SYNTHESIS AND ANTITUMOR ACTIVITY OF COPPER, NICKEL AND COBALT COORDINATION COMPOUNDS WITH 1-(2-HYDROXYPHENYL)ETHANONE N(4)-ALLYL-3 THIOSEMICARBAZONE

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The paper presents the synthesis of the ligand 1-(2-hydroxyphenyl)ethanone N(4)-allyl-3-thiosemicarbazone (H₂L) and six coordination compounds of copper, nickel and cobalt with this ligand. The structure of thiosemicarbazone H₂L was studied using ¹H and ¹³C NMR spectroscopy. The synthesized coordination compounds were studied using elemental analysis, gravimetric analysis of water content, molar conductivity, and magnetochemistry. For H₂L the antitumor activity towards human leukemia HL-60 cells and cervical cancer HeLa cells was determined. It was established that the substitution of hydrogen atom with methyl group in the azomethinic fragment leads to the growth of antitumor activity.

Keywords: complexes, 1-(2-hydroxyphenyl)ethanone, thiosemicarbazone, antitumor activity.

Introduction

Thiosemicarbazide derivatives contain a wide range of donor atoms and form with transition metal ions coordination compounds with various composition, structure, and properties [11]. Many of these coordination compounds possess antimicrobial, antifungal, antitumor and other kinds of biological activities [2,5,6]. 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.

Some 1-(2-hydroxyphenyl)ethanone 3-thiosemicarbazones are already described in scientific literature [3,9]. It was found that thiosemicarbazones and their coordination compounds possess antimicrobial activity [10]. The aim of this work is finding the conditions of synthesis, determination of the composition, structure and physicochemical properties of the copper, nickel, and cobalt coordination compounds with 1-(2-hydroxyphenyl)ethanone 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 [1,12]. 1-(2-hydroxyphenyl)ethanone (Sigma-Aldrich), metal salts were used as received. The NMR spectra of free ligand were determined in CDCl₃ at room temperature on a Bruker DRX-400 spectrometer.

Magnetochemical research was made at room temperature using Gouy method [18]. Quantitative analyses on copper, nickel, and cobalt were made using titration methods [15-17]. Melting point of the free ligand was measured using capillary method [14]. Molar conductivity values were determined in 10⁻³mol/L methanol solutions using slidewire bridge R-38.
Synthesis of the 1-(2-hydroxyphenyl)ethanone N(4)-allyl-3-thiosemicarbazone (H₂L)

1-(2-hydroxyphenyl)ethanone N(4)-allyl-3-thiosemicarbazone was synthesized by high-yielding Schiff base condensation reaction [4,8].

The solution of N(4)-allyl-3-thiosemicarbazide (1.31g, 0.01mol) in ethanol was added to the ethanolic solution of 1-(2-hydroxyphenyl)ethanone (1.36g, 0.01mol). 3 drops of glacial acetic acid were added. The obtained mixture was refluxed for 2h. 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.

Yield: 88%
Melting point: 105-106°C

\[ \text{1H NMR (CDCl}_3, \delta (ppm)): 10.84 \text{ (br, 1H, OH); 8.93 \text{ (br, 1H, NH); 7.49 \text{ (d, 1H, CH aromatic); 7.33 \text{ (t, 1H, CH aromatic); 6.99(d, 1H, CH aromatic); 6.96(t, 1H, CH aromatic); 6.92 (br, 1H, NH); 5.97 (m, 1H, CH from allyl moiety); 5.28 (m, 2H, CH}_2=C); 4.39 (m, 2H, CH}_2-N); 2.42 (s, 3H, CH}_3).} \]

\[ \text{13C NMR (CDCl}_3, \delta (ppm)): 177.88 \text{ (C=S); 157.50 \text{ (C-O); 153.96 \text{ (C=N); 132.73 \text{ (CH from allyl moiety); 132.02, 128.45, 119.79, 119.31, 117.64 \text{ (C aromatic); 117.56 (CH}_2=); 47.40 (CH}_2-N); 14.17 (CH}_3).} \]

