complexes have been obtained as colored solids and decompose at the temperature range between 209 and >380 °C without melting. As far as solubility is concerned, the metal complexes **2**, **4**, **6**, and **8** are fully soluble in most common organic solvents such as chloroform, methylene chloride, diethyl ether, and acetone, but other Ni(II) complexes **10**, **12**, and **14** and all Cu(II) complexes **1**, **3**, **5**, **7**, **9**, **11**, and **13** are insoluble in most common organic solvents except DMF and DMSO. The 10^{-3} M solutions of the complexes in DMSO show low molar conductance values in the range of 6.2–11.8 Ω^{-1} cm^2 mol^{-1} (Table 1). These values indicated that all synthesized complexes are nonelectrolytes.[30–32]

Our attempts to obtain single crystals of the compounds failed, so we had to characterize our ligands and their respective metal complexes with FT-IR, UV-Vis, ^{1}H and ^{13}C NMR, ESI-MS, molar conductance, and elemental analyses. The spectral data suggest that all complexes are formed as depicted in Scheme 2 and all assumptions are correct.



Scheme 1. Synthesis of hydrazone ligands [HL_n (n = 1–7)].



Scheme 2. Synthesis and proposed structures of complexes [$M(L_n)_2$] (n = 1–7; M = Ni, Cu).

Table 1. Analytical and physical data of ligands and their complexes.

Compound	Systematic name	Yield (%)	Color	MP (°C)	Found(calcd.) (%)			Λ_m^a (Ω^{-1} cm^2 mol^{-1})
					C	H	N	
HL_1	$C_{12}H_{15}N_3O_3$ (249.26)	97	White	164–166[33]	57.97(57.82)	6.10(6.07)	16.45(16.86)	-
HL_2	$C_{11}H_{13}N_3O_3$ (235.23)	97	White	178–180[33]	56.18(56.13)	5.49(5.57)	17.94(17.86)	-
HL_3	$C_{13}H_{17}N_3O_3$ (263.29)	98	White	146–147[34]	59.45(59.30)	6.62(6.51)	15.83(15.96)	-
HL_4	$C_{12}H_{13}N_3O_3$ (247.24)	96	White	165–166[34]	58.35(58.29)	5.21(5.30)	17.12(16.99)	-
HL_5	$C_{16}H_{15}N_3O_3$ (297.30)	96	White	187–189[33]	64.66(64.64)	5.04(5.09)	14.28(14.13)	-
HL_6	$C_{16}H_{15}N_3O_3$ (313.30)	53	White	142–143[34]	61.31(61.34)	4.85(4.83)	13.46(13.41)	-
HL_7	$C_{15}H_{13}N_3O_3$ (283.28)	96	Yellowish-white	246–247[34]	63.70(63.60)	4.75(4.63)	14.68(14.83)	-
$Cu(L_1)_2$ (**1**)	$C_{24}H_{28}CuN_6O_6$ (560.06)	55	Gray	230	51.28(51.47)	4.93(5.04)	14.89(15.01)	7.2
$Ni(L_1)_2$ (**2**)	$C_{24}H_{28}N_6NiO_6$ (555.21)	39	Yellow-orange	311	51.99(51.92)	4.95(5.08)	15.25(15.14)	6.4
$Cu(L_2)_2$ (**3**)	$C_{22}H_{24}CuN_6O_6$ (532.01)	56	Gray	246	49.70(49.67)	4.63(4.55)	15.84(15.80)	8.3
$Ni(L_2)_2$ (**4**)	$C_{22}H_{24}N_6NiO_6$ (527.16)	33	Yellow-orange	349	50.22(50.12)	4.71(4.59)	15.89(15.94)	10.4
$Cu(L_3)_2$ (**5**)	$C_{26}H_{32}CuN_6O_6$ (588.11)	54	Gray	210	53.15(53.10)	5.59(5.48)	14.18(14.29)	8.6
$Ni(L_3)_2$ (**6**)	$C_{26}H_{32}N_6NiO_6$ (583.26)	31	Yellow-orange	272	53.66(53.54)	5.42(5.53)	14.47(14.41)	6.2
$Cu(L_4)_2$ (**7**)	$C_{24}H_{24}CuN_6O_6$ (556.03)	55	Gray	209	51.89(51.84)	4.49(4.35)	15.06(15.11)	7.8
$Ni(L_4)_2$ (**8**)	$C_{24}H_{24}N_6NiO_6$ (551.18)	21	Yellow-orange	248	52.16(52.30)	4.57(4.39)	15.40(15.25)	11.8
$Cu(L_5)_2$ (**9**)	$C_{32}H_{28}CuN_6O_6$ (656.15)	60	Gray	250	58.64(58.58)	4.41(4.30)	12.69(12.81)	9.9
$Ni(L_5)_2$ (**10**)	$C_{32}H_{28}N_6NiO_6$ (651.29)	46	Yellow-orange	312	59.17(59.01)	4.26(4.33)	13.03(12.90)	9.6
$Cu(L_6)_2$ (**11**)	$C_{32}H_{28}CuN_6O_8$ (688.15)	53	Gray	218	55.66(55.85)	4.17(4.10)	12.29(12.21)	7.7
$Ni(L_6)_2$ (**12**)	$C_{32}H_{28}N_6NiO_8$ (683.29)	35	Yellow-orange	240	56.37(56.25)	4.07(4.13)	12.19(12.30)	11.2
$Cu(L_7)_2$ (**13**)	$C_{30}H_{24}CuN_6O_6$ (628.09)	52	Grayish brown	340	57.39(57.37)	3.88(3.85)	13.29(13.38)	10.6
$Ni(L_7)_2$ (**14**)	$C_{30}H_{24}N_6NiO_6$ (623.24)	48	Yellow	380	57.67(57.81)	3.70(3.88)	13.37(13.48)	9.1

2.1. Elemental analysis

The elemental analysis data of the synthesized ligands and their complexes are given in Table 1. The data show the formation of metal complexes in a 1:2 (M:L) molar ratio. We found that the elemental analysis of the ligands and their complexes were in agreement with the found values.

