SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF COPPER(II), NICKEL(II), COBALT(III) AND IRON(III) COORDINATION COMPOUNDS WITH 1-(2-HYDROXYPHENYL)ETHANONE N(4)-ALLYL-S-METHYLISOTHIOSEMICARBAZONE

Irina USATAIA
State University of Moldova

The paper presents the synthesis of the 1-(2-hydroxyphenyl)ethanone N(4)-allyl-S-methylisothiosemicarbazone (HL) and seven coordination compounds of copper, nickel, cobalt and iron with this pro-ligand. The newly obtained compounds were investigated by IR, 1H and 13C NMR spectroscopy, elemental analysis, molar electric conductivity and magnetic susceptibility. For the synthesized compounds the antibacterial and antifungal activities in vitro were studied on a series of standard strains, such as Staphylococcus aureus (ATCC 25923), Escherihia coli (ATCC 25922), Klebsiella pneumoniae and Candida albicans. The in vitro antiproliferative activity of the pro-ligand and complexes was screened on Hep-2, BxPC-3, RD cancer cells and normal MDCK cells. It was established that coordination compounds manifest better antiproliferative activity than the pro-ligand.

Keywords: coordination compounds, 1-(2-hydroxyphenyl)ethanone, isothiosemicarbazone, biological activity.

Introduction
Isothiosemicarbazones and their metal complexes manifest a wide range of biological properties such as antimicrobial, antifungal, antituberculous, cytotoxic activities [1-3]. However, there is a limited number of studies on S-alkylisothiosemicarbazones, in spite of their importance in selective biological activity. Most of the medicaments existing today have high toxicity, so the search for new substances with lower toxicity and more effective anticancer drugs are of great interest. Some 1-(2-hydroxyphenyl)ethanone 3-thiosemicarbazones are already described in scientific literature [4,5].

The aim of this work is finding the conditions of synthesis, determination of the composition and physico-chemical properties of the copper, nickel, cobalt and iron coordination compounds with 1-(2-hydroxyphenyl)ethanone N(4)-allyl-S-methylisothiosemicarbazone.

Experiment
Materials and methods
N(4)-allyl-3-thiosemicarbazide was synthesized by the reaction between allyl isothiocyanate and hydrazine hydrate [6]. 1-(2-hydroxyphenyl)ethanone (Sigma-Aldrich), metal salts were used as received.
The 1H and 13C NMR spectra were recorded on a Bruker DRX-400, using CDCl3 as a solvent. The chemical shifts (δ) in ppm were measured relative to tetramethylsilane (TMS). Infrared spectra of the compounds were recorded on a Bruker ALPHA FTIR spectrophotometer at room temperature in the range of 4000-400 cm−1. Magnetochemical research was made at room temperature using Gouy method [7].
The determination of metal content in the synthesized coordination compounds, was performed similarly to the literature procedures [8-11].

Melting point of the pro-ligand was measured using capillary method [12].

Molar conductivity values were determined in 10⁻³ mol/L methanol solutions using slidewire bridge R-38.

**Synthesis of the 4-allyl-3-thiosemicarbazide**

4-Alllyl-3-thiosemicarbazide (Scheme 1) was prepared similarly to the literature procedure [13].

White solid. Yield: 84%; m.p.: 91-93 °C; FW: 131.1994 g/mol;

Main IR peaks (cm⁻¹): ν(NH₂), 3339, 3270; ν(N³-H), ν(N⁴-H) 3189, 3162; ν(C=C allyl) 1641; ν(C=S) 1226. ¹H NMR (CDCl₃; δ, ppm): 8.71 (br, 1H, NH); 7.54 (br, 1H, NH); 5.73 (m, 1H, CH from allyl moiety); 5.25 (m, 2H, CH₂=C); 4.28 (m, 2H, CH₂-N); 3.78 (br, 2H, NH₂).

¹³C NMR (CDCl₃; δ, ppm): 177.51 (C=S); 132.43 (CH from allyl moiety); 116.15 (CH₂=); 46.77 (CH₂-N).