Synthesis of coordination compounds

The complexes (I-IV) were obtained by stirring a hot solution of H₂L in ethanol with the corresponding copper salts in 1:1 molar ratio: CuCl₂·2H₂O (I), CuBr₂ (II), Cu(NO₃)₂·3H₂O (III), Cu(ClO₄)₂·6H₂O (IV). Cobalt and nickel coordination compounds were synthesized similarly, but in 1:2 molar ratio: (CH₃COO)₂Ni·4H₂O (V), Co(NO₃)₂·6H₂O (VI). After cooling green (in case of complexes I-IV) or brown (in case of complexes V-VI) precipitates of corresponding coordination compounds were filtered, washed with small amounts of cold ethanol and dried.

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% CO₂ humidified atmosphere at 37°C. Cells were currently maintained twice a week by diluting the cells in RPMI 1640 medium containing 10% FBS.

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).

Cell proliferation assay of HL-60 cells. 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 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, 5% CO₂. 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).
Cell proliferation assay of HeLa cells

Cells were trypsinized Trypsin-ethylene-diaminetetraacetic acid (tryps-in-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 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, 2% CO₂. Compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare the stock solution of 10mM. These compounds and doxorubicin were 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 600nm 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}} \text{ sample}-\text{Abs}_{600\text{nm}} \text{ sample}}{\text{Abs}_{570\text{nm}} \text{ control}-\text{Abs}_{600\text{nm}} \text{ control}}\right) \times 100]\n
The IC₅₀ values were evaluated by program GraphPad Prism 6

Results and discussion

The Shiff base H₂L has been prepared by a known method [15,16]. The structure of H₂L was determined by ¹H and ¹³C NMR spectroscopy. All complexes were prepared by the direct reaction between the ligand H₂L and the corresponding metal salts. The obtained coordination compounds are microcrystalline solids and are stable in air. The elemental analyses on copper, nickel, and cobalt and gravimetric analysis of water content indicate the general formulae Cu(HL)X·nH₂O (X=Cl⁻, Br⁻, NO₃⁻, ClO₄⁻; n=1-2), Ni(HL)₂, and Co(HL)₂NO₃.

Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Formula</th>
<th>ηₕ, %</th>
<th>Found / calculated, water %</th>
<th>Found / calculated, metal %</th>
<th>µeff b, MB</th>
<th>λc c</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>Cu(HL)Cl·H₂O</td>
<td>C₁₂H₁₆CuClN₃O₂S</td>
<td>74</td>
<td>4.57/4.93</td>
<td>17.58/17.39</td>
<td>1,82</td>
<td>113</td>
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<tr>
<td>II</td>
<td>Cu(HL)Br·H₂O</td>
<td>C₁₂H₁₆CuBrN₃O₂S</td>
<td>65</td>
<td>3.98/4.39</td>
<td>15.79/15.51</td>
<td>1,79</td>
<td>120</td>
</tr>
<tr>
<td>III</td>
<td>Cu(HL)NO₃·2H₂O</td>
<td>C₁₂H₁₆CuN₄O₆S</td>
<td>68</td>
<td>9.12/8.78</td>
<td>15.23/15.50</td>
<td>1,78</td>
<td>126</td>
</tr>
<tr>
<td>IV</td>
<td>Cu(HL)ClO₄·2H₂O</td>
<td>C₁₂H₁₆CuClN₃O₇S</td>
<td>79</td>
<td>8.32/8.05</td>
<td>14.48/14.20</td>
<td>1,88</td>
<td>108</td>
</tr>
<tr>
<td>V</td>
<td>Ni(HL)₂</td>
<td>C₂₄H₂₈Ni₃N₆O₂S</td>
<td>73</td>
<td>0.15/0.00</td>
<td>10.86/10.57</td>
<td>2.92</td>
<td>13</td>
</tr>
<tr>
<td>VI</td>
<td>Co(HL)₂NO₃</td>
<td>C₂₄H₂₈Co₃N₇O₃S</td>
<td>76</td>
<td>0.32/0.00</td>
<td>9.32/9.54</td>
<td>dia d</td>
<td>137</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.