2.2. FT-IR spectra

Infrared spectroscopy is a very advantageous technique for shedding light on the structure of synthesized ligands and their complexes, and the enolization and subsequent bonding to the metal center has been successfully proven. The FT-IR spectra of the metal hydrazone complexes were compared with that of the free hydrazone ligands in the region of 4000–400 cm^{-1}. The spectrum of the free hydrazone ligands showed the characteristic absorption bands at 3192–3230, 1661, 1553–1589, and 1038–1069 cm^{-1} due to ν(N-H), ν(C=O), ν(C=N), and ν(N-N) vibrations, respectively (Table 2). The bands due to the ν(N-H) and ν(C=O) vibrations of the free ligands were absent for all the complexes **1**–**14**, thus indicating that enolization and deprotonation had taken place prior to coordination.[28,29] This view was confirmed by the detection of a new ν(C-O) band in the range of 1376–1383 cm^{-1} in all metal complexes. We observed that the stretching frequency of C=N slightly shifted to a lower wave number, which supports that the imine nitrogen is involved in coordination to the metal ion in all metal complexes. In addition, the characteristic ν(N-N) stretching frequencies of free ligands were shifted to a lower frequency (35–39 cm^{-1}) due to the involvement of one nitrogen atom of N-N moiety in bonding with metal. In the far FT-IR region, two new bands around 558–594 and 416–491 cm^{-1} in the complexes can

Table 2. FT-IR and UV-vis spectral data of hydrazone ligands and their complexes.

Compound	ν(N-H)	ν(C=O)	ν(C=N)	ν(C-O)	ν(N-N)	ν(M-O)	ν(M-N)	λ_{max} (nm)
HL_1	3200	1661	1569	-	1046	-	-	242, 266
HL_2	3207	1661	1561	-	1038	-	-	267
HL_3	3192	1661	1576	-	1053	-	-	268
HL_4	3223	1661	1553	-	1053	-	-	241, 268
HL_5	3230	1661	1553	-	1053	-	-	239, 267
HL_6	3207	1661	1569	-	1038	-	-	265, 282
HL_7	3207	1661	1571	-	1069	-	-	281, 308
$Cu(L_1)_2$ (**1**)	-	-	1532	1376	1018	590	456	266, 304
$Ni(L_1)_2$ (**2**)	-	-	1547	1377	1027	582	464	254, 294
$Cu(L_2)_2$ (**3**)	-	-	1529	1379	1005	594	460	266, 305
$Ni(L_2)_2$ (**4**)	-	-	1536	1377	1003	591	448	253, 298
$Cu(L_3)_2$ (**5**)	-	-	1523	1383	1012	594	434	265, 307
$Ni(L_3)_2$ (**6**)	-	-	1539	1379	1013	566	453	250, 301
$Cu(L_4)_2$ (**7**)	-	-	1531	1383	1007	577	416	266, 307
$Ni(L_4)_2$ (**8**)	-	-	1528	1379	1028	566	453	256, 307
$Cu(L_5)_2$ (**9**)	-	-	1535	1379	1014	590	491	267, 306
$Ni(L_5)_2$ (**10**)	-	-	1546	1376	1012	567	453	256, 297
$Cu(L_6)_2$ (**11**)	-	-	1543	1377	1013	584	479	269, 307
$Ni(L_6)_2$ (**12**)	-	-	1537	1378	1013	561	451	271, 352
$Cu(L_7)_2$ (**13**)	-	-	1532	1379	1030	581	490	267, 358
$Ni(L_7)_2$ (**14**)	-	-	1530	1379	1022	558	468	281, 318

ν in cm^{-1} ; λ in nm.

be assigned to ν(M-O) and ν(M-N), respectively. Infrared spectroscopy thereby suggests a bidentate ligand coordinating environment through its imine nitrogen and enolized carbonyl oxygen donors in all of the complexes studied. The FT-IR spectra of free hydrazone ligand HL_1 and its Cu(II) complex $Cu(L_1)_2$ **1** and its Ni(II) complex $Ni(L_1)_2$ **2** are shown in Figure 1.

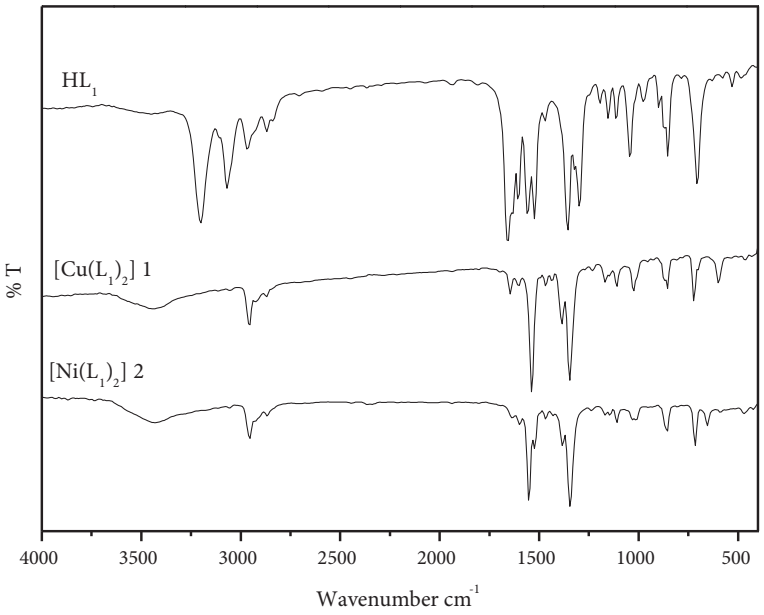


Figure 1. The FT-IR spectra of Schiff base ligand HL_1, $Cu(L_1)_2$ **1**, and $Ni(L_1)_2$ **2**.

