Synthesis and crystal structure of cytotoxic copper(II) complex with 1,10-phenanthroline-5,6-dione and isothiazole derivative

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Abstract. Oligopyridine based copper(II) complexes are of interest to scientists as possible anticancer agents due to promising cytotoxic and DNA binding/cleaving properties. In this study, copper(II) complex [Cu(phenanthroline)2(C2H5OH)2]·C2H2OH with 1,10-phenanthroline-5,6-dione (phenanthroline-cisplatin) and 4,5-dichloro-isothiazole-3-carboxylic acid (HL) was synthesized and characterized by elemental analysis, IR-spectroscopy, X-ray powder diffraction and single-crystal X-ray diffraction. According to X-ray diffraction data, obtained compound is mononuclear complex with square pyramidal coordination environment of the central atom which is surrounded by two isothiazolate molecules and one phenanthroline ligand. The X-ray diffraction data are confirmed by IR-spectroscopy data showing the presence of characteristic stretching vibration bands of the carboxyl and carboxyl groups of oligopyridine ligand and isothiazolate ions, respectively. Density functional theory (DFT) calculations for complex were carried out using the ADF software package to perform geometry optimization and frequency calculations that were in a good agreement with experimental IR spectrum. Cytotoxicity of complex and initial reagents was tested against HepG2 (human hepatocellular carcinoma) and MCF-7 (human breast adenocarcinoma) cell lines. The complex showed high dose-dependent cytotoxic activity with the IC50 values of 0.60±0.03 µM and 0.96±0.13 µM, respectively, which is higher than the activity of cisplatin against these cell lines. The activity of the complex is due to the presence of phenanthroline ligand, which exhibits a similar cytotoxic activity.

Key words: copper complex, isothiazole, crystal structure, phenanthroline, cytotoxicity

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Синтез и кристаллическая структура цитотоксического комплекса меди(II) с 1,10-фенантролин-5,6-дионом и производным изотиазола

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Аннотация. Комплексы меди(II) на основе олигопиридинов привлекают интерес исследователей в качестве возможных противопуховелых агентов ввиду их выдающихся цитотоксических свойств и способности связываться/расщеплять ДНК. В настоящем исследовании были получены и охарактеризованы с помощью ИК-спектроскопии, элементного, рентгенофазового и рентгеноструктурного анализа комплексы меди(II) с 1,10-фенантролин-5,6-дионом (phendione) и 4,5-дихлороизотиазол-3-карбоновой кислотой, [Cu(phendione)L]_2C_2H_5OH. Соответствующимым данным рентгеноструктурного анализа, полученное соединение является моноядерным, при этом две молекулы изотиазола и 1,10-фенантролин-5,6-дион образуют квадратно-пирамидальное окружение центрального атома. Данные рентгеноструктурного анализа согласуются с данными ИК-спектроскопии, указывающими на наличие характерных полос валентных колебаний карбонильной и карбоксильной групп. Расчеты, выполненные методом теории функционала плотности (DFT) с использованием программного пакета ADF, позволили оптимизировать геометрию комплекса и вычислить теоретический ИК-спектр, который хорошо согласуется с экспериментальным. Цитотоксичность комплексов и исходных реагентов исследована на клеточных линиях HepG2 (гепатоцеллюлярная карцинома) и MCF-7 (аденокарцинома молочной железы). Комплекс проявляет высокую дозозависимую цитотоксическую активность, при этом значения параметра IC₅₀ составляют 0,60±0,03 мМ (HepG2) и 0,96±0,13 мМ (MCF-7), что превышает активность цисплатина по отношению к данным клеточным линиям. Активность комплекса обусловлена присутствием лиганда phendione, который в свободном виде также обладает токсичностью.

Ключевые слова: комплекс меди, изотиазол, кристаллическая структура, фенантролин, цитотоксичность

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INTRODUCTION

Currently, mixed-ligand complexes of essential metals with 1,10-phenanthroline and its derivatives are being actively studied in vitro and in vivo for their biological properties (cytotoxic, antioxidant, antibacterial, antiviral and other activities) [1–7]. Essential metals include such microelements as iron, copper, zinc, cobalt, nickel, manganese and some other metals. Among the complexes of essential metals, the most interesting as anticancer agents are 1,10-phenanthroline based copper(II) complexes, since they are capable to interact and cleave DNA/RNA backbone [8, 9]. Moreover, oligopyridine and amino acid/acylacetonate based copper(II) complexes (Casiopéinas) have shown antiproliferative, genotoxic, and antineoplastic activity [10, 11]. The most promising complexes from the Casiopéinas series have completed preclinical trials [12]. Besides amino acids, N-donor systems (terpyridine, imidazole, benzimidazole, tetrazole), O-donor systems (salicylic acid, dicarboxylic acids), S-donor systems (thiosemicarbazones, thiocarbamates, thioureas), Schiff bases are often used as secondary ligand in synthesis of cytotoxic Casiopéinas-like complexes [6]. The isothiazole heterocycles and coordination compounds with isothiazoles have been shown to be bioactive substances with pesticidal, anticancer, anti-inflammatory and antiviral activities [13]. However, despite their promising properties, isothiazoles are negligibly used for the synthesis of bioactive oligopyridine based complexes with essential metals. Thus, the research aiming preparation of corresponding complexes may be indeed promising from the point of view of medicinal chemistry.

