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Short communication

Copper(II) and uranyl(II) complexes with acylthiosemicarbazide: Synthesis, characterization, antibacterial activity and effects on the growth of promyelocytic leukemia cells HL-60

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ABSTRACT

New chelates of N^1-[4-(4-X-phenylsulfonyl)benzoyl]-N^4-butyl-thiosemicarbazide (X = H, Cl, Br) with Cu^{2+} and UO_2^{2+} have been prepared and characterized by analytical and physico-chemical techniques such as magnetic susceptibility measurements, elemental and thermal analyses, electronic, ESR and IR spectral studies. Room temperature ESR spectra of Cu(II) complexes yield {*g*} values characteristic of distorted octahedral and pseudo-tetrahedral geometry. Infrared spectra indicate that complexes contain six-coordinate uranium atom with the ligand atoms arranged in an equatorial plane around the linear uranyl group. Effects of these complexes on the growth of human promyelocytic leukemia cells HL-60 and their antibacterial activity (against *Staphylococcus epidermidis* ATCC 14990, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 14579, *Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 11775 strains) were studied comparatively with that of free ligands.

1. Introduction

Complexes of thiosemicarbazide and 1,4-substituted thiosemicarbazide are of general interest as models for bioinorganic processes [1–3]. Numerous transition metal complexes of substituted thiosemicarbazides, particularly the 1,4-substituted derivatives, have been prepared and characterized and they have been found to possess a wide variety of biological activities against bacteria, fungi and certain type of tumours [4–10]. Acylthiosemicarbazide contains oxygen, sulphur and nitrogen as potential donor atoms and is liable to form deprotonated complexes by loss of hydrazinic proton via enolisation/thioenolisation, because it might present a lot of tautomeric forms (Fig. 1).

Keeping this observation in view, in this paper we have synthesized new complexes of Cu(II) and UO_2(II) with N^1-[4-(4-X-phenylsulfonyl)benzoyl]-N^4-butyl-thiosemicarbazide as ligand (Fig. 2) and we described a preliminary investigation of their structure and biological activity.

2. Chemistry

The ligands N^1-[4-(4-X-phenylsulfonyl)benzoyl]-N^4-butyl-thiosemicarbazide, X = H, Cl, Br were obtained earlier [11] according to Scheme 1.

For the synthesis of complexes were used Cu(II) chloride, nitrate, acetate and thiocyanate salts and UO_2(II) acetate, respectively, which were refluxed with stirring with a hot clear solution of the ligand in ethanol (1:1 metal:ligand ratio).

3. Biological activity

3.1. Antibacterial activity

The synthesized compounds were tested for their in vitro antibacterial activity against the Gram-positive and the Gram-negative bacteria: *Staphylococcus epidermidis* ATCC 14990, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 14579, *Pseudomonas aeruginosa*

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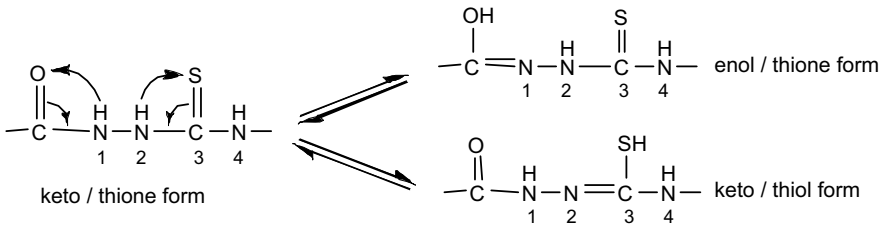


Fig. 1. Tautomeric forms for N^1-acyl-N^4-substituted thiosemicarbazide.

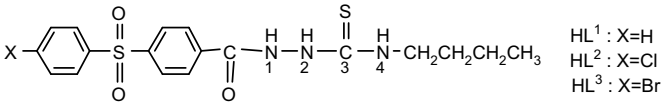


Fig. 2. Structure of the ligands.

ATCC 9027 and *Escherichia. coli* ATCC 11775, comparative with free ligands, using the paper disc diffusion method [12] (for the qualitative determination) and the serial dilutions in liquid broth method [13] for determination of MIC. Chloramphenicol was used as control drug.

3.2. Antitumor activity

The biochemical effects involved in the antitumor activity of ligands and some of newly synthesized complexes in human HL-60 promyelocytic leukemia cells were investigated using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS), which is converted to blue formazan by an enzyme present in metabolically active cells. This product can be completely solubilized in acidified isopropanol and detected by a microtiter plate reader [14].

