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SYNTHESIS, ANTIBACTERIAL, AND ANTIFUNGAL ACTIVITIES OF 8-QUINOLINEALDEHYDE CHALCOGENSEMICARBAZONES AND THEIR COPPER(II) COMPLEXES

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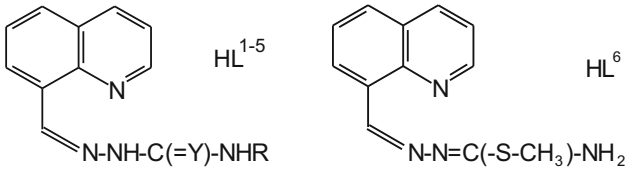
The antibacterial and antifungal activities of a series of 8-quinolinealdehyde chalcogensemicarbazones have been studied. Only the selenium analog shows activity against Gram-positive bacteria. 8-Quinolinealdehyde thiosemicarbazone becomes active against the indicated bacteria upon coordination to copper(II) ions. The antibacterial activity of the obtained complexes depends on neither the nature of the anion nor the form (molecular or anionic) of the ligand. Introduction of electron-accepting groups on the amide nitrogen atom of the thiosemicarbazide fragment leads to a substantial increase in the activity. 8-Quinolinealdehyde semicarbazone as well as its copper(II) complexes do not show appreciable antibacterial activity against the test species. All investigated compounds do not exhibit any antifungal properties.

Key words: 8-quinolinealdehyde thiosemicarbazone, synthesis, copper(II) complexes, antibacterial and antifungal activity.

Acquired resistance to antibiotics is currently steadily increasing in microorganisms. Therefore, much attention is being paid to the targeted synthesis of novel biologically active compounds. One group of such drugs comprises thiosemicarbazone derivatives and their metal coordination compounds, many of which exhibit a broad spectrum of biological activity. Antitumor [1], antiviral [2], antibacterial [3], antimalarial [4], and other types of biological activity are known for thiosemicarbazones. Complexation in many instances enhances their biological activity [5, 6]. Among the studied compounds, thiosemicarbazones of heterocyclic carbonyl pyridine derivatives occupy a special place [7 – 12]. In particular, the antibacterial and antifungal properties of 2-pyridinealdehyde thiosemicarbazone and certain of its complexes were studied [13 – 18]. Herein experimental results on the synthesis and the study of the bacteriostatic and bactericidal activities of certain derivatives of 8-quinolinealdehyde thiosemicarbazone (HL^{1-5} and HL^6) in addition to their copper coordination compounds of formula $Cu(HL^1)X_2 \cdot nH_2O$, where X = NO_3, $n = 2$ (**I**); Cl, $n = 1$ (**II**); ClO_4, $n = 3$ (**III**); $Cu(L^1)NO_3$ (**IV**); $Cu(L^2)NO_3 \cdot 0.25H_2O$ (**V**); $Cu(L^3)NO_3 \cdot H_2O$ (**VI**); $Cu(HL^4)(NO_3)_2 \cdot 2H_2O$ (**VII**), $Cu(HL^5)(NO_3)_2 \cdot 2H_2O$ (**VIII**), and $Cu(HL^6)(NO_3)_2 \cdot 2H_2O$ (**IX**) are presented in order to find new compounds with antibacterial activity. 8-Quinolinealdehyde is formally structurally similar to heterocyclic 2-pyridinealdehyde. However, it has several important differences. Copper complexes of HL^1 were first reported by Ablov, et al. [19]. Considering the processes occurring during purification of the final products obtained by the literature method [19], we improved the synthetic method for the copper coordination complexes with HL^1 [20]. The effects of various factors on the biological activity of these compounds were studied. These included coordination and deportonation of the ligand and the nature of the chalcogenide (Y), the substituent on the terminal N atom (R), and the anion (X).



HL^1 – Y=S, R=H; HL^2 – Y=S, R=C_6H_5; HL^3 – Y=S, R=*p*-F-C_6H_4; HL^4 – Y=O, R=H; HL^5 – Y=Se, R=H,

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TABLE 1. Elemental Analyses, % (Found/Calc.) and Effective Magnetic Moments (μ_{ef}) for Newly Synthesized **II**, **III**, **VI**, and **VIII**

Compound	Empirical formula	C	H	N	Cu	μ_{ef}, μ_B
II	$C_{11}H_{12}CuN_4OSCl_2$	34.38/34.52	3.09/3.16	14.72/14.64	16.47/16.60	1.82
III	$C_{11}H_{16}CuN_4O_{11}SCl_2$	24.02/24.16	2.88/2.95	10.34/10.25	11.51/11.62	1.85
VI	$C_{17}H_{14}CuFN_5O_4S$	43.57/43.73	2.87/3.02	14.87/15.00	13.52/13.61	1.87
VIII	$C_{11}H_{14}CuN_6O_8Se$	26.25/26.38	2.75/2.82	19.61/16.78	12.61/12.69	1.79