![Scheme 1. Synthesis of the 4-allyl-3-thiosemicarbazide.](image)

**Synthesis of the 1-(2-hydroxyphenyl)ethanone N(4)-allyl-S-methylisothiosemicarbazone (HL)**

1-(2-Hydroxyphenyl)ethanone N(4)-allyl-S-methylisothiosemicarbazone (HL) (Scheme 2) was prepared according to the modification of the procedure described in the literature [14].

4-Alllyl-3-thiosemicarbazide (1.31 g, 10 mmol) was dissolved in 20 mL of ethanol with constant stirring. After that iodomethane (1.56 g, 11 mmol) was added. The mixture was stirred at room temperature for 2 hours and 1-(2-hydroxyphenyl)ethanone (1.36 g, 10 mmol) was added. The solution was stirred at 80 °C for 30 min. After the reaction mixture was cooled to room temperature, the yellow solid was isolated by filtration, washed with ethanol and dried in vacuo.

![Scheme 2. Synthesis of 1-(2-hydroxyphenyl)ethanone 4-allyl-S-methylisothiosemicarbazone hydroiodide.](image)

Sodium carbonate (1.06 g, 10 mmol) was added to the solution of 1-(2-hydroxyphenyl)ethanone 4-allyl-S-methylisothiosemicarbazone hydroiodide (3.91 g, 10 mmol).

After the reaction mixture was cooled to room temperature, the 1-(2-hydroxyphenyl)ethanone 4-allyl-S-methylisothiosemicarbazone was extracted with chloroform from the reaction mixture. After evaporation yellow solid was obtained (Scheme 3).

![Scheme 3. Neutralization of 1-(2-hydroxyphenyl)ethanone 4-allyl-S-methylisothiosemicarbazone hydroiodide.](image)
Yellow solid. Yield: 80%; m.p.: 50-52 °C; FW: 263.36 g/mol.
Main IR peaks (cm⁻¹): v(OH) 3387, v(C=C allyl) 1644, v(C=N) 1595, v(C–O) 1245, v(CH₂–S) 1067, v(C=C) 622.

1st tautomeric form (HL(A) on Scheme 4): ¹H NMR (CDCl₃; δ, ppm): 13.51 (br, 1H, OH); 7.50 (d, 1H, CH aromatic); 7.26 (t, 1H, CH aromatic); 6.96 (d, 1H, CH aromatic); 6.86(t, 1H, CH aromatic); 5.87 (m, 1H, CH from allyl moiety); 5.52 (br, 1H, NH); 5.19 (m, 2H, CH₂=C); 3.9 (m, 2H, CH₂-N); 2.49 (s, 3H, CH₃-S); 2.41 (s, 3H, CH₃). ¹³C NMR (CDCl₃; δ, ppm): 159.71 (C-S); 163.77, 134.06, 128.25, 120.43, 118.74, 117.11 (C aromatic); 159.40 (CH₂-C’=N); 130.83 (CH from allyl moiety); 116.80 (CH₂=C); 45.80 (CH₂-N); 13.77 (CH₃); 13.19 (CH₂-S).

2nd tautomeric form (HL(B) on Scheme 4): ¹H NMR (CDCl₃; δ, ppm): 13.51 (br, 1H, OH); 7.51 (d, 1H, CH aromatic); 7.23 (t, 1H, CH aromatic); 6.98 (d, 1H, CH aromatic); 6.86 (t, 1H, CH aromatic); 5.98 (m, 1H, CH from allyl moiety); 5.22 (m, 2H, CH₂=C); 4.60 (br, 1H, NH); 4.08 (m, 2H, CH₂-N); 2.50 (s, 3H, CH₃-S); 2.40 (s, 3H, CH₃). ¹³C NMR (CDCl₃; δ, ppm): 159.54 (C-S); 161.77, 134.14, 127.97, 120.27, 118.40, 117.28 (C aromatic); 159.31 (CH₂-C’=N); 130.46 (CH from allyl moiety); 117.05 (CH₂=C); 46.20 (CH₂-N); 13.56 (CH₃); 13.23 (CH₂-S).