2.3. UV-Vis spectra

The UV-Vis spectra of hydrazone ligands [HL_n (n=1–7)] and their complexes (**1**–**14**) were recorded in DMF (10^{-5} M) at room temperature (Table 2). The bands observed in the range of 239–308 nm in the spectra of hydrazone ligands are assigned to the intraligand $\pi \rightarrow \pi^*$ transitions. After complexation, the bands seen in the range of 294–358 nm can be assigned to the n $\rightarrow \pi^*$ transition band of the ligand metal charge transfer transitions.[35,36]

2.4. ^{1}H and ^{13}C NMR spectra

The NMR spectra of the Cu(II) complexes could not be recorded due to the paramagnetic nature of the compounds. The NMR spectra of diamagnetic Ni(II) complexes were recorded in $CDCl_3$ (**2**, **4**, **6**, and **8**) and DMSO-d_6 solutions (**10**, **12**, and **14**) using tetramethylsilane (TMS) as the internal standard. The NMR spectra of the hydrazone ligands were recorded in DMSO-d_6 as the solvent. The chemical shifts in the ^{1}H and ^{13}C NMR spectra of the ligands and corresponding Ni(II) complexes are reported in Table 3. The ^{1}H NMR spectra of all hydrazone ligands [HL_n (n=1–7)] showed one singlet at 11.67–11.70 ppm corresponding to the –NH proton. For the Ni(II) complexes [$Ni(L_n)_2$ (n=1–7)] the disappearance of the –NH proton signals showed that the –NH group of the ligand deprotonates during complex formation. The aromatic ring proton signals of hydrazone ligands appeared as doublet-doublet due to p-substituted phenyl ring protons in the range of 8.52–8.04 ppm. For the Ni(II) complexes, the signals of the aromatic region showed an upfield shift on

Table 3. ^{1}H and ^{13}C NMR spectral data of hydrazone ligands and their nickel(II) complexes.

Compound	^{1}H NMR δ in ppm	^{13}C NMR δ in ppm
HL_1	11.67 (s, 1H), 8.32 (d, J = 10.0 Hz, 2H), 8.08 (d, J = 10.0 Hz, 2H), 7.76 (t, J = 5.0 Hz, 1H), 2.17 (t, J = 7.5 Hz, 2H), 1.86 (sept, J = 6.8 Hz, 1H), 0.94 (d, J = 5.0 Hz, 6H)	161.8, 153.8,149.9, 139.9, 129.7, 124.2, 41.5, 26.9, 22.9
HL_2	11.67 (s, 1H), 8.31 (d, J = 10.0 Hz, 2H), 8.04 (d, J = 10.0 Hz, 2H), 7.68 (d, J = 6.0 Hz, 1H), 2.64–2.52 (m, 1H), 1.12 (d, J = 6.8 Hz, 6H)	164.7, 156.2, 151.3, 140.1, 130.1, 124.7, 33.0, 20.1
HL_3	11.67 (s, 1H), 8.30 (d, J = 8.8 Hz, 2H), 8.04 (d, J = 8.8 Hz, 2H), 7.68 (t, J = 8.8 Hz, 1H), 2.34–2.29 (m, 2H), 1.75–1.69 (m, 2H), 1.50–1.46 (m, 4H), 0.82 (t, J = 7.6 Hz, 3H)	164.6, 156.4, 151.3, 140.1, 130.1, 124.7, 34.8, 29.2, 25.3, 21.8, 13.4
HL_4	11.67 (s, 1H), 8.31 (d, J = 8.8 Hz, 2H), 8.04 (d, J = 8.8 Hz, 2H), 7.66 (t, J = 6.0 Hz, 1H), 5.68–5.66 (m, 1H), 4.91–4.85(m, 2H), 2.69 (q, J = 5.0 Hz, 2H), 2.26 (q, J = 5.0 Hz, 2H)	164.8, 154.2, 141.3, 129.9, 124.9, 117.7, 117.6, 31.51, 29.42
HL_5	11.70 (s, 1H), 8.34 (d, J = 8.8 Hz, 2H), 8.08 (d, J = 8.8 Hz, 2H), 7.79 (t, J = 5.2 Hz, 1H), 7.32–7.16 (m, 5H), 2.86–2.82 (m, 2H), 2.63–2.58 (m, 2H)	161.1, 152.7, 149.1, 140.8, 139.1, 129.9, 128.3, 128.3, 125.9, 123.5, 33.7, 31.8
HL_6	11.70 (s, 1H), 8.34 (d, J = 8.8 Hz, 2H), 8.08 (d, J = 8.8 Hz, 2H), 7.76 (t, J = 5.6 Hz, 1H), 7.30–7.15 (m, 5H), 4.59 (s, 2H), 4.15 (d, J = 10.0 Hz, 2H)	161.2, 152.3, 148.8, 140.2, 138.9, 129.1, 128.2, 128.0, 126.3, 123.3, 71.6, 66.9
HL_7	11.70 (s, 1H), 8.52 (s, 1H), 8.42 (d, J = 8.8 Hz, 2H), 8.21 (d, J = 8.8 Hz, 2H), 7.70 (d, J = 8.8 Hz, 2H), 7.33 (d, J = 8.8 Hz, 2H), 3.14 (s, 3H)	161.5, 149.8, 140.3, 131.8, 130.1, 129.7, 127.8, 124.3, 21.8
$Ni(L_1)_2$ (**2**)	8.12 (d, J = 15.0 Hz, 4H), 7.95 (d, J = 15.0 Hz, 4H), 6.75 (t, J = 5.0 Hz, 2H), 2.66 (t, J = 7.5 Hz, 4H), 1.97 (septet, J = 5.0 Hz, 2H), 1.00 (d, J = 5.0 Hz, 12H)	171.5, 163.8, 148.2, 135.1, 128.3, 122.2, 36.9, 25.8, 21.7
$Ni(L_2)_2$ (**4**)	8.12 (d, J = 10.0 Hz, 4H), 7.96 (d, J = 10.0 Hz, 4H), 6.53 (d, J = 10.0 Hz, 2H), 3.5 (septet, J = 5.0 Hz, 2H), 1.13 (d, J = 5.0 Hz, 12H)	171.5, 169.4, 148.2, 135.1, 128.4, 122.2, 27.2, 18.5
$Ni(L_3)_2$ (**6**)	8.12 (d, J = 5.0 Hz, 4H), 7.96 (d, J = 10.0 Hz, 4H), 6.73 (t, J = 10.0 Hz, 2H), 2.75 (q, J = 10 Hz, 4H), 1.60–1.54 (m, 4H), 1.35–1.32 (m, 8H), 0.88 (t, J = 5.0 Hz, 6H)	171.5, 164.6, 148.2, 135.1, 128.4, 122.2, 30.6, 28.2, 24.7, 21.3, 12.9
$Ni(L_4)_2$ (**8**)	8.11 (d, J = 5.0 Hz, 4H), 7.95 (d, J = 10.0 Hz, 4H), 6.73 (t, J = 5.0 Hz, 2H), 5.89–5.87 (m, 2H), 5.12–5.06 (m, 4H), 2.87 (q, J = 5.0 Hz, 4H), 2.34 (q, J = 5.0 Hz, 4H)	172.9, 164.7, 149.5, 136.5, 129.7, 123.5, 116.7, 116.5, 30.1, 28.6
$Ni(L_5)_2$ (**10**)	8.32 (d, J = 5.0 Hz, 4H), 8.08 (d, J = 5.0 Hz, 4H), 7.31–7.26 (m, 10H), 7.19 (t, J = 5.0 Hz, 2H), 2.83 (t, J = 7.4 Hz, 4H), 2.60 (q, J = 5.0 Hz, 4H)	172.5, 163.9, 149.8, 135.4, 128.9, 128.6, 128.2, 126.5, 123.5, 29.3, 24.9
$Ni(L_6)_2$ (**12**)	8.21 (d, J = 5.0 Hz, 4H), 8.02 (d, J = 5.0 Hz, 4H), 7.32–7.16 (m, 10H), 6.68 (t, J = 5.0 Hz, 2H), 4.65 (s, 4H), 4.23 (d, J = 10.0 Hz, 4H)	172.9, 163.8, 149.9, 134.7, 128.5, 128.4, 128.1, 127.1, 123.3, 72.7, 67.1
$Ni(L_7)_2$ (**14**)	8.47 (s, 2H), 8.36 (d, J = 5.0 Hz, 4H), 8.16 (d, J = 10.0 Hz, 4H), 7.64 (d, J = 5.0 Hz, 4H), 7.29 (d, J = 5.0 Hz, 4H), 3.17 (s, 6H)	172.3, 162.3, 149.9, 140.9, 131.9, 130.2, 129.9, 127.9, 124.3, 21.7