4. Results and discussions

4.1. Chemistry

The elemental analysis data along with some physical properties of the complexes are reported in Table 1. The composition and molecular formulas of the complexes under investigation were further confirmed from the values of molar conductance. The molar conductances in DMSO are in 2.5–8.6 $ohm^{-1}\,cm^2\,mol^{-1}$ range. The data suggests that the complexes behave as nonelectrolytes [15].

4.1.1. IR spectra

Relevant IR data is given in Table 2. In order to study the binding mode of thiosemicarbazides to the metal ion complexes, the IR spectrum of the free ligand was compared with the spectra of the complexes.

All the ligands having three vibrational frequency from 3425 to 3178 cm^{-1} which are assigned to the stretching vibration of the NH groups, a strong band at the regions of 1679–1672 cm^{-1} corresponding to ν(C=O) stretching vibration [11,16].

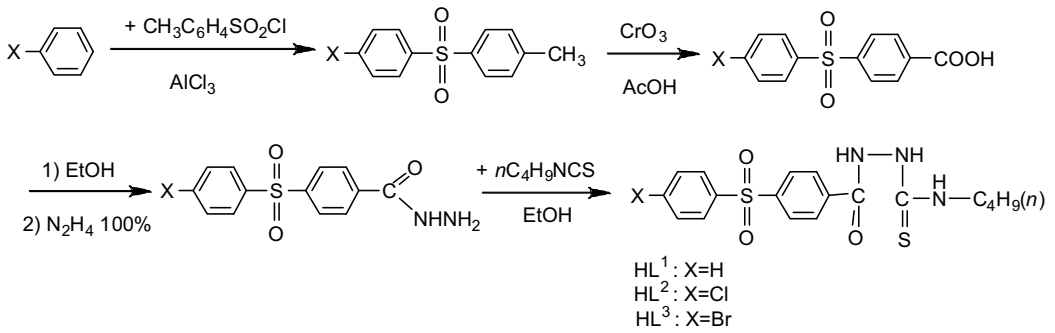
In all IR spectra of complexes the ν(C=O) mode of ligand is not observed, but the presence of a new band at 1578–1559 cm^{-1}, due to the ν(N=C) of N=C–O$^-$ group, indicates the enolisation of C=O group followed by deprotonation and complexation with metal ions. This is further supported by the presence of band at 1246–1242 cm^{-1} corresponding to C=S stretching vibrations in free ligands which have not presented considerable change in the complexes and ruling out the possibility of bonding through the >C=S group. Furthermore, the Cu(II) complexes show extra IR bands in the 532–427 cm^{-1} and 477–412 cm^{-1} regions assigned to ν(M–O) and ν(M–N), respectively [17,18].

The complex combinations **1a**, **1b**, **5a**, **5b**, **9a**, **9b** obtained using $CuCl_2$ and $Cu(NO_3)_2$ exhibit υ(OH) and $\rho(H_2O)$ bands in the 3400–3420 and 680–690 cm^{-1} regions which are indicative of coordinated water in the complexes [19,20].

The presence of $\nu(CH_3COO)$ absorption bands at 1534 cm^{-1} and 1422 cm^{-1} in the IR spectra of complexes **2**, **6** and **10** suggests that the acetate group is coordinated to Cu(II) ion in ring bridge, in a bidentate form [17]. In the IR spectrum of **3**, **7** and **11** (prepared from CuSCN), two absorption bands in the region 684–670 cm^{-1} and 443–430 cm^{-1}, respectively, specific to the S-coordinated thiocyanate group are detected [17]. No absorption is noted in the 870–780 cm^{-1} range, so the N-coordination of the thiocyanate is ruled out.

In the uranyl complexes **4**, **8** and **12**, the bands which are observed near 1530 and 1435 cm^{-1} attributed to ν_{as}(OCO) and ν_s(OCO) of the acetate group, respectively, indicate asymmetric bidentate coordination of this group (Δ(OCO) = ν_{as}(OCO) − ν_s(OCO) < 100 cm^{-1}) [17]. Also, the uranyl complexes show strong IR band near 839 cm^{-1} assigned to $\nu_{as}(UO_2)$ [21–23].

The overall IR spectral data suggests that the thiosemicarbazide derivatives act as mononegative bidentate ligands and coordinate



Scheme 1. Synthesis of the ligands.

Table 1
Analytical data and physical properties of N^1-[4-(4-X-phenylsulfonyl)benzoyl]-N^4-butyl-thiosemicarbazide (X = H, Cl, Br) complexes.