The molar conductivity values of the copper complexes (I-IV) are in the range 108 - 126 Ω⁻¹·cm²·mol⁻¹ that indicates that complexes (I-IV) represent 1:1 electrolytes. [7] The corresponding anion (Cl⁻, Br⁻, NO₃⁻, ClO₄⁻) 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 molar conductivity value of the cobalt complex (VI) also corresponds to 1:1 electrolyte. [13] The molar conductivity value of the nickel complex (V) is very low (13 Ω⁻¹·cm²·mol⁻¹), suggesting that the complex is non-electrolyte.

The magnetochemical research showed that the synthesized copper coordination compounds (I-IV) have monomeric structure because their effective magnetic moments are close to the spin value for one unpaired electron. The nickel and cobalt coordination compounds have octahedral structure. The cobalt complex (VI) is diamagnetic that indicates that cobalt(II) is oxidized by oxygen from air to cobalt(III) during the synthesis. It also shows that the H₂L represents a strong-field ligand. It was supposed that the synthesized thiosemicarbazone H₂L behaves as mono-deprotonated tridentate ligand with O, N, S set of donor atoms. It coordinates to the central ions with deprotonated phenolic oxygen atom, azomethinic nitrogen atom, and sulfur atom forming five- and six-membered metallacycles. The proposed distribution of chemical bonds in the coordination compounds are shown in scheme 2.
In order to find out the biological properties it was studied the antitumor activity of the 1-(2-hydroxyphenyl)ethanone N(4)-allyl-3-thiosemicarbazone (H2L). The proliferation of human leukemia HL-60 cells in presence of H2L and structurally related substances is shown in scheme 3.
From the obtained data it can be seen that absence of hydroxyl group leads to the loss of antitumor activity. The comparison of the 2-hydroxybenzaldehyde and 1-(2-hydroxyphenyl)ethanone N(4)-allyl-3-thiosemicarbazones shows that the substitution of the hydrogen atom with a methyl group in the azomethinic fragment leads to the growth of antitumor activity. H₂L inhibits the proliferation of human promyelocytic leukemia HL-60 cells at the concentration 10μM by 15%. It loses antitumor activity at lower concentration (1 μM).

It was also studied antitumor activity towards HeLa cells. The results are shown in scheme 4.

The obtained data corroborates previously found dependence. The substitution of the hydrogen atom with a methyl group in the azomethinic fragment leads to the growth of antitumor activity towards HeLa cells. H₂L inhibits the proliferation of HeLa cells at the concentration 100μM by 100%. At concentrations 10μM, 1μM, 0.1μM it inhibits 32, 26 and 16% of HeLa cells, respectively. The antitumor activity towards HeLa cells is more pronounced than towards HL-60 cells.

It was also studied cytotoxicity on healthy cells MDCK. The experiment showed that H₂L inhibits proliferation of these cells only at 100μM concentration by 16%. The IC₅₀ value for MDCK exceed 100μM, whereas IC₅₀ value for HeLa is 12 μM. On this basis it is presupposed that H₂L has lower cytotoxic effect on healthy cells of human organism.

**Conclusions**

In this work 1-(2-hydroxyphenyl)ethanone N(4)-allyl-3-thiosemicarbazone was synthesized and studied using NMR spectroscopy. This ligand was used for synthesis of six coordination compounds of copper, nickel, and cobalt. These compounds were studied using elemental analysis, gravimetric analysis of water content, molar conductivity, and magnetochemistry. The copper coordination compounds (I-IV) have monomeric structure. The nickel (V) and cobalt (VI) complexes are octahedral. The ligand H₂L inhibits the proliferation of the human leukemia HL-60 cells at the concentration 10μM by 15%. It also inhibits proliferation of HeLa cells at concentrations 0.1-100 μM by 16-100%. The substitution of the hydrogen atom with a methyl group in the azomethinic fragment leads to the growth of antitumor activity. H₂L has lower cytotoxic effect on healthy cells MDCK than on tumor cells.
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