complexation compared with the free ligands. After complex formation, electron delocalization of the ligand backbone alters essentially and consequently some coupling constant (J) values of Ni(II) complexes are high for aromatic protons. In the aromatic region, the differences between the ligand and the complex were observed to be significant due to the changing electronic nature of the structures**.** The proton signals of (=N-CH) groups were observed at 7.79–7.76 ppm as triplet signals for the ligands HL_1, HL_3, HL_4, HL_5, and HL_6. From the ^{1}H NMR spectral data (Table 3) for the Ni(II) complexes **2**, **6**, **8**, **10**, and **12** the proton signals of the azomethine group of the above-mentioned ligands were shifted upfield due to the coordination of the azomethine nitrogen.[37] For the HL_2 hydrazone ligand, the proton signal of the azomethine group was observed as a doublet signal at 7.58 ppm. The proton signal for the (=N-CH) group in the complex $Ni(L_2)_2$ **4** showed an upfield shift at 6.53 ppm on complexation compared with the free ligand HL_2. The signal of azomethine proton of HL_6 was observed at 8.52 ppm. This signal in the corresponding complex $Ni(L_6)_2$ **14** appeared at 8.47 ppm.

In the ^{13}C NMR spectrum of hydrazone ligands, carbonyl carbon signals appeared in the range of 164.8–161.1 ppm. The signals of azomethine carbons were observed in the range of 156.4–149.8 ppm. For all the Ni(II) complexes, the signals of carbonyl and azomethine carbons showed a downfield shift on complexation with nickel metal ion compared with the free ligands. Representative ^{1}H and ^{13}C NMR spectra for the HL_1 and $Ni(L_1)_2$ (**2**) are shown in Figures 2 and 3. FT-IR and ^{1}H and ^{13}C NMR spectral data agree well with the suggested structures of the Ni(II) complexes, respectively.

2.5. Mass spectra

The mass spectral studies for the metal complexes were investigated. ESI-(+) mass spectrometry of all complexes indicates that there are M^+ and M^++2 isotope peaks, which are consistent with the proposed structures (Table 4). Examples of mass spectra of $Cu(L_6)_2$ **11** and $Ni(L_6)_2$ **12** are provided in Figure 4.

Table 4. The mass fragmentations of complexes.