Composition and structure of copper coordination complexes with HL. Reaction of HL^1 with copper nitrate in acidic medium formed $Cu(HL^1)(NO_3)_2{\cdot}2H_2O$ (**I**). Mixing solutions of Cu(II) chloride or perchlorate and HL^1 in the appropriate acid (HCl or $HClO_4$) isolated $Cu(HL^1)Cl_2{\cdot}H_2O$ (**II**) and $Cu(HL^1)(ClO_4)_2{\cdot}3H_2O$ (**III**). Compounds with similar ratios of components were formed by reaction of 2-pyridinealdehyde thiosemicarbazone with the corresponding Cu(II) salts [21, 22]. Slow crystallization of an aqueous solution of **I** formed $Cu(L^1)NO_3$ (**IV**), which was the hydrolysis product of **I** according to the scheme

$$Cu(HL^1)(NO_3)_2 + H_2O \leftrightarrow Cu(L^1)NO_3 + H_3O^+ + NO_3^-.$$

The enhanced protolytic properties of HL^1 were an effect of coordination. An x-ray structure of **IV** [20] showed that the organic ligand was planar and bonded to the central atom, as expected, through N, N, and S donor atoms. The resulting Cu–S coordination bond strongly polarized the NH bond of the five-membered metallocycle and created conditions that were favorable for loss of the proton. The shift of the equilibrium to the right side was also responsible for the lower solubility of **IV** as compared with **I**. When an electron-accepting substituent ($C_6H_5^-$ or p-$FC_6H_4^-$) was added to the terminal N

TABLE 2. Antimicrobial Activity of Studied Compounds (MIC*/MBC**) (μg/mL)

Compound	*S.aureus*					*S. saprophyticus*	*Str.gr. A*	*Str.gr. B*	*Str.gr. G*	*E.faecalis*	*E.coli* (str. 0 – 111)	*Kl.pneumoniae*	*S.typhimurium*	*S.enteritidis*	*Pr.vulgaris*	*Pr.mirabilis*	*Ps.aeruginosa*
	str. Wood	*str. Smith*	*str. 209-P*	*str. gem.*	*str. from a patient*												
HL^1			>300							>300	>300				>300		>300
			>300							>300	>300				>300		>300
HL^4			>300							>300	>300				>300		>300
			>300							>300	>300				>300		>300
HL^5			2.34							1.17	>300				300		>300
			4.68							75	>300				300		>300
HL^6			>300							>300	>300				>300		>300
			>300							>300	>300				>300		>300
I	2.34	2.34	4.68	2.34	2.34	4.68	4.68	1.17	0.29	<4.68	150	300	150	150	75	75	400
	2.34	2.34	4.68	2.34	2.34	9.37	9.37	1.17	0.29	150	150	300	300	150	75	150	400
II	2.34	2.34	4.68	2.34	4.68	4.68	9.37	<0.145	0.29	<4.68	150	150	150	150	37.5	75	300
	2.34	2.34	4.68	2.34	4.68	4.68	37.5	<0.145	1.17	150	150	150	300	150	37.5	75	300
III			4.68							4.68	75				150		300
			9.37							300	75				150		300
IV			4.68							4.68	37.5				300		300
			4.68							150	37.5				300		300
V	0.29		0.145							0.145	150	>300	>300	>300	300	300	>300
	0.58		0.290							150	150	>300	>300	>300	300	300	>300
VI	2.34		1.17							2.34	>300	>300	>300	>300	300	>300	>300
	2.34		2.34							300	>300	>300	>300	>300	300	>300	>300
VII			>300							300	>300				>300		>300
			>300							300	>300				>300		>300
VIII			2.34							9.37	300				300		300
			2.34							9.37	300				300		300
IX			9.37							37.5	75				37.5		150
			9.37							300	75				37.5		300
Furacilin X	9.35	9.35	18.7			9.35				37.5	18.7	>300	75	9.35	150	150	>300
	18.7	9.35	37.5			18.7				75	37.5	>300	150	9.35	300	300	>300

* MIC, minimum concentration of compound inhibiting growth and proliferation of bacteria (bacteriostatic activity)
** MBC, minimum bactericidal concentration (bactericidal activity).