Compd.	Molecular formula	MW (%)	Colour	Yield	Elemental analysis (%) calcd. (found)			Λ ($ohm^{-1}\,cm^2\,mol^{-1}$)	μ (BM)
					C	N	M		
1a	$[Cu(L^1)_2(H_2O)_2]$ ($C_{36}H_{44}CuN_6O_8S_4$)	880.57	Green	82	49.10 (49.02)	9.54 (9.47)	7.22 (7.15)	5.3	2.15
1b	$[Cu(L^1)_2(H_2O)_2]$ ($C_{36}H_{44}CuN_6O_8S_4$)	880.57	Green	68	49.10 (49.00)	9.54 (9.43)	7.22 (7.23)	6.1	2.25
2	$[Cu_2(L^1)_2(AcO)_2(H_2O)_4]$ ($C_{40}H_{54}Cu_2N_6O_{14}S_4$)	1098.24	Dark green	75	43.75 (43.70)	7.65 (7.59)	11.57 (11.65)	4.7	1.25
3	$[Cu(L^1)(SCN)(H_2O)]$ ($C_{19}H_{22}CuN_4O_4S_3$)	530.14	Green	83	43.05 (42.98)	10.57 (10.50)	11.99 (12.01)	2.5	1.75
4	$[UO_2(L^1)(AcO)]$ ($C_{20}H_{23}N_4O_7S_2U$)	719.57	Dark red	79	33.38 (33.31)	5.84 (5.78)	33.08 (33.22)	8.6	1.50
5a	$[Cu(L^2)_2(H_2O)_2]$ ($C_{36}H_{42}Cl_2CuN_6O_8S_4$)	949.46	Green	69	45.54 (45.48)	8.85 (8.79)	6.69 (6.73)	7.5	2.20
5b	$[Cu(L^2)_2(H_2O)_2]$ ($C_{36}H_{44}CuN_6O_8S_4$)	949.46	Green	71	45.54 (45.50)	8.85 (8.81)	6.69 (6.61)	6.5	2.23
6	$[Cu_2(L^2)_2(AcO)_2(H_2O)_4]$ ($C_{40}H_{52}Cl_2Cu_2N_6O_{14}S_4$)	1167.13	Dark green	80	41.16 (41.08)	7.20 (7.15)	10.89 (10.96)	4.1	1.34
7	$[Cu(L^2)(SCN)(H_2O)]$ ($C_{19}H_{21}ClCuN_4O_4S_3$)	564.58	Green	78	40.42 (40.43)	9.92 (10.01)	11.26 (11.35)	3.6	1.80
8	$[UO_2(L^2)(AcO)]$ ($C_{20}H_{22}ClN_4O_7S_2U$)	754.02	Dark red	65	31.86 (31.78)	5.57 (5.46)	31.57 (31.48)	5.2	1.50
9a	$[Cu(L^3)_2(H_2O)_2]$ ($C_{36}H_{42}Br_2CuN_6O_8S_4$)	1038.36	Green	78	41.64 (41.58)	8.09 (8.02)	6.12 (6.19)	2.8	2.18
9b	$[Cu(L^3)_2(H_2O)_2]$ ($C_{36}H_{42}Br_2CuN_6O_8S_4$)	1038.36	Green	81	41.64 (41.60)	8.09 (8.05)	6.12 (6.18)	3.7	2.22
10	$[Cu_2(L^3)_2(AcO)_2(H_2O)_4]$ ($C_{40}H_{52}Br_2Cu_2N_6O_{14}S_4$)	1256.03	Dark green	82	38.25 (38.17)	6.69 (6.61)	10.12 (10.18)	4.0	1.50
11	$[Cu(L^3)(SCN)(H_2O)]$ ($C_{19}H_{21}BrCuN_4O_4S_3$)	609.04	Green	68	37.47 (37.42)	9.20 (9.23)	10.43 (10.50)	3.2	1.75
12	$[UO_2(L^3)(AcO)]$ ($C_{20}H_{22}BrN_4O_7S_2U$)	798.46	Dark red	75	30.08 (30.02)	5.26 (5.30)	29.81 (29.88)	4.4	1.70

through deprotonated enolic carbonyl oxygen (=C–O$^-$) and hydrazinic N^{2}H nitrogen atoms and form a five-membered chelate ring.