Compounds	Mass spectra (ESI) m/z
$Cu(L_1)_2$ (**1**)	560.37 (M^+, 100%), 562.39 (M^++2, 60%)
$Ni(L_1)_2$ (**2**)	555.38 (M^+, 100%), 557.67 (M^++2, 57%)
$Cu(L_2)_2$ (**3**)	532.0 (M^+, 100%), 534.20 (M^++2, 30%)
$Ni(L_2)_2$ (**4**)	527.0 (M^+, 100%), 529.0 (M^++2, 41%)
$Cu(L_3)_2$ (**5**)	588.0 (M^+, 74%), 590.0 (M^++2, 30%)
$Ni(L_3)_2$ (**6**)	583.07 (M^+, 100%), 585.09 (M^++2, 42%)
$Cu(L_4)_2$ (**7**)	556.15 (M^+, 100%), 558.11 (M^++2, 31%)
$Ni(L_4)_2$ (**8**)	551.10 (M^+, 100%), 553.10 (M^++2, 43%)
$Cu(L_5)_2$ (**9**)	702.15 (M^++2Na, 100%), 703.14 (47%)
$Ni(L_5)_2$ (**10**)	651.10 (M^+, 100%), 653.10 (M^++2, 48%)
$Cu(L_6)_2$ (**11**)	687.90 (M^+, 100%), 689.90 (M^++1, 44%)
$Ni(L_6)_2$ (**12**)	683.0 (M^+, 100%), 685.0 (M^++2, 48%)
$Cu(L_7)_2$ (**13**)	628.10 (M^+, 100%), 630.20 (M^++2, 56%)
$Ni(L_7)_2$ (**14**)	623.13 (M^+, 100%), 625.10 (M^++2, 43%)

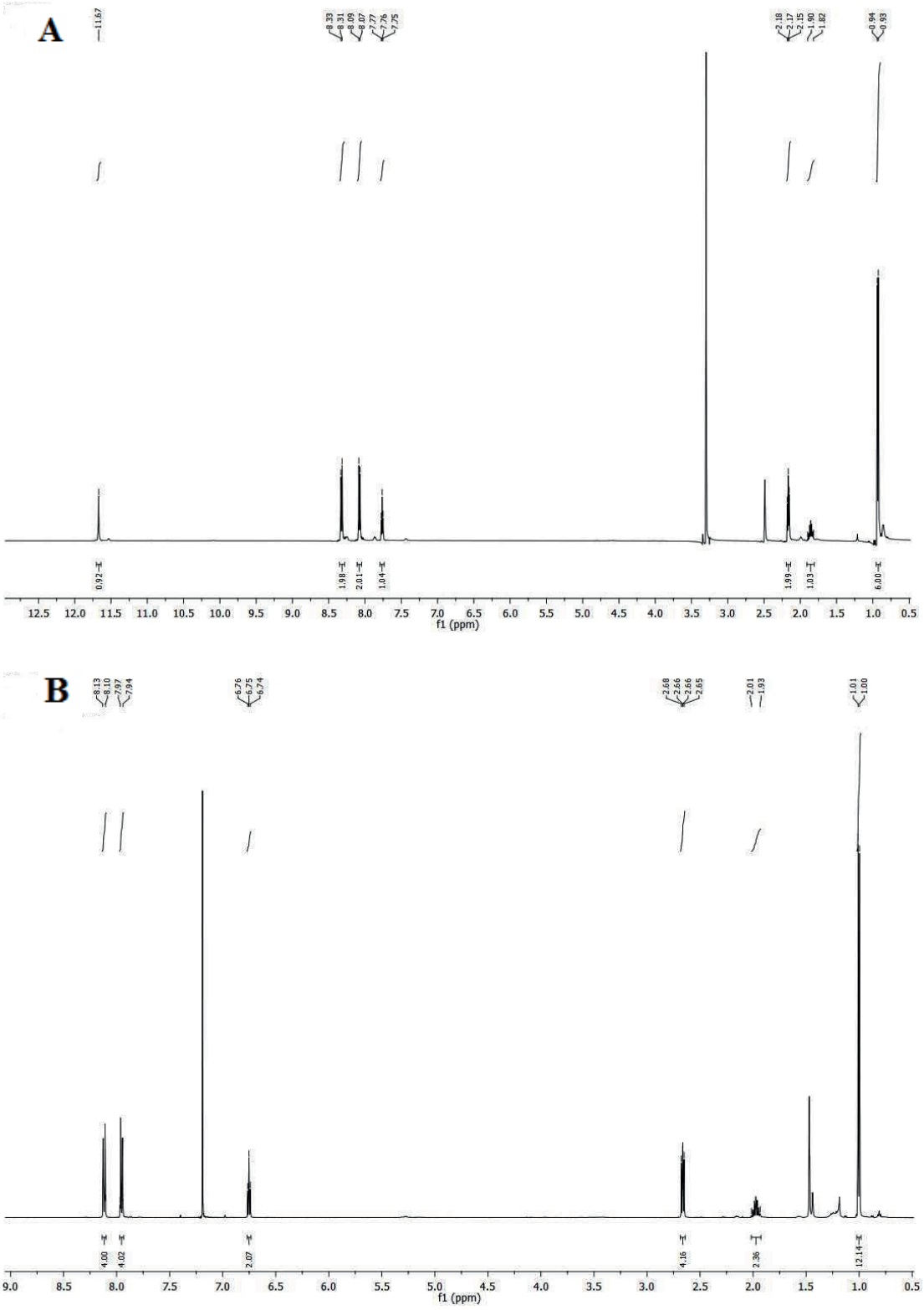


Figure 2. The ^{1}H NMR spectra of the HL_1 (**A**) and its Ni(II) complex $Ni(L_1)_2$ (**2**) (**B**).