atom, the acid–base properties were so strongly enhanced that complexes with the coordinated neutral thiosemicarbazone became impossible to prepare. Complexes $Cu(L^2)NO_3$ (**V**) and $Cu(L^3)NO_3$ (**VI**) in which the ligand is the anionic form of the corresponding thiosemicarbazone were isolated even from acidic solution. Changing the thiosemicarbazide S atom to O or Se formed $Cu(HL^4)(NO_3)_2{\cdot}2H_2O$ (**VII**) and $Cu(HL^5)(NO_3)_2{\cdot}2H_2O$ (**VIII**), where the organic ligand was also coordinated as the neutral species. The ligands in **I** – **VIII** were tridentate and coordinated to the Cu ion regardless of the state of deprotonation through N, N, and Y donors (Y = O, S, Se) [20, 23]. The ligand with a methylated S (HL^6) in complex **IX** was also tridentate but coordinated through 3 N atoms, forming one six-membered and one five-membered metallocycle. Regardless of the starting metal–ligand ratio and the order of mixing the solutions, products with a Cu–ligand ratio of 1:1 were always obtained. The effective magnetic moments at room temperature for the previously synthesized [19, 20, 23, 24] and newly synthesized complexes (Table 1) fell in the range 1.79 – 1.85 μ_B and indicated that the Cu had oxidation state +2 and that there was no exchange interaction between the paramagnetic centers. EPR spectra of **I** – **III** showed biaxial anisotropy for the *g*-factors with $g_{\|} = 2.206$, 2.194, and 2.209 and $g_{\perp} = 2.059$, 2.052, and 2.057, respectively. Hyperfine structure was not resolved in the parallel *g*-factor. The spectrum of **VI** was isotropic and had a broad line with $g_{iso} = 2.114$. Because $g_{\|} > g_{\perp} > 2.0032$, it was assumed that the ground state was the $d_{x^2-y^2}$ orbital.

EXPERIMENTAL CHEMICAL PART

All HL were synthesized by the literature method [23] in order to prepare 8-quinolinealdehyde semicarbazone starting with 8-quinolinealdehyde and the corresponding chalcogensemicarbazide. The purity of the products was checked by chromatography on Silufol plates, PMR spectra, and elemental analyses for C, H, and N.

Compounds **I**, **IV**, **V**, **VIII**, and **IX** were prepared by the literature methods [20, 23, 24].

(8-Quinolinidenethiosemicarbazide)copper(II) chloride, $Cu(HL^1)Cl_2{\cdot}H_2O$ (II). A solution of $CuCl_2{\cdot}2H_2O$ (0.34 g, 2 mmol) in HCl solution (10 mL, 0.1 M) was treated with a solution of HL^1 (0.46 g, 2 mmol) in the same acid (15 mL). The dark-green crystals that formed after a few minutes were filtered off and washed with H_2O acidified with HCl, EtOH, and Et_2O. The compound was very soluble in water and DMF, less soluble in EtOH, and insoluble in Et_2O. Yield 0.7 g.

(8-Quinolinidenethiosemicarbazide)copper(II) perchlorate, $Cu(HL^1)(ClO_4)_2{\cdot}3H_2O$ (III). A solution of $Cu(ClO_4)_2{\cdot}6H_2O$ (0.37 g, 1 mmol) in $HClO_4$ solution (7 mL, 0.1 M) was treated with HL^1 (0.23 g, 1 mmol) in $HClO_4$ (8 mL, 0.1 M). The dark-green crystals that formed were filtered off and washed with H_2O acidified with $HClO_4$, EtOH, and Et_2O. The compound was very soluble in water, EtOH, and DMF and insoluble in Et_2O. Yield 0.36 g.

[8-Quinolinidene-4-(3′-fluorophenyl)thiosemicarbazidato]copper(II) nitrate, $Cu(L^3)NO_3{\cdot}H_2O$ (VI). The compound was prepared by mixing a warm (~50°C) solution containing $Cu(NO_3)_2{\cdot}3H_2O$ (0.24 g) in EtOH (10 mL) and HL^3 (0.31 g, 1 mmol) in HNO_3 (10 mL) and EtOH (30 mL) at the same temperature. The green crystalline needle-like precipitate that formed on cooling was filtered off, washed with EtOH and Et_2O, and dried at room temperature. The compound was slightly soluble in water and EtOH, very soluble in DMF, and insoluble in Et_2O. Yield 0.43 g.

(8-Quinolinideneselenosemicarbazide)copper(II) nitrate, $Cu(HL^5)(NO_3)_2{\cdot}2H_2O$ (VIII). The compound was prepared by mixing solutions of HL^5 (0.28 g, 1 mmol) in HNO_3 (5 mL, 0.1 M) and $Cu(NO_3)_2{\cdot}3H_2O$ (0.24 g, 1 mmol) in the same volume of HNO_3 (0.1 M). The crystalline precipitate that formed after 4 h was filtered off and washed with H_2O acidified with HNO_3, EtOH, and Et_2O. The compound was very soluble in water and DMF, less soluble in EtOH, and insoluble in Et_2O. Yield 0.42 g.

Table 1 presents the elemental analyses and effective magnetic moments for the newly synthesized complexes.

