

Cu(II) Complexes with Nitrogen-Oxygen Donor Ligands: Synthesis and Biological Activity

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Abstract: The ligand N'-acetyl-4-(4-X-phenylsulfonyl)benzohydrazide (**I**), X=H, Cl, Br, forms complexes [Cu(L-H)₂] and [Cu(L-H)₂(NH₃)₂] which have been characterized by elemental analyses, magnetic moments, molar conductance, electronic, ESR and IR spectral measurements. Coordination area and metal ion geometry varies with the working conditions: different salts of Cu(II), pH medium and solvents. Room temperature ESR spectra of Cu(II) complexes yield $\langle g \rangle$ values characteristic of distorted octahedral and square planar geometry. The new compounds were also assayed by the agar disk diffusion method for antibacterial action against *S. epidermidis*, *B. subtilis*, *B. cereus*, *P. aeruginosa* and *E. coli*.

Keywords: diacylhydrazines, Cu(II) complexes, spectral data, antibacterial activity

1. Introduction

The coordination chemistry of nitrogen-oxygen donor ligands is an interesting area of research [1]. Complexes of substituted hydrazine such as hydrazides, thiosemicarbazides, hydrazones and diacylhydrazines are of general interest as models for bioinorganic processes [2-6]. A search of literature reveals that no more work has been done on complexation of diacylhydrazines. It is well known that these compounds containing two amide moieties which have a strong ability to form metal complexes (Fig. 1). This ligand system shows the keto-enol tautomerism and can act as mononegative bidentate or mononegative tridentate [7].

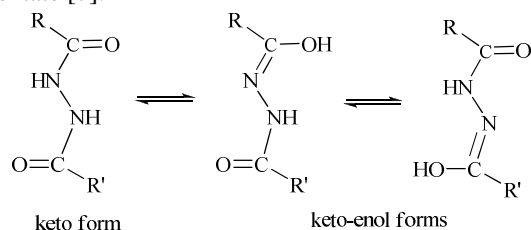


Figure 1. Keto-enol tautomerism of diacylhydrazine

Keeping this observation in view, in this paper we have synthesized new complexes of Cu(II) with N'-acetyl-4-(4-X-phenylsulfonyl)benzohydrazide (**I**), X=H, Cl, Br as ligand (Fig. 2) and we described a preliminary investigation of their structure.

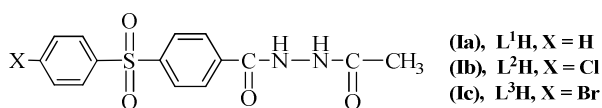


Figure 2. Structure of the ligand

2. Experimental

2.1. Synthesis of the complex

We prepared the Cu(II) complexes with anhydrous CuCl₂ and CuSCN salts. In the first case, an ethanolic solution of metallic ion salt (1 mmol/5 mL ethanol) was mixed with stirring with a hot clear ethanolic solution of the ligand (**I**) (2 mmol/20 mL ethanol). After refluxing the solution for 2 h, Na₂CO₃ was added until the pH=8-8.5 and the obtained precipitate (**1**), (**3**), or (**5**) was filtered under vacuum, washed successively with water, hot ethanol, cold ethanol and diethylether and finally dried under vacuum.

In the second case, a blue solution of CuSCN (1 mmol) in 5 mL NH₃ was mixed with stirring with a hot clear ethanolic solution of the ligand (**I**) (2 mmol/20 mL ethanol). The obtained mixture was refluxed 2.5-3 h and the obtained precipitate (**2**), (**4**), or (**6**) was filtered under vacuum, washed successively with water, hot ethanol, cold ethanol and diethylether and finally dried under vacuum.

2.2. Physical measurements

The reagent used in this work were commercial products (Merck or Chimopar Bucharest). Chemical elemental analyses were done with Carlo-Erba La-118 microdosimeter (for C, H and N) and an AAS-1N Carl-Zeiss-Jena spectrometer (for metallic ions).