4.1.2. Electronic spectra and magnetic studies

The nature of the ligand field around metal ion and the geometry of the metal complexes have been deduced from the electronic spectra and magnetic moment data of the complexes. The room temperature magnetic moment (Table 1) of the Cu(II) complexes **1a**, **1b**, **5a**, **5b**, **9a** and **9b** was found in the range 2.15–2.25 BM, indicative of one unpaired electron per Cu(II) ion. The UV–VIS spectra for these complexes (Table 3) shows d–d bands in the regions 13,003–12,345, 14,970–14,045 and 16,325–15,822 cm^{-1} which may be assigned to $d_{x^2-y^2} \rightarrow d_{z^2}$, $d_{x^2-y^2} \rightarrow d_{xy}$, and $d_{x^2-y^2} \rightarrow d_{xz,yz}$ transitions, respectively, due to the distorted octahedral geometry (D_{4h}) around Cu(II) [24].

The electronic spectra for **2**, **6** and **10** exhibit three bands observed at 12,562–12,484 cm^{-1}, 14,447–14,347 cm^{-1} and 16,835–16,121 cm^{-1} which may be assigned to $d_{x^2-y^2} \rightarrow d_{z^2}$, , $d_{x^2-y^2} \rightarrow d_{xy}$, and $d_{x^2-y^2} \rightarrow d_{xz,yz}$, transitions, respectively, due to the octahedral

Table 2
IR bands and their assignments for N^1-[4-(4-X-phenylsulfonyl)benzoyl]-N^4-butyl-thiosemicarbazide and its complexes **1**–**12**.

Compd.	υ_{N^4H}	υ_{N^2H}	υ_{N^1H}	$\upsilon_{C=O}$	$\upsilon_{C=N}$	$\upsilon_{C=S}$	$\upsilon_{N^1-N^2}$	$\upsilon^{as,s}_{acetate}$	$\upsilon^{as,s}_{SO_2}$	υ_{C-X} (X = Cl, Br)	υ_{M-O}, υ_{M-N} [17]
HL^1	3425	3290	3196	1672	–	1242	1010	–	1324	–	–
									1159		
1a	3287	3108	–	–	1561	1238	1015	–	1326	–	532
									1160		419
1b	3267	3103	–	–	1559	1236	1015	–	1324	–	531
									1160		419
2	3290	3110	–	–	1562	1238	1012	1534	1331	–	520
								1421	1160		428
3	3282	3104	–	–	1564	1240	1020	–	1323	–	518
									1154		423
4	3332	3243	–	–	1577	1238	1016	1531	1327	–	839
								1436	1157		(Uranyl abs.)
HL^2	3420	3340	3188	1672	–	1242	1010	–	1326	635	–
									1160	580	
5a	3284	3104	–	–	1560	1243	1013	–	1335	623	525
									1161	577	473
5b	3253	3141	–	–	1576	1240	1013	–	1331	622	528
									1162	581	477
6	3286	3158	–	–	1561	1240	1013	1534	1330	623	527
								1421	1160	577	473
7	3278	3242	–	–	1565	1240	1014	–	1329	624	529
									1157	570	471
8	3336	3242	–	–	1578	1230	1014	1529	1335	626	838
								1435	1162	581	(Uranyl abs.)
HL^3	3420	3341	3178	1679	–	1246	1011	–	1324	616	-
									1160	575	
9a	3283	3154	–	–	1570	1243	1011	–	1331	615	434
									1160	576	417
9b	3256	3143	–	–	1574	1243	1010	–	1330	614	438
									1161	577	413
10	3291	3175	-	-	1569	1243	1010	1534	1331	617	430
								1422	1160	574	418
11	3239	3141	–	–	1577	1245	1010	–	1326	616	427
									1156	574	412
12	3336	3244	–	–	1575	1239	1011	1528	1333	617	839
								1435	1162	575	(Uranyl abs.)

Table 3
Electronic spectra (cm^{-1}) for the Cu(II) complexes.

