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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NICKEL AND COPPER COORDINATION COMPOUNDS OF 5-NITROFURAN-2-CARBALDEHYDE N(4)-ALLYL-3-THIOSEMICARBAZONE

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The paper presents the synthesis of the ligand 5-nitrofuran-2-carbaldehyde N(4)-allyl-3-thiosemicarbazone (HL) and eight coordination compounds of copper(II) and nickel(II) with this ligand. The structure of thiosemicarbazone HL was studied using ^{1}H and ^{13}C NMR spectroscopy. For the synthesized compounds their antimicrobial and antifungal activity was studied on a series of standard strains. For HL and $Cu(HL)_2(NO_3)_2$ the antitumor activity towards human leukemia HL-60 cells was estimated. It was established that coordination compounds manifest better activity than free ligand.

Keywords: *complexes, 5-nitrofuran-2-carbaldehyde, thiosemicarbazone, biological activity.*

SINTEZA ŞI ACTIVITATEA BIOLOGICĂ A COMPUŞILOR COMPLECŞI AI NICHELULUI ŞI CUPRULUI CU N(4)-ALIL-3-TIOSEMICARBAZONA 5-NITROFURAN-2-CARBALDEHIDEI

Lucrarea conţine descrierea sintezei N(4)-alil-3-tiosemicarbazonei 5-nitrofuran-2-carbaldehidei (HL) şi a opt compuşi coordinativi ai cuprului (II) şi nichelului (II) cu acest ligand. Structura tiosemicarbazonei HL a fost stabilită în baza datelor spectroscopiei RMN ^{1}H şi ^{13}C. A fost studiată activitatea antimicrobiană şi antifungică a compuşilor sintetizaţi faţă de un spectru larg de tulpini standard. Cercetarea proprităţilor antiproliferative a arătat că compuşii HL şi $Cu(HL)_2(NO_3)_2$ inhibă creşterea şi multiplicarea celulelor leucemiei mieloide umane HL-60. S-a stabilit că compuşii coordinativi manifestă o activitate mai bună decât ligandul.

Cuvinte-cheie: *complecşi, 5-nitrofuran-2-carbaldehidă, tiosemicarbazonă, activitate biologică.*

Introduction

Thiosemicarbazide derivatives are widely used in medicine in treating various types of diseases [1]. All of them have a wide range of reactive atoms and form with metal ions coordination compounds with various composition, structure, and properties [2]. Many of these coordination compounds are biologically active substances [3]. They can be used as base materials for creating new antimicrobial, antifungal, and antitumor drugs, as well as for selective microbiologic nutritional media, disinfectants, antiseptics. Therefore, the synthesis and study of new coordination compounds of biometals with thiosemicarbazones is both of scientific and practical interest. 5-nitro-2-furaldehide moiety can be found in some antimicrobial drugs, such as nitrofural, nitrofurantoin, furazolidone [4].

The aim of this work is finding the conditions of synthesis, determination of the composition, structure, physicochemical, antimicrobial and antitumor properties of the coordination compounds of nickel and copper with 5-nitrofuran-2-carbaldehyde N(4)-allyl-3-thiosemicarbazone.

Experimental

Materials and methods

N(4)-allyl-3-thiosemicarbazide was synthesized by the reaction between allyl isothiocyanate and hydrazine hydrate [5]. 5-nitrofuran-2-carbaldehyde (Sigma-Aldrich) was used as received.

The NMR spectra of free ligand were determined in acetone-d^6 at room temperature on a Bruker DRX-400 spectrometer.

Magnetochemical research was made at room temperature using Gouy method [6].

Quantitative analyses on copper and nickel were made using titration methods [7,8].

Melting point of the free ligand was measured using capillary method [9].