Molar conductivities of the complexes were measured in DMSO (3·10⁻³M), at room temperature using OK-102/1 Radelkis conductivity instrument.

Electronic spectra were recorded using a Jasco V-550 spectrophotometer, in diffuse reflectance mode, using MgO dilution matrices.

IR spectra (KBr pellets) were recorded in the 4000-400 cm^{-1} region with BioRad FTS 135, spectrophotometer. ESR spectra were registered on a ART-6-IFIN type spectrophotometer, equipped with a field modulation nit at 100 kHz. The measurements were done in the X band, on micro-crystalline powder at room temperature using DPPH as standard.

The melting points were determinate with Boetius apparatus and are uncorrected.

3. Results and discussion

All synthesized complex combinations have melting points higher than 300°C. The elemental analyses data (wich confirm the molar combination report M:L 1:2) along with some physical properties of the complexes are reported in Table 1.

All the solid complexes are stable in air, are soluble in DMF and DMSO, but insoluble in other organic solvents.

The molar conductivities of the complexes in 10^{-3} M DMSO were found to be 2.74-4.70 $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$ (Table 1) and suggesting their non-electrolytic nature [8].

3.1. Infrared spectra

The comparative IR spectral study of the ligands L^1H , L^2H and L^3H and their Cu(II) complexes reveals the coordination mode of the ligand during the complex formation. The IR spectrum of the ligands (Table 2) shows bands at 3222-3440 cm^{-1} due to the presence of two NH groups and at 1527-1711 cm^{-1} which are assigned to amide I, II, III vibrations [9]. The disappearance of one ν_{NH} band of ligand in the IR spectra of complexes (1), (3) and (5) and the appearance of new band in the 1569 - 1585 cm^{-1} range

assignable to C=N group suggesting removal of the hydrazinic proton via enolisation and participation of the enolic oxygen in bonding. In the spectra of the complexes (2), (4), (6) the broad band at $\sim 3400 \text{ cm}^{-1}$, together with new band at $\sim 693 \text{ cm}^{-1}$ indicating the presence of coordinating NH_3 . The nature of the metal-ligand bonding is confirmed by the newly formed bands at $\sim 530 \text{ cm}^{-1}$ and 420 cm^{-1} in the spectra of complexes, which is tentatively assigned to Cu-O and Cu-N vibrations [8-10].

3.2. Electronic spectra

The electronic spectra of the Cu(II) complexes (Table 3) are compared with those of the ligands. Two bands appeared at 38610-39840 cm^{-1} and 28735-30487 cm^{-1} , which can be assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively, in all the ligands [11]. The complexes (1), (3) and (5) show two bands in the region 17980-18835 cm^{-1} and 15960-16339 cm^{-1} which can be assigned to d-d transitions of the metal ions (${}^2B_{1g} \rightarrow {}^2A_{1g}$; ${}^2B_{2g}$) and which strongly favour square-planar geometry around the central metal ion [12]. In addition, the μ_{eff} values for this compounds, in range 1.74-1.84 BM, indicative of one unpaired electron per Cu(II) ion and suggesting that the square-planar geometry [13]. The electronic spectra of complexes (2), (4), (6) showed two or three low-energy bands at 10437-16420 cm^{-1} and a strong high-energy at 23310-28328 cm^{-1} . The low-energy band in the position typically is expected for an octahedral distorted configuration and may be assigned to the transitions $d_{x^2-y^2} \rightarrow d_{xz,yz}$; d_{z^2} ; d_{xy} [12, 14]. The strong high-energy band is assigned to metal \rightarrow ligand charge transfer. The observed magnetic moment values for the complexes (2), (4), (6) are 2.18, 2.21 and 2.14 BM, respectively and supporting the D_{4h} geometry.