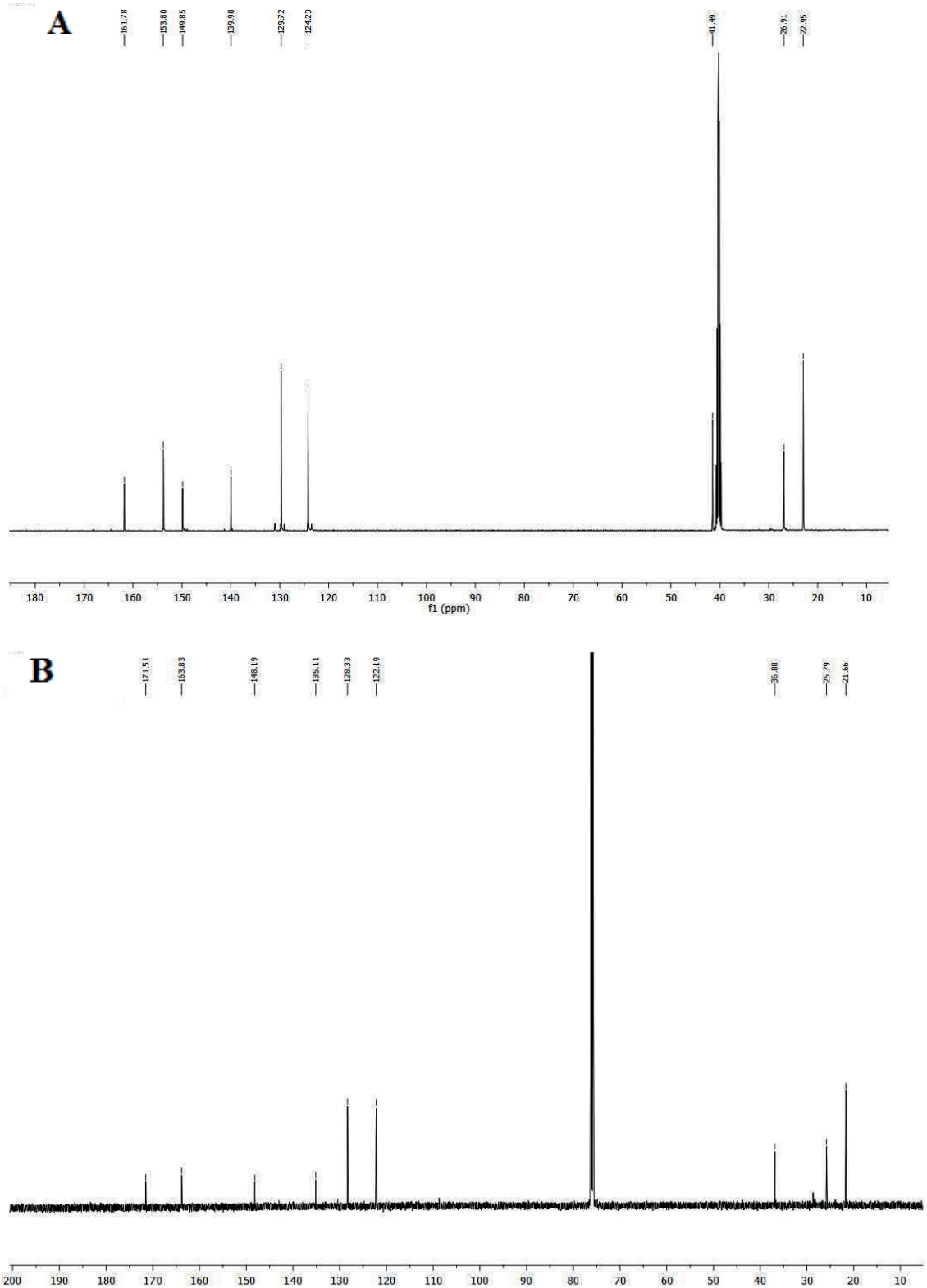


Figure 3. The ^{13}C NMR spectra of the HL_1 (**A**) and its Ni (II) complex $Ni(L_1)_2$ (**2**) (**B**).