Synthesis of the 5-nitrofuran-2-carbaldehyde N(4)-allyl-3-thiosemicarbazone (HL)

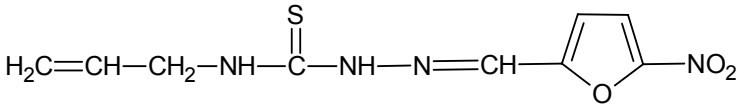
5-nitrofuran-2-carbaldehyde N(4)-allyl-3-thiosemicarbazone was synthesized by refluxing an equimolar mixture of N(4)-allyl-3-thiosemicarbazide with 5-nitrofuran-2-carbaldehyde. The solution of N(4)-allyl-3-thiosemicarbazide (1.31g, 0.01mol) in ethanol was added to the ethanolic solution of 5-nitrofuran-2-carbaldehyde

(1,41g, 0.01mol) and refluxed for 1h. After cooling and slow evaporation at room temperature the yellow precipitate of the synthesized substance appeared. It was filtered out from the solution and dried.

Melting point: 169-171^{0}C

^{1}H NMR (acetone-d^6, δ (ppm)): 10.93 (br, 1H, NH), 8.52 (br, 1H, NH), 8.12 (s, 1H, CH=N), 7.62(d, 1H, CH from furan moiety, J=3.9Hz), 7.21 (d, 1H, CH from furan moiety, J=3.9Hz), 5.97 (m, 1H, CH from allyl moiety), 5.18 (m, 2H, CH_2=C), 4.35 (m, 2H, CH_2-N).

^{13}C NMR (acetone-d^6, δ (ppm)): 178.47 (C=S), 152.27, 134.26, 129.28, 115.60, 113.82, 113.36, 46.33.



Scheme 1. HL ligand

Synthesis of coordination compounds

The complexes (I-VIII) were obtained by stirring a hot solution of HL in ethanol with the corresponding copper and nickel salts in 2:1 molar ratio: $CuCl_2 \cdot 2H_2O$ (I), $CuBr_2$ (II), $Cu(NO_3)_2 \cdot 3H_2O$ (III), $CuSO_4 \cdot 5H_2O$ (IV), $Cu(ClO_4)_2 \cdot 6H_2O$ (V), $NiCl_2 \cdot 6H_2O$ (VI), $Ni(NO_3)_2 \cdot 6H_2O$ (VII), $(CH_3COO)_2Ni \cdot 4H_2O$ (VIII). After cooling the brown precipitates of corresponding coordination compounds were filtered, washed with small amounts of cold ethanol and dried.

Antibacterial bioassay

The antibacterial activity of complexes was determined under liquid nutritive environment [2% of peptonate bullion (pH 7.0)] using successive dilutions method. *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (GISK 8035), *Escherihia coli* (ATCC 25922), *Salmonella abony* (GISK 03/03) standard stems were used as reference culture for in vitro experiment. The dissolution of studied substances in dimethylformamide, microorganisms' cultivation, suspension obtaining, determination of minimal inhibition concentration (MIC) and minimal bactericide concentration (MBC) were carried out according to the previously reported method.

Antifungal bioassay

Antimycotic properties of the synthesized substances were investigated in vitro on laboratory stems of *Candida albicans*. The activity was determined in liquid Sabouroud nutritive environment (pH 6.8). The inoculates were prepared from fungi stems which were harvested during 3–7 days. Their concentration in suspension is (2–4) or 10^6 colonies forming units/mh. Sowings for levures and micelles were incubated at 37^{0}C during 7 and 14 days, respectively.

Cell culture. Human promyelocytic leukemia cells HL-60 (ATCC, Rockville, MD, USA) were routinely grown in suspension in 90% RPMI-1640 (Sigma, Saint Louis, USA) containing *L*-glutamine (2 nM), antibiotics (100 IU penicillin/ml, 100 µg streptomycin/ml) and supplemented with 10% (v/v) foetal bovine serum (FBS), in a 5% CO_2 humidified atmosphere at 37°C. Cells were currently maintained twice a week by diluting the cells in RPMI 1640 medium containing 10% FBS.