Compound	Band max (cm^{-1})	Transitions	Geometry
1a	38,023; 29,940; 27,397	Intraligand	Distorted
	24,691	Charge transfer	Octahedral (D_{4h})
	16,325	$d_{x^2-y^2} \rightarrow d_{xz,yz}$	
	14,970	$d_{x^2-y^2} \rightarrow d_{xy}$	
1b	37,987; 29,914; 27,365	Intraligand	Distorted
	24,687	Charge transfer	Octahedral (D_{4h})
	14,903	$d_{x^2-y^2} \rightarrow d_{xy}$	
	12,345	$d_{x^2-y^2} \rightarrow d_{z^2}$	
2	38,103; 29,967; 27,485	Intraligand	Distorted
	24,691	Charge transfer	Octahedral (D_{4h})
	16,121	$d_{x^2-y^2} \rightarrow d_{xz,yz}$	
	14,347	$d_{x^2-y^2} \rightarrow d_{xy}$	
	12,484	$d_{x^2-y^2} \rightarrow d_{z^2}$	
3	37,287; 30,094	Intraligand	Pseudo-
	24,154; 20,375	Charge transfer	Tetrahedral
	14,026	$d_{xy} \rightarrow d_{z^2}$; $d_{x^2-y^2}$	
5a	38,561; 29,787; 27,302	Intraligand	Distorted
	23,981	Charge transfer	Octahedral (D_{4h})
	15,822	$d_{x^2-y^2} \rightarrow d_{xz,yz}$	
	14,388	$d_{x^2-y^2} \rightarrow d_{xy}$	
	12,391	$d_{x^2-y^2} \rightarrow d_{z^2}$	
5b	38,260; 29,568; 27,300	Intraligand	Distorted
	23,484	Charge transfer	Octahedral (D_{4h})
	15,873	$d_{x^2-y^2} \rightarrow d_{xz,yz}$	
	14,814	$d_{x^2-y^2} \rightarrow d_{xy}$	
6	38,602; 29,805; 27,326	Intraligand	Distorted
	22,870	Charge transfer	Octahedral (D_{4h})
	16,187	$d_{x^2-y^2} \rightarrow d_{xz,yz}$	
	14,447	$d_{x^2-y^2} \rightarrow d_{xy}$	
	12,562	$d_{x^2-y^2} \rightarrow d_{z^2}$	
7	37,814; 30,101	Intraligand	Pseudo-
	24,205; 20,526	Charge transfer	Tetrahedral
	13,880	$d_{xy} \rightarrow d_{z^2}$; $d_{x^2-y^2}$	
9a	30,087; 29,714; 25,009	Intraligand	Distorted
	21,682	Charge transfer	Octahedral (D_{4h})
	16,103	$d_{x^2-y^2} \rightarrow d_{xz,yz}$	
	14,045	$d_{x^2-y^2} \rightarrow d_{xy}$	
9b	30,015; 29,684; 24,999	Intraligand	Distorted
	21,240	Charge transfer	Octahedral (D_{4h})
	15,873	$d_{x^2-y^2} \rightarrow d_{xz,yz}$	
	14,326	$d_{x^2-y^2} \rightarrow d_{xy}$	
	13,003	$d_{x^2-y^2} \rightarrow d_{z^2}$	
10	30,114; 29,812; 25,126	Intraligand	Distorted
	20,214	Charge transfer	Octahedral (D_{4h})
	16,835	$d_{x^2-y^2} \rightarrow d_{xz,yz}$	
	14,388	$d_{x^2-y^2} \rightarrow d_{xy}$	
11	30,003; 27,980	Intraligand	Pseudo-
	23,614; 20,835	Charge transfer	Tetrahedral
	14,280	$d_{xy} \rightarrow d_{z^2}$; $d_{x^2-y^2}$	

(D_{4h}) geometry around Cu(II) [24,25]. For these complexes $\mu_{eff} < 2$ (1.25–1.50 BM) suggests an antiferromagnetic interaction in the dinuclear compounds [26].

In the electronic spectrum of complexes with thiocyanate, the broad band was observed at 14,280–13,880 cm^{-1} region due to $d_{xy} \rightarrow d_{z^2}$; $d_{x^2-y^2}$ transition, suggesting a pseudo-tetrahedral configuration around the central metal ion [24]. This complexes show two extra absorption bands at 24,205–23,614 and 20,835–20,375 cm^{-1} respectively, which are attributed to ligand–metal charge transfer. The complexes were found paramagnetic with magnetic moments in 1.75–1.80 BM range, which is normal for Cu(II) with pseudo-tetrahedral stereochemistry [24].

The UV–VIS spectrum of uranyl complexes **4**, **8**, **12** (Table 4) shows one or two bands in addition to the ligand bands. The first band observed at >590 nm due to electronic transitions from apical oxygen atom to the f-orbitals of uranyl atom is characteristic of the uranyl moiety [24]. The second band observed at 694 nm in **8** corresponding to charge transfer from equatorial donor atoms of the ligand to the uranyl ion, which is imparting the complex its red colour [24].

4.1.3. ESR spectra

The tensor value {*g*} of copper complexes (Table 5) can be used to derive the ground state.