TABLE 1. Elemental analysis data and some physical characteristics of the complexes (1)-(6)

No.	Compound	Molecular formula (M, uam)	Colour	Yield (%)	Elemental analyses calc. (found)				Λ ($\Omega^{-1}\text{cm}^2\text{mol}^{-1}$)	μ_{eff} (BM)
					% C	% H	% N	% Cu		
(1)	[Cu(L ¹ -H) ₂]	CuC ₃₀ H ₂₆ N ₄ O ₈ S ₂ (698.22)	green	79	51.61 (51.66)	3.75 (3.80)	8.02 (8.07)	9.10 (9.03)	3.70	1.81
(2)	[Cu(L ¹ -H) ₂ (NH ₃) ₂]	CuC ₃₀ H ₃₂ N ₆ O ₈ S ₂ (732.28)	dark green	78	49.20 (49.27)	4.40 (4.45)	11.48 (11.54)	8.68 (8.62)	2.74	2.18
(3)	[Cu(L ² -H) ₂]	CuC ₃₀ H ₂₄ Cl ₂ N ₄ O ₈ S ₂ (767.11)	green	65	46.97 (47.02)	3.15 (3.21)	7.30 (7.34)	8.28 (8.20)	4.20	1.84
(4)	[Cu(L ² -H) ₂ (NH ₃) ₂]	CuC ₃₀ H ₃₀ Cl ₂ N ₆ O ₈ S ₂ (801.17)	dark green	83	44.97 (45.02)	3.77 (3.81)	10.49 (10.53)	7.93 (7.86)	3.89	2.21
(5)	[Cu(L ³ -H) ₂]	CuC ₃₀ H ₂₄ Br ₂ N ₄ O ₈ S ₂ (856.01)	green	81	42.08 (42.13)	2.83 (2.88)	6.55 (6.59)	7.42 (7.37)	3.67	1.74
(6)	[Cu(L ³ -H) ₂ (NH ₃) ₂]	CuC ₃₀ H ₃₀ Br ₂ N ₆ O ₈ S ₂ (890.07)	dark green	69	40.48 (40.53)	3.40 (3.44)	9.44 (9.49)	7.14 (7.07)	4.70	2.14

TABLE 2. IR bands and their assignments for *N'*-acetyl-4-[(4-*X*-phenyl)sulfonyl]benzohydrazide (Ia-c) and its complexes (1)-(6)

No.	Compound	ν_{NH}	ν_{CH} (aryl)	ν_{CH_3} as, sym	amide I	amide II	amide III	$\nu_{\text{C=N}}$	ν_{SO_2} as, sym	$\nu_{\text{C-O}}$	$\nu_{\text{N-N}}$	$\nu_{\text{C-X}}$	$\nu_{\text{Cu-O}}$ Cu-N
(Ia)	(L ¹ H)	3440 3255	3091	2925 2854	1711	1653	1527	-	1325 1297 1160	-	1017	-	-
(1)	[Cu(L ¹ -H) ₂]	3347	3089	2916 2858	1622	1515	1487	1585	1320 1295 1154	1105	1013	-	529 417
(2)	[Cu(L ¹ -H) ₂ (NH ₃) ₂]	3446 3348	3086	2927 2853	1635	1516	1488	1575	1319 1154 1293	1034	1015	-	529 427
(Ib)	(L ² H)	3353 3222	3091	2923	1692	1652	1544	-	1326 1304 1157	-	1013	764	-
(3)	[Cu(L ² -H) ₂]	3214	3093	2922 2860	1615	1532	1491	1569	1321 1284 1159	1091	1014	768	527 412
(4)	[Cu(L ² -H) ₂ (NH ₃) ₂]	3460 3282	3088	2921	1617	1527	1490	1569	1321 1285 1159	983	1014	768	531 430
(Ic)	(L ³ H)	3423 3283	3091	2920 2881	1690	1646	1531	-	1327 1292 1157	-	1009	568	-
(5)	[Cu(L ³ -H) ₂]	3268	3086	2923 2854	1635	1517	1487	1572	1322 1295 1154	1104	1010	576	529 417
(6)	[Cu(L ³ -H) ₂ (NH ₃) ₂]	3446 3290	3089	2917 2896	1628	1515	1487	1572	1322 1295 1153	978	1010	576	529 417