EXPERIMENTAL BIOLOGICAL PART

Antibacterial activity was studied using double serial dilutions [25] in liquid growth medium in meat-peptone bullion. The studied compounds were dissolved in DMF (10 mg/mL). Successive dilutions to the desired concentration were made using meat-peptone bullion.

The test microbes for the *in vitro* experiment were Gram-positive and Gram-negative microorganisms. The Gram-positive microbes included five strains of *Staphylococcus aureus*, three of which were standard (Wood, Smith, 209-P) and two of which were from patients (gem+ and a strain from a patient) and standard strains *S. saprophyticus*, *Streptococcus* (gr. A, B, G) and *Entrecoccus faecalis* (strain from a patient). The Gram-negative microbes were standard strains *Escherichia coli* (str. 0 – 111), *Klebsiella pneumoniae*, *Salmonella typhimurium*, *S. enteritidis*, *Proteus vulgaris*, *P. mirabilis*, and *Pseudomonas aeruginosa*.

Tubes with given concentrations of compounds in growth medium were inoculated with test cultures using 18-hour agar cultures. The seeding dose was 500,000 microbes per mL of medium. The microbe mixture was prepared using the optical turbidity standard.

The activities of the compounds were determined as the minimum concentration inhibiting growth and proliferation of the microorganisms upon exposure for 1 d in a thermostat at 37°C.

Antifungal properties were studied using double serial dilutions in Sabureau bullion using standard strains *Aspergillus niger*, *A. fumigatus*, *Penicillium*, and *Candida albicans* (strain from a patient). Innoculates were prepared

from 7-day fungus cultures. The density of fungus suspensions was determined using bacterial turbidity standard taking into account that the number of fungi was approximately ten times greater than the number of bacteria.

The studied compounds were dissolved in DMF (10 mg/mL). Successive dilutions to the desired concentration were made in Sabureau bullion. Fungistatic activity was determined visually from the lack of fungus growth in growth medium for 14 d at 28°C. Fungicidal activity was determined from the lack of growth after repeated seeding of fungus on Sabureau agar and subsequent incubation for 7 d at 28°C.

Table 2 shows that 8-quinolinealdehyde thiosemicarbazone (HL^1) had practically no antibacterial properties. Methylation of the thiosemicarbazide on the S atom (HL^6) or replacing the S by O (HL^4) did not improve their biological activity against the studied microorganisms. Only for Y = Se (HL^5) did substantial activity against Gram-positive bacteria appear.

Coordination of the thiosemicarbazones HL changed the activity more substantially. Table 2 shows that coordinating HL^1 to Cu (**I** – **III**) caused a substantial increase of the antibacterial activity against Gram-positive bacteria. These compounds differed in the nature of the anion (NO_3^-, Cl^-, ClO_4^-). However, a comparison of their antibacterial activities could not confirm that the biological activities of this group of compounds depended on the nature of the anion. The activity of **IV**, which differed from **I** by the degree of ligand protonation, was at the same level. Considering the aforementioned protolytic equilibrium and the similarity of the antibacterial activities of **I** and **IV**, it could be assumed that the active species was the complex with the anionic form of the ligand. Complexes **V** and **VI** also contained a deprotonated ligand but were characterized by greater bacteriostatic activity than that of the unsubstituted analog **IV**. This indicated that an electron-accepting substituent on the terminal N atom had a favorable influence on the biological activity. This phenomenon was reported previously for the related 2-pyridinealdehyde thiosemicarbazone [14]. The organic ligand in **I** – **VI** was bound to the central Cu ion by an N,N,S donor set. It seemed interesting to determine how changing the bonding mode of the organic ligand affected the biological activity of the corresponding copper complexes. A convenient subject for this was coordination compound **IX**, in which the Cu:ligand:nitrate ratio remained at 1:1:2, like in **I**. The only difference was that the ligand was coordinated through an N,N,N donor set [24]. Table 2 shows that the bacteriostatic activity of **IX** (against *S. aureus* str. 209-P and *E. faecalis*) was lower than that of **I**. It should be noted that **IX** typically had slightly greater bacteriostatic and bactericidal activity against Gram-negative bacteria although its activity against Gram-positive bacteria was lower.

Although the biological activity of HL^1 changed sharply after complexation to Cu(II), the corresponding semicarbazone HL^4 (**VII**) remained inactive.

The bactericidal activity against *S. aureus* (str. 209-P) practically did not change upon complexation of selenosemicarbazone HL^5 to Cu(II). However, complexation did inhibit somewhat the activity of **VIII** against *E. faecalis* (Table 2).

The study of the antifungal activity of chalcogensemicarbazones HL^{1-6} and their copper complexes **I** – **X** showed that they were slightly active against the fungi *Candida albicans*, *A. niger*, *A. fumigatus*, and *Penicillium* (minimum fungicidal concentration ≥ 600 μg/mL).

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