Cell proliferation assay. The cell proliferation assay was performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Cell Titer 96 Aqueous, Promega, USA), which allowed us to measure the number of viable cells. In brief, triplicate cultures of 1 x 10^4 cells in a total of 100 µl medium in 96-well microtiter plates (Becton Dickinson and Company, Lincoln Park, NJ, USA) were incubated at 37°C, 5% CO_2. Compounds were dissolved in ethanol to prepare the stock solution of 1 x 10^{-2} M. These compounds and doxorubicin (Novapharm, Toronto, Canada) was diluted at multiple concentrations with culture media, added to each well and incubated for 3 days. Following each treatment, 20 µl MTS was added to each well and incubated for 4 h. MTS is converted to water-soluble colored formazan by a dehydrogenase enzyme present in metabolically active cells. Subsequently, the plates were read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA).

Results and discussion

The Shiff base HL has been prepared by a known method [10]. The structure of HL was determined by ^{1}H and ^{13}C NMR spectroscopy. All complexes were prepared by the direct reaction between the ligand HL and the corresponding metal salts. The obtained coordination compounds are microcrystalline solids and are stable in air. The elemental analyses on copper and nickel suggest the general formulae $M(HL)_2X_2$ ($M=Cu^{2+}$, Ni^{2+}; $X=Cl^-$, Br^-, NO_3^-, ClO_4^{2-}, $½SO_4^{2-}$) and NiL_2.

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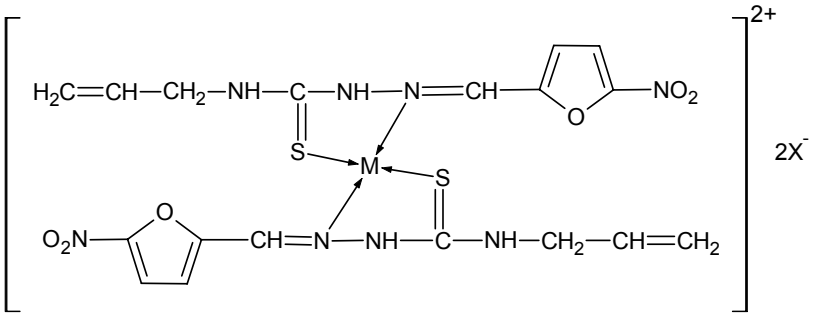
Table 1

Physical and analytical data of the metal complexes (I-VIII)

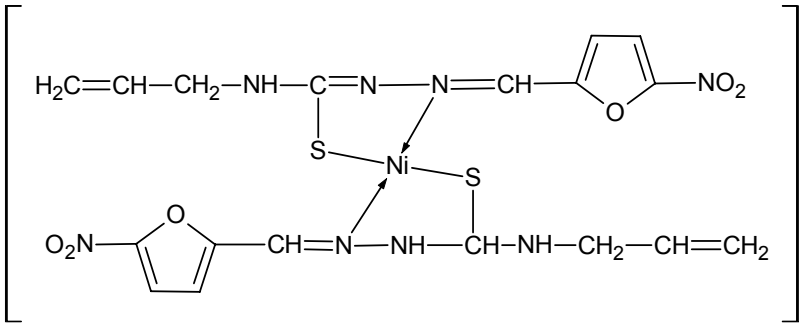
No.	Compound	Formula	Found / calculated, Cu %	μ_{eff} MB
I	$Cu(HL)_2Cl_2$	$C_{18}H_{20}CuCl_2N_8O_6S_2$	9,69/9,88	1,81
II	$Cu(HL)_2Br_2$	$C_{18}H_{20}CuBr_2N_8O_6S_2$	8,73/8,68	2,20
III	$Cu(HL)_2(NO_3)_2$	$C_{18}H_{20}CuN_{10}O_{12}S_2$	9.32/9,13	1,78
IV	$Cu(HL)_2SO_4$	$C_{18}H_{20}CuN_8O_{10}S_3$	9,44/9,51	1,82
V	$Cu(HL)_2(ClO_4)_2$	$C_{18}H_{20}CuCl_2N_8O_{14}S_2$	8.65/8.24	1,92
VI	$Ni(HL)_2Cl_2$	$C_{18}H_{20}NiCl_2N_8O_6S_2$	9,37/9,20	diamagnetic
VII	$Ni(HL)_2(NO_3)_2$	$C_{18}H_{20}NiN_{10}O_{12}S_2$	8,58/8,49	diamagnetic
VIII	NiL_2	$C_{18}H_{20}NiN_8O_6S_2$	9,97/10.35	diamagnetic

The magnetochemical research showed that the synthesized coordination compounds of copper are monomeric, coordination compounds of nickel have a square-planar structure. It was supposed that the synthesized thiosemicarbazone HL behaves as neutral or mono-deprotonated bidentate ligand with N, S set of donor atoms. It coordinates to the central ions with azomethinic nitrogen atom and sulfur atom forming a five-membered metallacycle. The proposed structures of the coordination compounds are shown in scheme 2.