TABLE 3. Electronic absorption spectral data of *N'*-acetyl-4-[(4-*X*-phenyl)sulfonyl]benzohydrazide Cu(II) complexes (1)-(6)

No.	Compound	Band max (cm ⁻¹)	Assignments	Geometry
(1)	[Cu(L ¹ -H) ₂]	38910, 28735 23923 17980, 15960	intraligand charge transfer ² B _{1g} → ² A _{1g} ; ² B _{2g}	square-planar (D _{2h})
(2)	[Cu(L ¹ -H) ₂ (NH ₃) ₂]	39062, 30487 28328 16284, 14265	intraligand charge transfer d _{x²-y²} → d _{xz} ; d _{z²}	distorted octahedral (D _{4h})
(3)	[Cu(L ² -H) ₂]	39840, 30210 23923 18361, 16181	intraligand charge transfer ² B _{1g} → ² A _{1g} ; ² B _{2g}	square-planar (D _{2h})
(4)	[Cu(L ² -H) ₂ (NH ₃) ₂]	38167, 30120 23310 16420, 13950	intraligand charge transfer d _{x²-y²} → d _{xz} ; d _{z²}	distorted octahedral (D _{4h})
(5)	[Cu(L ³ -H) ₂]	39840, 28735 23640 18335, 16339	intraligand charge transfer ² B _{1g} → ² A _{1g} ; ² B _{2g}	square-planar (D _{2h})
(6)	[Cu(L ³ -H) ₂ (NH ₃) ₂]	38610, 29411 23474 16129, 14104, 10437	intraligand charge transfer d _{x²-y²} → d _{xz} ; d _{z²} ; d _{xy}	distorted octahedral (D _{4h})

3.3. Electron paramagnetic resonance study

The ESR spectra of copper complexes provide information of importance in studying the metal ion environment. The X-band ESR spectra of the Cu(II) complexes (1), (3), (5) and (4) recorded in the solid state, are shown in the figures 3 and 4.

From the observed *g* values of Cu(II) complexes (1), (3) and (5) at room temperature (Table 4), it is evident that the unpaired electron is localized in the d_{x²-y²} orbital and the ground state is ²B_{1g}. The *g*_∥ < 2.3 value

confirms the covalent character of the metal-ligand bond. The axial symmetry parameter, *G*, is less than four and indicates considerable exchange interaction in the solid complex [15].

The room temperature ESR spectra of the polycrystalline Cu(II) complexes (2), (4), (6) exhibit an isotropic signal, without any hyperfine splitting, with *g*_{iso} = 2.099-2.106. The *g* values obtained in the present study when compared to the *g* value of a free electron, 2.0023, indicates an increase of the covalent nature of the bonding between the metal ion and the ligand molecule [13].