**Synthesis of coordination compounds**

The complexes (I-IV) were obtained by stirring a hot solution of HL in ethanol with the corresponding copper and nickel salts in 1:1 molar ratio: CuCl₂·2H₂O (I), Cu(NO₃)₂·3H₂O (II), Cu(CH₃COO)₂·H₂O (III), Ni(CH₃COO)₂·2H₂O (IV). Cobalt and iron coordination compounds (V-VII) were synthesized similarly, but in 1:2 molar ratio: Co(NO₃)₂·6H₂O (V), CoBr₂·6H₂O (VI), Fe(NO₃)₂·6H₂O (VII). After cooling green (in case of complexes I-III) or brown (in case of complexes IV-VII) precipitates of corresponding coordination compounds were filtered, washed with small amounts of cold ethanol and dried.

**Biological studies**

**Antibacterial bioassay**

The antibacterial activity of complexes was determined under liquid nutritive environment [2% of peptone bullion (pH 7.0)] using successive dilutions method. *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* standard stems were used as reference culture for in vitro experiment. The dissolution of studied substances in dimethyl sulfoxide, 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 Sabouraud nutritive environment (pH 6.8). The inoculums were prepared from fungi stems which were harvested during 3-7 days. Their concentration in suspension is (2-4) or 10⁶ colonies forming units/ml. Sowings for levers and micelles were incubated at 37 °C during 7 and 14 days, respectively.

**Cell proliferation assay of Hep-2, BxPC-3, RD cells**

Cells were trypsinized Trypsin-ethylenediaminetetraacetic acid (trypsin-EDTA) 0.05% (Invitrogen) and counted under an inverted microscope (OLYMPUS). The cell proliferation assay was performed using resazurin (7-hydroxy-3H-phenoxazin-3-one-10-oxide sodium salt) (SIGMA), which allowed us to measure the number of viable cells.

In brief, plate out, in triplicate of 1·10⁴ 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, 2% CO₂. Compounds were dissolved in dimethyl sulfoxide to prepare the stock solution of 10mM. These compounds and doxorubicin was diluted at multiple concentrations with culture media, added to each well and incubated for 24 hours. Following each treatment, 20 µL resazurin indicator solution was added to each well and incubated for 4 hours. Subsequently, the absorbance was read with 570 nm and 600 nm filters. The measurement was made by imaging hybrid reader (Synergy H1, Biotek).

The percentage inhibition was calculated according to the formula:

\[
100 - \left( \frac{\text{Abs}_{570\text{nm sample}} - \text{Abs}_{600\text{nm sample}}}{\text{Abs}_{570\text{nm control}} - \text{Abs}_{600\text{nm control}}} \right) \times 100
\]

The IC₅₀ values were evaluated by statistical software.
MDCK cell culture

Madin-Darby canine kidney cells of line MDCK (ATCC) p.3-4 and epitheloid cervix carcinoma cells of line HeLa p.4-6 (SIGMA) were used. They were cultured as monolayers in Dulbecco’s Modified Eagle Medium (D-MEM) high glucose (Invitrogen) containing L-glutamine, bovine albumin fraction (V7.5%) 0.2% v/v (Invitrogen), HEPES buffer (N-2 hydroxyethylpiperazine–N’-2-ethane sulfonic acid) 20mM (Invitrogen), antibiotics penicillin-streptomycin (final concentration 100 U/mL penicillin and 100 μg/mL streptomycin sulfate) (Invitrogen) and supplemented with fetal bovine serum (FBS-irradiated) 10% v/v (Cambrex) in culture conditions (2% CO₂, 78% air in humidified chamber at 37°C).

Results and discussion

The pro-ligand HL and seven new metal complexes were synthesized in ethanol in good yield. The structure and purity of HL were determined by 1H and 13C NMR spectroscopy. All complexes were prepared by the direct reaction between the pro-ligand HL and the corresponding metal salts. The obtained coordination compounds are microcrystalline solids and are stable in air. The elemental analyses on copper, nickel, cobalt and iron suggest the general formulae M(L)X (M=Cu²⁺, Ni²⁺; X=Cl⁻, Br⁻, NO₃⁻, CH₃COO⁻) and M(L)₂X (M=Co³⁺, Fe³⁺; X=Br⁻, NO₃⁻).

Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Formula</th>
<th>ηε, %</th>
<th>Found/calculated, metal %</th>
<th>µeff, MB</th>
<th>λ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Cu(L)Cl</td>
<td>C₁₀H₁₈ClCuN₃O₅S</td>
<td>75</td>
<td>17.23/17.59</td>
<td>1.96</td>
<td>88</td>
</tr>
<tr>
<td>II</td>
<td>Cu(L)NO₃</td>
<td>C₁₀H₁₈CuN₃O₅S</td>
<td>89</td>
<td>16.72/16.38</td>
<td>1.78</td>
<td>101</td>
</tr>
<tr>
<td>III</td>
<td>Cu(L)OAc</td>
<td>C₁₀H₁₈CuN₃O₅S</td>
<td>71</td>
<td>16.88/17.04</td>
<td>1.83</td>
<td>102</td>
</tr>
<tr>
<td>IV</td>
<td>Ni(L)OAc</td>
<td>C₁₀H₁₈NiN₂O₅S</td>
<td>74</td>
<td>15.20/15.44</td>
<td>dia⁴</td>
<td>85</td>
</tr>
<tr>
<td>V</td>
<td>Co(L)₂NO₃</td>
<td>C₂₀H₂₆CoN₃O₇S₂</td>
<td>80</td>
<td>8.92/9.13</td>
<td>dia⁴</td>
<td>98</td>
</tr>
<tr>
<td>VI</td>
<td>Co(L)₂Br</td>
<td>C₂₀H₂₆BrCoN₆O₂S₂</td>
<td>83</td>
<td>8.59/8.88</td>
<td>dia⁴</td>
<td>109</td>
</tr>
<tr>
<td>VII</td>
<td>Fe(L)₂NO₃</td>
<td>C₂₀H₂₆FeN₃O₇S₂</td>
<td>88</td>
<td>8.15/8.69</td>
<td>5.75</td>
<td>75</td>
</tr>
</tbody>
</table>

a – yield; b – effective magnetic moments at room temperature (293K); c – molar conductivity in methanol at room temperature, Ω⁻¹ cm² mol⁻¹; d – diamagnetic.

In the NMR spectra all peaks of isothiosemicarbazone HL are double [15]. It indicates the presence of tautomer forms of isothiosemicarbazone in solution. The integral ratio between two tautomeric forms is 1:3.3 (HL(A):HL(B)). The presence of tautomer forms can be caused by syn/anti isomerism around C=N¹ double bond, and cis/trans (Z/E) isomerism around C=N² double bond (Scheme 4).

Scheme 4. The tautomeric forms of the pro-ligand HL.

The molar conductivity values of the synthesized complexes (I-VII) are in the range 75 - 109 Ω⁻¹ cm² mol⁻¹ that indicates that complexes (I-VII) represent 1:1 electrolytes [16, 17]. The corresponding anion (Cl⁻, Br⁻, NO₃⁻, CH₃COO⁻) can be either in the outer sphere or in the inner sphere as it can be easily substituted by the solvent molecule during dissolution process.

The magnetochemical research showed that the synthesized copper coordination compounds (I-III) have monomeric structure because the effective magnetic moments for the synthesized complexes I-III vary in the range of 1.78-1.96 μµ which are close to the spin value for one unpaired electron. The nickel complex (IV) is diamagnetic that indicates that this coordination compound has square-planar coordination geometry. The cobalt and iron coordination compounds have octahedral structure. The cobalt complexes (V-VI) are diamagnetic that indicates that cobalt (II) is oxidized by oxygen from air to cobalt (III) during the synthesis.
The synthesis of the complexes is reproducible and the isothiosemicarbazone HL coordinates as a mono-negative tridentate ligand with ONN-set of donor atoms. Type of the ligand coordination with the central ions was elucidated from comparative analysis of IR spectra of complexes (I-VII) and the pro-ligand HL. It coordinates to the central ions by deprotonated phenolic oxygen atom, azomethinic and thiocarbamide nitrogen atoms forming five- and six-membered metallacycles. The proposed distribution of chemical bonds in the coordination compounds is shown in scheme 5.