$M=Cu^{2+}, Ni^{2+}$

$X=Cl^-, Br^-, NO_3^-, ClO_4^-, {}^1/_2\ SO_4^{2-}$



Scheme 2. Proposed structures of the metal complexes.

In order to find out the biological properties of complexes it was studied their antimicrobial and antifungal activity. The results are shown in table 2.

Table 2

The minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) (mg/mL)

Compound	*Escherichia coli*, ATCC 25922		*Salmonella abony* GISK 03/03		*Staphylococcus aureus*, ATCC 25923		*Bacillus cereus* GISK 8035		*Candida albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
HL	>10,0	>10,0	>10,0	>10,0	>10,0	>10,0	>10,0	>10,0	>10,0	>10,0
I	0,5	1.0	0,25	0,5	0,25	0,5	0,25	0,5	0,5	2.0
II	0.25	0.5	0.25	0.25	0.25	0.5	0.25	0.5	0.5	0.5
III	0.5	1.0	0.25	0.5	0.25	0.5	0.5	1.0	0.5	0.5
IV	1.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0
V	1.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0
VI	0.25	0.5	0.5	0.5	0.5	1.0	0.5	1.0	0.5	1.0
VII	1.0	1.0	1.0	2.0	1.0	0.5	1.0	1.0	1.0	1.0
VIII	0.12	0.5	0.25	0.5	0.12	0.25	0.25	0.5	0.12	0.25

It was found, that the thiosemicarbazone HL doesn't manifest antimicrobial and antifungal activities in the studied range of concentrations. On the other hand, the coordination compounds show selective antimicrobial and antifungal activity towards a series of standard strains *Staphylococcus aureus (ATCC 25923), Bacillus cereus (GISK 8035), Escherihia coli (ATCC 25922), Salmonella abony (GISK 03/03)*, and *Candida albicans* in the range of concentration 0.12-2.0 mg/mL. It was shown that the nature of the acid residue has an influence on the antimicrobial activity of these complexes. For the homotypic complexes the minimal bacteriostatic and bactericidal concentrations diminish in the following way: $ClO_4^- \approx SO_4^{2-} > NO_3^- > Cl^- > Br^-$.

It was also studied the antitumor activity of two of these substances. The percent of inhibited human leukemia HL-60 cells is shown in the table 3.

Table 3

Percent of inhibited human leukemia HL-60 cells[a], %

Compound	Concentration, mol/L		
	10^{-5}	10^{-6}	10^{-7}
HL	57.7	0	0
$Cu(HL)_2(NO_3)_2$	66.0	0	0

[a] - SEM < ± 4% of a single experiment in triplicate

It is determined that the studied copper coordination compound is more active than the free ligand at the concentration 10^{-5}mol/L. Both ligand and coordination compound lose antitumor activity at lower concentrations (10^{-6} and 10^{-7} mol/L).

Conclusions

In this work 5-nitrofuran-2-carbaldehyde N(4)-allyl-3-thiosemicarbazone was synthesized and studied using NMR spectroscopy. This ligand was used for synthesis of eight coordination compounds of copper and nickel. These compounds were studied using elemental analysis, magnetochemistry, and biological testing. It was determined, that the coordination compounds show antibacterial and antifungal activities. Coordination compound $Cu(HL)_2(NO_3)_2$ and free ligand HL show antitumor activity. They inhibit the proliferation of the human leukemia HL-60 cells at the concentration 10^{-5}mol/L by 66.0 and 57.7%, respectively. Coordination compounds are more active than the free ligand.

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