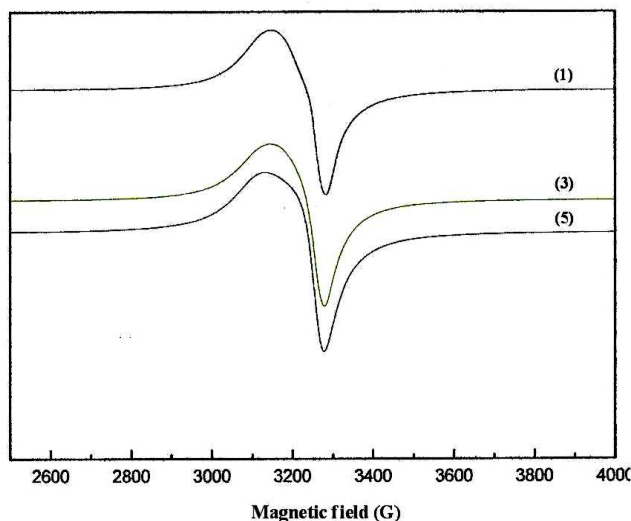


Figure 3. ESR spectra of the Cu(II) complexes (1), (3), (5) at 300K

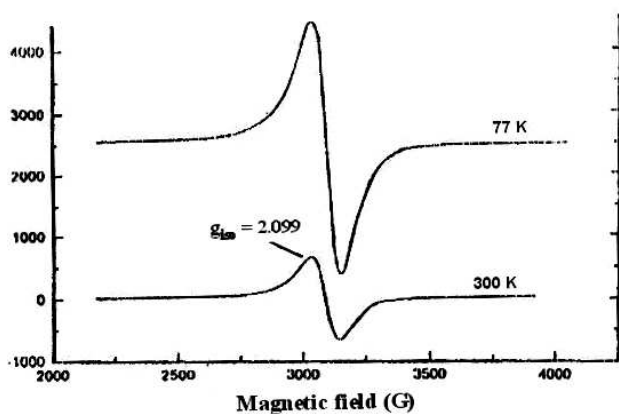


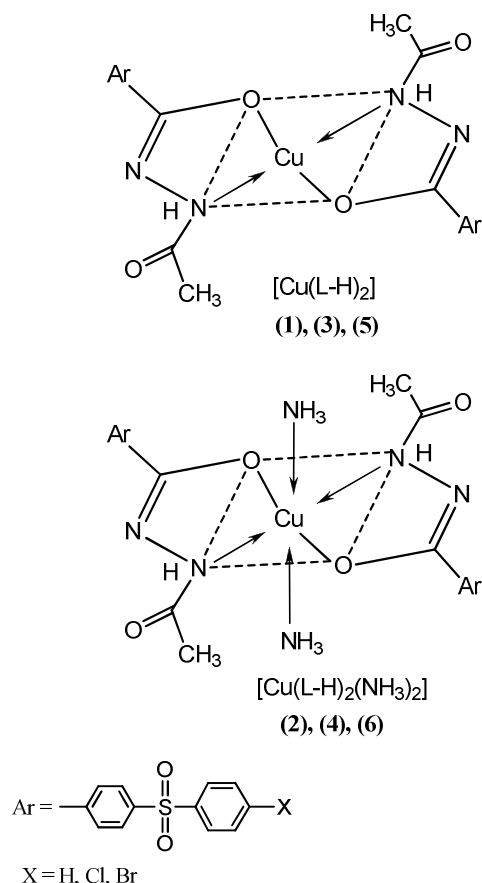
Figure 4. ESR spectra of the Cu(II) complex (4) at 300 and 77K

TABLE 4. The $\{g\}$ parameter values for the *N'*-acetyl-4-[(4-*X*-phenyl)sulfonyl]benzohydrazide Cu(II) complexes (1)-(6)

No.	Compound	g_{iso}	g_{\parallel}	g_{\perp}	G
(1)	$[Cu(L^1-H)_2]$	-	2.127	2.050	2.54
(2)	$[Cu(L^1-H)_2(NH_3)_2]$	2.106	-	-	-
(3)	$[Cu(L^2-H)_2]$	-	2.128	2.053	2.42
(4)	$[Cu(L^2-H)_2(NH_3)_2]$	2.099	-	-	-
(5)	$[Cu(L^3-H)_2]$	-	2.139	2.053	2.62
(6)	$[Cu(L^3-H)_2(NH_3)_2]$	2.103	-	-	-

$$G = (g_{\parallel} - 2)/(g_{\perp} - 2)$$

Correlating the experimental data we can estimate that the stereochemistry of the prepared complexes (Fig. 5):

Figure 5. The proposed structural formula *N'*-acetyl-4-(4-*X*-phenylsulfonyl)benzohydrazide (1), *X*=H, Cl, Br complexes

3.4. Antibacterial activity

The potential antimicrobial activity of the ligands and their newly complexes towards five standard bacterial strains (*Staphylococcus epidermidis* (*Se*) ATCC 14990; *Bacillus subtilis* (*Bs*) ATCC 6633; *Bacillus cereus* (*Bc*) ATCC 14579; *Pseudomonas aeruginosa* (*Pa*) ATCC 9027; *Escherichia coli* (*Ec*) ATCC 11775) was investigated.