For the synthesized compounds the antibacterial and antifungal activities in vitro were studied on a series of standard strains. The study of antibacterial and antifungal activities (Table 2) showed that HL and its coordination compounds possess bacteriostatic and bactericidal activities. The activity of the synthesized compounds towards gram-negative microorganisms is less pronounced than towards gram-positive bacteria and fungi.

### Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Escherichia coli, ATCC 25922</th>
<th>Staphylococcus aureus, ATCC 25923</th>
<th>Klebsiella pneumoniae</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td>HL·HI</td>
<td>0.5</td>
<td>0.5</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>I</td>
<td>0.12</td>
<td>0.12</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>II</td>
<td>0.03</td>
<td>0.06</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>III</td>
<td>0.12</td>
<td>0.12</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>IV</td>
<td>0.5</td>
<td>0.05</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>V</td>
<td>0.5</td>
<td>0.05</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>VI</td>
<td>0.5</td>
<td>0.05</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>VII</td>
<td>0.5</td>
<td>0.5</td>
<td>0.03</td>
<td>0.06</td>
</tr>
</tbody>
</table>
The HL-HI and coordination compounds show selective antimicrobial and antifungal activity towards a series of standard strains *Staphylococcus aureus* (ATCC 25923), *Escherihia coli* (ATCC 25922), *Klebsiella pneumoniae*, and *Candida albicans* in the range of concentration 0.03-0.5 mg/mL. It was found that the copper complexes are the most active ones. The comparison of antibacterial and antifungal activities of these compounds against the selected types of bacteria indicates that the activity of the coordination compounds decreases in the following way Cu(II) > Fe(III) ≈ Co(III) ≈ Ni(II).

It was also studied the antitumor activity of the pro-ligand and complexes on Hep-2, BxPC-3, RD cancer cells and normal MDCK cells.

In order to find out the biological properties it was studied the antitumor activity of the 1-(2-hydroxyphenyl)ethanone N(4)-allyl-S-methylisothiosemicarbazone (HL) and its coordination compounds. The IC₅₀ values of these substances are shown in scheme 6.

![Scheme 6. IC₅₀ values of tested substances.](image)

It was determined that the studied copper, cobalt and iron coordination compounds are more active than the pro-ligand and nickel complex. The complexes I and III manifest better activity than doxorubicine that is used in medical practice.

The study of the influence of synthesized compounds on healthy MDCK cells, showed that they have lower cytotoxic effect on healthy cells of human organism. The cobalt complexes (V-VI) showed promising antiproliferative activity and low toxicity.

**Conclusions**

In this work 1-(2-hydroxyphenyl)ethanone N(4)-allyl-S-methylisothiosemicarbazone was synthesized and studied using NMR spectroscopy. This pro-ligand was used for synthesis of seven coordination compounds of copper, nickel, cobalt and iron. These compounds were studied using elemental analysis, molar conductivity, and magnetochemistry. The copper coordination compounds (I-III) have monomeric structure. The nickel (IV) complex is square-pyramidal, cobalt (V-VI) and iron (VII) complexes are octahedral. It was determined, that the coordination compounds show antibacterial and antifungal activities.

The synthesized compounds selectively inhibit the Hep-2, BxPC-3, RD cancer cells growth in the range of concentrations 10⁻⁵-10⁻⁷ mol/L. The cobalt complexes (V-VI) inhibit proliferation of cancer cells and does not affect the growth of normal cells.
References:


Acknowledgements:
We would like to thank Olga Garbuz, Moldova State University, for determination of the antitumor activity of our substances.

Date despre autor:
Irina USATAIA, doctorandă, Școala doctorală Științe Chimice, Universitatea de Stat din Moldova.

Prezentat la 20.11.2017