The shape of ESR spectra for (**1a**, **b**), (**5a**, **b**) and (**9a**, **b**) is very similar, leading to close values of the {*g*} tensor, which are specific to an octahedral geometry of the Cu(II) ion. The parameter $G = (g_{\|} - 2)/(g_{\perp} - 2) < 4$ (*G* value is in 1.90–2.94 range) indicates an exchange interaction between the Cu(II) ions and further supports the dinuclear nature of the complexes [25–27].

The room temperature ESR spectra of Cu(II) complexes obtained with $Cu(CH_3COO)_2$ exhibit an isotropic signal, with $g_{iso} = 2.089$ (**2**); 2.086 (**6**) and 2.090 (**10**), which confirms the octahedral geometry for metallic ion [28,29]. In pseudo-tetrahedral complexes (**3**), (**7**) and (**11**) the unpaired electron lies in the d_{xy} orbital giving $^2B_1$ as the ground state. The *g* values obtained in case of these complexes indicate an increase of the covalent nature of the bonding between the metal ion and the ligand molecule. The ESR parameters of the complexes coincide well with related systems which suggests that the complex has pseudo-tetrahedral geometry [24,30].

4.1.4. Thermogravimetric analysis

The TG thermograms of complexes are characterized by three or four degradation steps, the first and/or the second weight loss endothermic step corresponds to the release of the coordinated small fragments (H_2O, SCN). Always, the ligand supports two stepwise decomposition between 275–480 °C (~60% ligand loss) and 500–680 °C (~40% ligand loss). For the complexes **2**, **4**, **6**, **8**, **10**, **12** the last step of decomposition is attributed to the removal of the acetate species. Up to 700 °C, the final residue (CuO, UO_3) is formed.

Thus, based on these analytical and physico-chemical data, the proposed structures for the complexes are shown in Fig. 3.

4.2. Biological activity

4.2.1. Antibacterial activity

The free ligands and their metal complexes were screened for their antibacterial activities. The results (Table 6) indicated that the ligands do not have any significant activity, whereas their complexes showed more activity against the some organisms under identical experimental conditions. This would suggest that the chelation could facilitate the ability of a complex to cross a cell membrane and can be explained by Tweedy's chelation theory [31]. Chelation considerably reduces the polarity of the metal ion mainly because of partial sharing of its positive charge with the donor group and possible electron delocalization over the whole chelate ring. Such chelation could also enhance the lipophilic character of the central metal atom, which subsequently favours its permeation through the lipid layer of the cell membrane [32].

Most of the Cu(II) tested compounds are less active towards studied microorganisms in comparison to the control drug.

Table 4
UV–VIS spectral data (nm) for the uranyl complexes **4**, **8**, **12**.

Compd.	$\pi \rightarrow \pi^*$; $n \rightarrow \pi^*$and charge transfer transitions	d → d transitions
HL[1]	266; 337; 365	-
4	263; 366	592
HL[2]	260; 336; 367	–
8	260; 373	590; 694
HL[3]	263; 337; 378	–
12	261; 362	594

Table 5
The {g} parameter values for the Cu(II) complexes.

Compound	g_{iso}	$g_{\|}$	$g_{\perp}$	g_1	g_2	g_3
1a	–	2.128	2.050	–	–	–
1b	–	2.128	2.053	–	–	–
2	2.089	–	–	–	–	–
3	–	–	–	2.024	2.094	2.219
5a	–	2.135	2.071	–	–	–
5b	–	2.149	2.071	–	–	–
6	2.086	–	–	–	–	–
7	–	–	–	2.036	2.089	2.195
9a	–	2.127	2.050	–	–	–
9b	–	2.162	2.055	–	–	–
10	2.090	–	–	–	–	-
11	–	–	–	2.040	2.088	2.246

Thiocyanato and acetato Cu(II) complexes inhibit the growth of *B. subtilis*, similar to chloramphenicol. The UO_2 derivatives are more active than free ligands and have no significant effect against *P. aeruginosa*, *S. epidermidis* and *B. cereus* comparative with control drug.

4.2.2. Cell viability determination (MTS assay)

The in vitro cytotoxicity of the ligands HL^1, HL^2, HL^3 and some of their Cu(II) and UO_2(II) complexes, **1a**, **3**, **6**, **7**, **8**, **9a**, **11**, **12** on human promyelocytic leukemia cells HL-60 was determined by an MTS-based assay [14]. Doxorubicin was used as reference. None of the

Table 6
Antibacterial activities of ligands HL^1, HL^2, HL^3 and Cu(II) and UO_2(II) complexes **1**–**12** as MIC values (μg/mL).