Qualitative determination of antimicrobial activity was done using the disk diffusion method [16]. Suspensions in sterile peptone water from 24 h cultures of microorganisms were adjusted to 0.5 McFarland. Muller-Hinton Petri dishes of 90 mm were inoculated using these suspensions. Paper disks (6 mm in diameter) containing 10 μ L of the substance to be tested (at a concentration of 2048 μ g/mL in DMSO) were placed in a circular pattern in each inoculated plate. Incubation of the plates was done at 37°C for 18-24 hours. Reading of the results was done by measuring the diameters of the inhibition zones generated by the tested substances using a ruler.

Determination of MIC was done using the serial dilutions in liquid broth method [17, 18]. The materials used were 96-well plates, suspensions of microorganism (0.5 McFarland), Muller-Hinton broth (Merck), solutions of the substances to be tested (2048 $\mu\text{g/mL}$ in DMSO). The following concentrations of the substances to be tested were obtained in the 96-well plates: 1024; 512; 256; 128; 64; 32; 16; 8; 4; 2 $\mu\text{g/mL}$. After incubation at 37°C for 18-24 hours, the MIC for each tested substance was determined by macroscopic observation of microbial growth. It corresponds to the well with the lowest concentration of the tested substance where microbial growth was clearly inhibited. Chloramphenicol was used as control drug.

TABLE 5. Antibacterial activities of compounds (Ia-c) and (1)-(6) as MIC values ($\mu\text{g/mL}$)

No.	Compound	Gram-positive bacteria			Gram-negative bacteria	
		<i>Se</i>	<i>Bs</i>	<i>Bc</i>	<i>Pa</i>	<i>Ec</i>
(Ia)	(L ¹ H)	1024	512	1024	1024	1024
(Ib)	(L ² H)	512	128	512	1024	512
(Ic)	(L ³ H)	512	128	1024	1024	512
(1)	[Cu(L ¹ -H) ₂]	512	512	512	512	512
(2)	[Cu(L ¹ -H) ₂ (NH ₃) ₂]	128	128	256	256	128
(3)	[Cu(L ² -H) ₂]	128	64	1024	1024	512
(4)	[Cu(L ² -H) ₂ (NH ₃) ₂]	512	512	256	512	128
(5)	[Cu(L ³ -H) ₂]	128	128	256	512	64
(6)	[Cu(L ³ -H) ₂ (NH ₃) ₂]	64	64	1024	1024	512
	Chloramphenicol	64	64	128	64	128

From the data presented in Table 5 it may be seen that the free ligands were not active against the gram-positive and gram-negative bacteria. Upon complexation the activity against the tested micro-organisms increased. Such increased activity of metal chelate can be explained on the basis of the chelation theory. According this, on chelation, the polarity of the metal ion will be reduced to a greater extent due to overlap of ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of π -electrons over the whole chelate ring and enhances the lipophilicity of complexes [19]. This increased lipophilicity enhances the penetration of complexes into the lipid membranes and blocks the metal binding sites in enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism [20].

4. Conclusions

This study reports the successful synthesis and characterization, through analytical and physicochemical

techniques, of new complexes of Cu(II) with N'-acetyl-4-(4-X-phenylsulfonyl)benzohydrazide, X=H, Cl, Br as ligand. In all complexes diacylhydrazine acts as mononegative bidentate ligand bonding through the carbonyl/enolic oxygen and the hydrazinic NH nitrogen. Coordination area and geometry for the metal ions varies with the working conditions: if used anhydrous salts of Cu(II) in alcohol, obtained complex is square-planar and alcohol molecules did not coordinate; if used CuSCN dissolved in ammonia, obtained complex was distorted octahedral and ammonia molecules coordinate. All the complexes precipitated in basic medium. The antibacterial data given for the compounds presented in this paper allowed us to state that the (phenylsulfonyl)phenyl group bonded to a hydrazine moiety is not main cause for the appearance of antibacterial activity. Presence of the metallic ion could be responsible for the variation of the antibacterial activity. Other investigations are in progress.

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