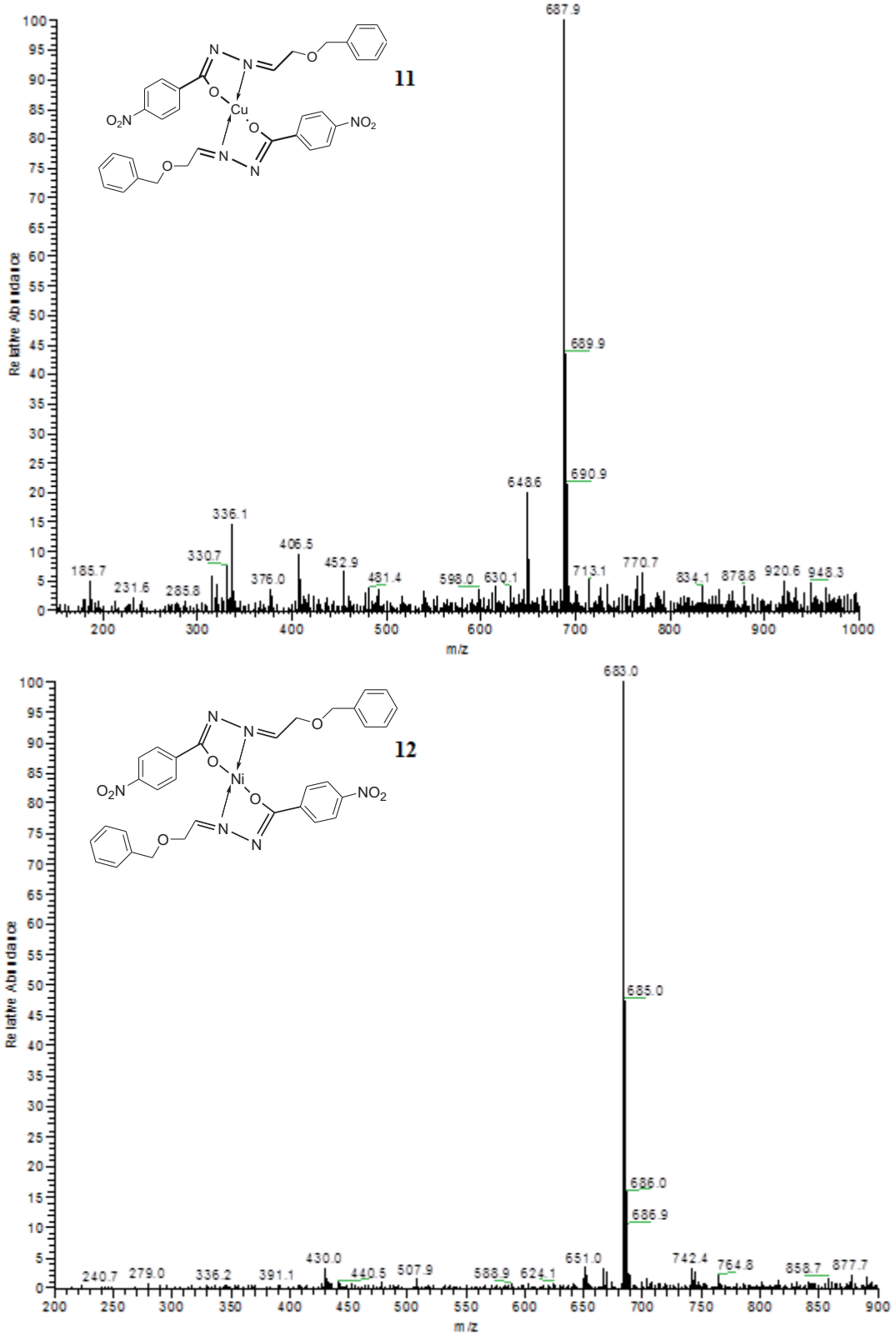


Figure 4. The ESI-MS spectra of $Cu(L_6)_2$ (**11**) and $Ni(L_6)_2$ (**12**) complexes.

2.6. Antimicrobial results

To investigate the relationships between chemical structure and antimicrobial activity, we have designed and synthesized novel Cu(II) and Ni(II) complexes. The minimum inhibitory concentration (MIC) of the synthesized compounds was determined against bacteria and the yeast *C. albicans* using a standard broth dilution technique. All the MIC results of the tested compounds are given in Table 5. According to the results, compounds were able to show inhibition against the selected microorganisms with MIC values of between 39.06 and 625 mg/L.

Among the synthesized compounds, $Cu(L_4)_2$ (**7**) and $Cu(L_7)_2$ (**13**) for *S. epidermidis* and HL_7 and $Cu(L_7)_2$ (**13**) for *E. faecalis* were found to be the most active derivatives at MIC values of 39.06 mg/L. Additionally, for gram-negative rods, the most significant antibacterial activity with MIC value of 312.5 mg/L was found against *E. coli*. The compounds $Ni(L_5)_2$ (**10**), $Cu(L_7)_2$ (**13**), and $Ni(L_7)_2$ (**14**) showed good antifungal effect against *C. albicans* at MIC values of 39.06 mg/L. From the results (Table 5), the metal complexes **7** and **13** for *S. epidermidis*, **13** for *E. coli*, and **10**, **13**, and **14** for *C. albicans* are found to be more effective than their free ligands under identical experimental conditions. Among the fourteen new complexes synthesized, complex $Cu(L_4)_2$ (**7**) containing direct aromatic moiety in the ligand (HL_7) was found to be most active against various test microorganisms as compared to the other complexes synthesized.

2.7. Conclusions

In this study, the synthetic procedure, spectral characterization, and biological activity of seven hydrazone-based Schiff base ligands and their new Cu(II) and Ni(II) complexes are reported. This article reports fourteen new complexes of a previously synthesized 4-nitrobenzoylhydrazide ligand series and spectral characterization results are harmonious with the assumed structures. The novelty of our study is that we used 4-nitrobenzoylhydrazone derivatives as the ligand to have a tetradentate fashion without using extra donor groups from the substituents. In all of those cases, the bonding mode is straightforward, and enolization and subsequent coordination of the ligand are obvious. Ligands and their complexes were tested as new antibacterial and antifungal agents. Among these complexes, $Cu(L_4)_2$ (**7**) was found to be the most active as compared to other complexes.

The syntheses of 4-H-benzoylhydrazide Schiff base complexes are underway, but the first observation about them was that it was harder to synthesize the relevant complexes without a nitro group, which seemingly facilitates the formation of complexes.

3. Experimental

3.1. Materials and methods

All of the reagents were obtained from commercial suppliers unless otherwise stated. The solvents utilized for chromatography were of technical grade and distilled prior to use. Thin layer chromatography (TLC) was carried out on Merck aluminum support plates (silica gel 60 F_{254}). Visualization was achieved under UV light (254 and 330 nm). Column chromatography was performed using Merck 60 silica gel (particle size 0.2–0.063 mm). ^{1}H NMR spectra were recorded at 500 MHz on a Varian Inova 500 spectrometer. ^{13}C NMR spectra were similarly recorded at 125 MHz. ^{1}H NMR and ^{13}C NMR chemical shifts (δ) were reported in parts per million (ppm) relative to respective residual solvent signals in $CDCl_3$ (δ = 7.26 ppm, 77.16 ppm), or in DMSO-d_6 (δ = 2.5 ppm, 39.52 ppm). Coupling constants (J) are reported in hertz (Hz) and refer to apparent multiplicities. The following abbreviations are used for the multiplicities: s: singlet, d: doublet, t: triplet, q: quartet. Mass spectra (MS-ESI, 70 eV) were conducted on a Thermo Finnigan spectrometer. FT-IR spectra were recorded

Table 5. In vitro antibacterial activity of synthesised ligands and their complexes.

