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 ȘI ACTIVITATEA BIOLOGICĂ A COMPUȘILOR COMPLEȘI AI NICHELULUI ȘI CUPRULUI CU N(4)-ALLYL-3-THIOSEMICARBAZONA 5-NITROFURAN-2-CARBALDEHIDEI

Lucrarea conține descrierea sintezei N(4)-allyl-3-thiosemicarbazonei 5-nitrofuran-2-carbaldehydei (HL) și a opt compuși coordinativi ai cuprului (II) și nichelului (II) cu acest ligand. Structura tiosemicarbazonei HL a fost stabilită în baza datelor spectroscopicii RMN $^1$H și $^{13}$C. A fost studiată activitatea antimicrobiană și antifungică a compușilor sintetizați față de un spectru larg de tulpi de standard. Cercetarea proprietăților antiproliferative a arătat că compușii HL și Cu(HL)$_2$(NO$_3$)$_2$ inhibă creșterea și multiplicarea celulelor leucemiei mieloide umane HL-60. S-a stabilit că compușii coordinativi manifestă o activitate mai bună decât ligandul.

**Cuvinte-cheie:** compuși, 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-furaldehyde moiety can be found in some antimicrobial drugs, such as 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.

Magnetocochemical 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°C

\( ^1H \) NMR (acetone-\( d_6 \), \( \delta \) (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).

\( ^13C \) NMR (acetone-\( d_6 \), \( \delta \) (ppm)): 178.47 (C=S), 152.27, 134.26, 129.28, 115.60, 113.82, 113.36, 46.33.

\[
\text{Scheme 1. HL ligand}
\]

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\(_2\)·2H\(_2\)O (I), CuBr\(_2\) (II), Cu(NO\(_3\))\(_2\)·3H\(_2\)O (III), CuSO\(_4\)·5H\(_2\)O (IV), Cu(ClO\(_4\))\(_2\)·6H\(_2\)O (V), NiCl\(_2\)·6H\(_2\)O (VI), Ni(NO\(_3\))\(_2\)·6H\(_2\)O (VII), (CH\(_3\)COO)\(_2\)Ni·4H\(_2\)O (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°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 \(^1H\) and \(^13C\) 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)\(_2\)X\(_2\) (M=Cu\(^{2+}\), Ni\(^{2+}\); X=Cl\(^-\), Br \(^-\), NO\(_3\)-, ClO\(_4\)-, \(\frac{1}{2}\)SO\(_4\)-) and NiL\(_2\).
Table 1

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Formula</th>
<th>Found / calculated, Cu %</th>
<th>µ_eff MB</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Cu(HL)₂Cl₂</td>
<td>C₁₈H₂₀CuCl₂N₈O₆S₂</td>
<td>9,69/9,88</td>
<td>1,81</td>
</tr>
<tr>
<td>II</td>
<td>Cu(HL)₂Br₂</td>
<td>C₁₈H₂₀CuBr₂N₈O₆S₂</td>
<td>8,73/8,68</td>
<td>2,20</td>
</tr>
<tr>
<td>III</td>
<td>Cu(HL)₂(NO₃)₂</td>
<td>C₁₈H₂₀CuN₁₀O₁₂S₂</td>
<td>9,32/9,13</td>
<td>1,78</td>
</tr>
<tr>
<td>IV</td>
<td>Cu(HL)₂SO₄</td>
<td>C₁₈H₂₀CuN₈O₁₀S₃</td>
<td>9,44/9,51</td>
<td>1,82</td>
</tr>
<tr>
<td>V</td>
<td>Cu(HL)₂(ClO₄)₂</td>
<td>C₁₈H₂₀CuCl₂N₈O₁₄S₂</td>
<td>8,65/8,24</td>
<td>1,92</td>
</tr>
<tr>
<td>VI</td>
<td>Ni(HL)₂Cl₂</td>
<td>C₁₈H₂₀NiCl₂N₈O₆S₂</td>
<td>9,37/9,20</td>
<td>diamagnetic</td>
</tr>
<tr>
<td>VII</td>
<td>Ni(HL)₂(NO₃)₂</td>
<td>C₁₈H₂₀NiN₁₀O₁₂S₂</td>
<td>8,58/8,49</td>
<td>diamagnetic</td>
</tr>
<tr>
<td>VIII</td>
<td>NiL₂</td>
<td>C₁₈H₂₀NiN₈O₆S₂</td>
<td>9,97/10,35</td>
<td>diamagnetic</td>
</tr>
</tbody>
</table>

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.

![Scheme 2. Proposed structures of the metal complexes.](image)

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Escherichia coli, ATCC 25922</th>
<th>Salmonella abony GISK 03/03</th>
<th>Staphylococcus aureus, ATCC 25923</th>
<th>Bacillus cereus GISK 8035</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
</tr>
<tr>
<td>HL</td>
<td>&gt;10,0</td>
<td>&gt;10,0</td>
<td>&gt;10,0</td>
<td>&gt;10,0</td>
<td>&gt;10,0</td>
</tr>
<tr>
<td>I</td>
<td>0.5</td>
<td>1.0</td>
<td>0.25</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>II</td>
<td>0.25</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>III</td>
<td>0.5</td>
<td>1.0</td>
<td>0.25</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>IV</td>
<td>1.0</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>V</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>VI</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>VII</td>
<td>0.12</td>
<td>0.5</td>
<td>0.25</td>
<td>0.5</td>
<td>0.12</td>
</tr>
</tbody>
</table>

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), *Escherichia 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: $\text{ClO}_4^->\text{SO}_4^{2-}>\text{NO}_3^->\text{Cl}^->\text{Br}^-$. 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

<table>
<thead>
<tr>
<th>Percent of inhibited human leukemia HL-60 cells*, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration, mol/L</strong></td>
</tr>
<tr>
<td><strong>10^{-5}</strong></td>
</tr>
<tr>
<td>HL</td>
</tr>
<tr>
<td>Cu(HL)$_2$(NO$_3$)$_2$</td>
</tr>
</tbody>
</table>

* - 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.

**Acknowledgements**

We would like to thank Professor Donald Poirier from the Oncology and Molecular Endocrinology Research Center CHUL, Laval University for determination of the antitumor activity of our substances.

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References:


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