Compd.	Gram-positive bacteria[a]			Gram-negative bacteria[b]	
	Se	*Bs*	*Bc*	*Pa*	*Ec*
HL^1	1024	512	1024	1024	1024
1a	512	512	512	512	512
1b	512	512	512	512	512
2	256	512	512	512	512
3	512	256	1024	1024	1024
4	256	256	512	512	512
HL^2	512	128	512	512	512
5a	256	128	512	512	256
5b	256	128	512	512	256
6	128	128	256	512	256
7	256	64	512	256	512
8	256	64	256	128	128
HL^3	512	128	1024	1024	512
9a	256	128	512	512	256
9b	256	128	512	512	256
10	128	64	512	512	256
11	128	64	512	1024	256
12	256	256	512	512	256
Control	64	64	128	64	128

Control = chloramphenicol.
[a] *Se* (*Staphylococcus epidermidis* ATCC 14990); *Bs* (*Bacillus subtilis* ATCC 6633); *Bc* (*Bacillus cereus* ATCC 14579).
[b] *Pa* (*Pseudomonas aeruginosa* ATCC 9027); *Ec* (Escherichia coli ATCC 11775).

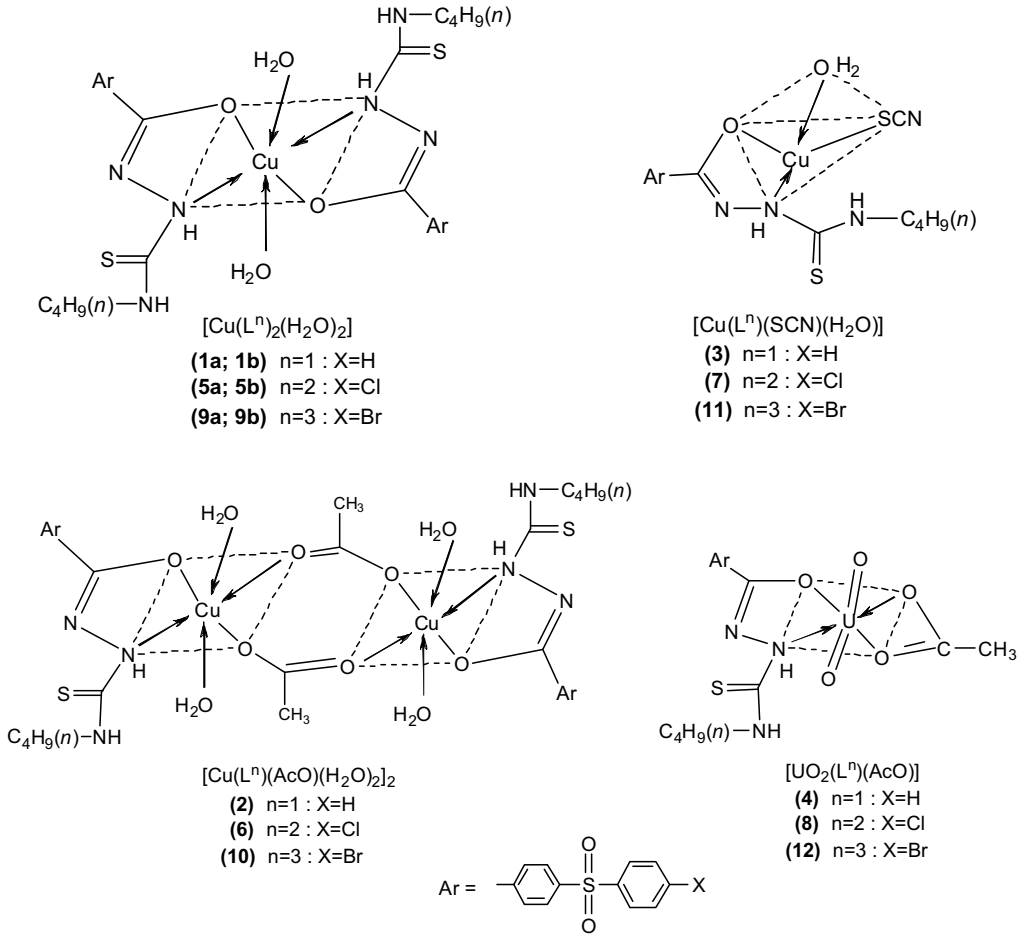


Fig. 3. Proposed structures of the complexes **1**–**12**.

compounds in the tested concentration inhibited cell proliferation by 50% and therefore IC_{50} values could not be calculated.