	MIC Values (mg/L)										
	1	$Cu(L_1)_2$	$Ni(L_1)_2$	HL_2	$Cu(L_2)_2$	$Ni(L_2)_2$	HL_3	$Cu(L_3)_2$	$Ni(L_3)_2$	HL_4	$Cu(L_4)_2$
S. aureus ATCC 29213	625	-	625	625	625	625	625	625	625	312.5	625
S .epidermidis ATCC 12228	-	312.5	625	625	156.2	312.5	-	625	-	625	39.06
E. faecalis ATCC 29212	625	625	625	-	625	625	-	625	625	625	625
P. aeruginosa ATCC 27853	-	-	-	-	-	-	-	-	-	-	-
E.coli ATCC 25922	625	-	625	625	625	625	625	625	-	625	312.5
K. pneumoniae ATCC 4352	-	625	-	-	-	-	-	-	-	625	-
P. mirabilis ATCC 14153	-	625	625	-	-	-	-	-	625	-	625
C. albicans ATCC 10231	312.5	-	-	312.5	-	-	312.5	312.5	312.5	312.5	-

Table 5. Continued.

	MIC Values (mg/L)										
	$Ni(L_4)_2$	HL_5	$Cu(L_5)_2$	$Ni(L_5)_2$	HL_6	$Cu(L_6)_2$	$Ni(L_6)_2$	HL_7	$Cu(L_7)_2$	$Ni(L_7)_2$	Reference antibiotics (mg/L)
S. aureus ATCC 29213	625	625	625	-	625	312.5	625	312.5	312.5	312.5	Cefuroxime-Na 1.2
S .epidermidis ATCC 12228	312.5	-	312.5	312.5	312.5	312.5	78.1	312.5	39.06.	156.2	Cefuroxime 9.8
E. faecalis ATCC 29212	625	625	312.5	625	625	312.5	78.1	39.06	39.06	78.1	Amikacin 128
P. aeruginosa ATCC 27853	-	-	-	-	-	312.5	-	-	-	-	Ceftazidime 2.4
E.coli ATCC 25922	312.5	625	-	-	625	625	625	-	312.5	625	Cefuroxime-Na 4.9
K. pneumoniae ATCC 4352	-	-	-	-	625	-	-	312.5	625	-	Cefuroxime-Na 4.9
P. mirabilis ATCC 14153	625	625	625	625	-	312.5	312.5	-	-	-	Cefuroxime-Na 2.4
C. albicans ATCC 10231	78.1	312.5	-	39.06	156.2	-	312.5	-	39.06	39.06	Clotrimazole 4.9

on a Mattson 1000 spectrometer in the 4000–400 cm^{-1} range, in the form of KBr pellets at room temperature. The molar conductance of 10^{-3} M solutions of the complexes in DMSO was measured at room temperature on a digital WPA CMD 750 conductivity meter. UV-Vis spectra were recorded using a Shimadzu UV-1650 PC spectrophotometer. Melting points were measured with a Büchi Melting Point B-540 apparatus. The elemental analyses were determined on a Thermo Finnigan Flash EA 1112 Series Elemental Analyzer.

3.2. Syntheses

3.2.1. Synthesis of hydrazones

The hydrazone ligands HL_n (n = 1–7) were prepared as follows: the aldehyde (1.20 equiv., 6.0 mmol) was added to a solution of 4-nitrobenzoylhydrazide (1.0 equiv., 5.0 mmol) in anhydrous N,N-dimethylformamide (20 mL) at room temperature and the mixture was stirred for 18 h at the same temperature as shown in Scheme 1. Then the reaction mixture was treated with water and extracted with ethyl acetate (3 × 40 mL). The combined organic layers were washed with water (2 × 80 mL) and saturated brine (40 mL) and dried over anhydrous sodium sulfate. Crude hydrazone obtained after removing ethyl acetate in vacuo was purified by flash column chromatography using ethyl acetate and hexane to obtain pure hydrazones.

3.2.2. Synthesis of metal complexes (1–14)

The complexes were synthesized by reacting 5 mL of methanolic solutions of each metal(II) salt ($CuSO_4.5H_2O$ or $NiCl_2.6H_2O$; 1 mmol), sodium acetate (2 mmol), and 10 mL of methanolic solutions of the ligand (2 mmol) separately in a 1:2 molar ratio (M:L) in a round-bottomed flask (Scheme 2). The Ni(II) complexes were formed as insoluble precipitates after refluxing the reaction mixture for 1 h, whereas the Cu(II) complexes were precipitated immediately during stirring of the reaction mixture on a magnetic stirrer at room temperature. The resulting precipitates were filtered off, washed several times with ethanol, and dried under vacuum. The purity of the product was determined by TLC and elemental analysis.

3.3. Antimicrobial activity

Antimicrobial activities against *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, *Enterococcus faecalis* ATCC 29212, and *Candida albicans* ATCC 10231 were determined by the microbroth dilution technique using the Clinical Laboratory Standards Institute (CLSI) recommendations.[38,39] Mueller–Hinton broth for bacteria and RPMI-1640 medium for the yeast strain were used as the test media. Serial twofold dilutions ranging from 5000 mg/L to 4.8 mg/L were prepared in the media. The inoculum was prepared using a 4–6 h broth culture of each bacteria and 24 h culture of yeast strains adjusted to a turbidity equivalent of a 0.5 McFarland standard, diluted in broth media to give a final concentration of 5×10^5 cfu/mL for bacteria and 0.5×10^3 to 2.5×10^3 cfu/mL for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation. The trays containing Mueller–Hinton broth were incubated at 35 °C for 18–20 h while the trays containing RPMI-1640 medium were incubated at 35 °C for 46–50 h. The MIC was defined as the lowest concentration of compound giving complete inhibition of visible growth. As a control, antimicrobial effects of the solvents were investigated against test microorganisms. The results were evaluated according to the values of the controls.

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