5. Conclusions

The coordination ability of the N^1-[4-(4-X-phenylsulfonyl)benzoyl]-N^4-butyl-thiosemicarbazide (X = H, Cl, Br) has been proved in complexation reaction with Cu(II) and UO_2(II) ions. The analytical and physico-chemical analyses confirmed the composition and the structure of the newly obtained complex combinations.

In all complexes the ligand acts as mononegative bidentate bonding through the carbonyl/enolic oxygen and the hydrazinic N^2H nitrogen. Room temperature ESR spectra of Cu(II) complexes yield {*g*} values characteristic of distorted octahedral and pseudo-tetrahedral geometry. Infrared spectra indicate that complexes contain six-coordinate uranium atom with the ligand atoms arranged in an equatorial plane around the linear uranyl group.

The antibacterial data given for the compounds presented in this paper allowed us to state that the (phenylsulfonyl)phenyl group is not the main cause for the appearance of antibacterial activity and the metal ion could be responsible for the variation of the antibacterial activity. Results of antitumoral screening indicate that both the ligands and their complexes have not presented cytotoxic effects in micro-molar concentrations.

Other investigations are in progress for this class of complex combinations with acylthiosemicarbazides.

6. Experimental protocols

6.1. Chemistry

The content of metallic ions was determined by atomic absorption spectroscopy with AAS-1N spectrometer Carl Zeiss Jena; C, H, and N were done with a Carlo Erba microdosimeter, after drying the complexes at 105 °C. The molar conductivity was determined in DMSO (3×10^{-3} M), at room temperature, using OK-102/1 Radelkis conductometer. Electronic spectra were recorded by the diffuse-reflectance technique, using MgO as diluting matrix, on a JASCO V-550 spectrophotometer. IR spectra were recorded with a BioRad FTS 135 spectrophotometer in the 4000–400 cm^{-1} region using KBr pellets. The thermal analysis of the complexes was carried out in static air atmosphere, with a sample heating rate of 10 °C/min using DuPont 2000 ATG thermo-balance. ESR spectra were recorded with an ART-6, model IFA-Bucharest, X-band spectrometer (9.01 GHz) online with a PC equipped with a 100 KHz field modulation unit, on polycrystalline powders at room temperature. The melting points were determinated with Boetius apparatus and are higher than 250 °C for all complexes. The required chemicals were purchased from Merck and Chimopar, Bucharest and all manipulations were performed using materials as received.

6.1.1. Synthesis of the ligands

N^1-[4-(4-X-phenylsulfonyl)benzoyl]-N^4-butyl-thiosemicarbazides (HL^1, HL^2, HL^3, X = H, Cl, Br) used as ligands were synthesized by the method described in reference [11].

6.1.2. Synthesis of the complexes

All the Cu(II) complexes were synthesized by the following general procedure. The corresponding copper(II) salt ($CuCl_2$, $Cu(NO_3)_2$, or $Cu(CH_3COO)_2$) and the appropriate thiosemicarbazide in equimolar ratio were dissolved in ethanol and mixed together (CuSCN was dissolved in 25% NH_3). The reaction mixture was boiled under refluxing state for 1–2 h. After cooling at room temperature, a microcrystalline complex was separated. It was filtered under vacuum and the crystals were washed with cold ethanol and anhydrous diethylether and kept in a desiccator over fused $CaCl_2$. When we used $CuCl_2$ and $Cu(NO_3)_2$ the same complexes were obtained (**1a**, **b**; **5a**, **b**; **9a**, **b**). For uranyl(II) complexes it was used $UO_2(CH_3COO)_2$ and the work procedure was analogous.

6.2. Antibacterial activity

Qualitative determination of antibacterial activity was done using the disk diffusion method. Suspensions in sterile peptone water from 24 h cultures of microorganisms were adjusted to 0.5 McFarland. These suspensions were inoculated in Muller–Hinton Petri dishes of 90 mm. Paper disks (6 mm in diameter) containing 10 μL of the substance to be tested (at a concentration of 2048 μg/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 h. Reading of the results was done by measuring the diameters of the inhibition zones generated by the tested substances using a ruler. Chloramphenicol was used as a reference substance.

Determination of MIC was done using the serial dilutions in liquid broth method. The materials used were 96-well plates, suspensions of microorganism (0.5 McFarland), Muller–Hinton broth (Merck) and solutions of the substances to be tested (2048 μg/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 μg/mL. After incubation at 37 °C for 18–24 h, the MIC for each tested substance was determined by macroscopic observation of microbial growth. It corresponds well with the lowest concentration of the tested substance where microbial growth was clearly inhibited.

Cytotoxicity of the tested compounds was determined by standard MTS assay [14].

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