In continuation of our work [14] in order to expand the number of available cytotoxic oligopyridine based complexes with isothiazole as secondary ligand, the present study set out to synthesise new mixed-ligand copper(II) complex with 1,10-phenanthroline-5,6-dione and 4,5-dichloro-isothiazole-3-carboxylic acid and evaluate its biological activity.

EXPERIMENTAL SECTION

1,10-Phenanthroline-5,6-dione (phenidine) was acquired from ABCR (Germany). 4,5-Dichloro-isothiazole-3-carboxylic acid (HL) was synthesized as previously described [15, 16]. Solvents and reagents were used as purchased without any further purification. Elemental analysis (C, H, N) was performed using Euro EA 3000 analyzer. IR absorption spectra were recorded on SCIMITAR FTS 2000 and VERTEX-80 spectrophotometers at 4000–400 cm$^{-1}$ (in fluorinated oil – in the region of 4000–1500 cm$^{-1}$, in vaseline oil – 1800–400 cm$^{-1}$). Powder XRD analysis of complexes was performed on a Shimadzu XRD-7000 diffractometer (CuKa radiation, Ni filter, 3–40° 26 range, 0.03° 26 step, room temperature).

Quantum-chemical calculations were carried out on the computational cluster of NIIС SB RAS using the Amsterdam Density Functional (ADF) program by dint of density functional theory (DFT)1. The generalized gradient approximation (GGA), the density functional PBE (Perdew–Burke–Ernzerhof) [17] in combination with the all electron basis set TZ2P [18] were employed. The calculations were carried out for non-solvent system.

 Bruker D8 Venture diffractometer with the graphite-monochromated MoKα radiation (λ = 0.71073 Å) was utilized to collect single-crystal XRD data for copper(II) complex. All measurements were carried out at 150 K, and the φ- and ω-scan techniques were applied. Absorption corrections were applied with the SADABS program2. The crystal structure was solved and refined by means of the SHELXTL [19] and SHELXL [20] programs using OLEX2 GUI [21]. Atomic thermal displacement parameters for non-hydrogen atoms were refined anisotropically. Positions of hydrogen atoms were calculated according to their geometrical conditions and refined by dint of the riding model. The crystallographic data and details of the structure refinements are shown in Table 1. CCDC 2121029 contains the supplementary crystallographic data for this paper. These information can be obtained free of charge from The Cambridge Crystallographic Data Center at https://www.ccdc.cam.ac.uk/structures/

Synthesis of [Cu(phenidine)L$_2$]$_2$C$_2$H$_2$OH, [Cu(H$_2$)O]L$_2$ was synthesized as previously described [14]. The precipitate of [Cu(H$_2$)O]L$_2$ (0.048 g, 0.10 mmol) was resuspended in mixture of ethanol (2 ml) and dichromomethane (3 ml). Solid phenidine (0.025 g, 0.12 mmol) was added to suspension with stirring. The reaction mixture became clear, precipitate dissolved. The solution was left for slow evaporation at room temperature. Polycrystalline phase was obtained after a week. Green crystals were filtered out, washed with ethanol and dried in air. Yield: 0.064 g (90%). Elemental analysis (%): Calc. for C$_{22}$H$_{15}$Cl$_2$CuN$_2$O-$S_2$: C, 37.0; H, 1.7; N, 7.8. Found: C, 37.3; H, 1.8; N, 7.7.

Cytotoxic activity. Human hepatocellular carcinoma (HepG2) and human breast adenocarcinoma (MCF-7) cell lines were cultured in lscove’s Modified Dulbecco’s Medium (IMDM) supplemented with a 10% fetal bovine serum under a humidified atmosphere (5% CO$_2$ and 95% air) at 37°C. Cell viability was evaluated by Hoechst/Pi staining as previously described [22]. Hep2 cells were cultured to 96-well plates at a density 5·10$^4$ cells per well. After 24 hours cells were treated with complex dissolved in DMSO and incubated for 48 hours. Serial dilutions were prepared in IMDM medium in the concentration range of 0.1–25 μM. For identification of live, apoptotic and dead cells, treated cells and control cells were stained with a mixture of fluorescent dyes.

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Green crystals of copper(II) complex [Cu(phendione)L₂]·C₂H₅OH were obtained after slow evaporation of ethanol/dichloromethane (1:1.5 by volume) solution containing [Cu(H₂O)L₂] and phendione ligand in 1:1.2 molar ratio. Obtained complex is soluble in DMSO, CH₂CN, sparingly soluble in ethanol, CH₂Cl₂ and practically insoluble in water. The elemental analysis results for [Cu(phendione)L₂]·C₂H₅OH are consistent with the proposed formula of the complex. The presence of solvent ethanol molecule was confirmed by IR-spectroscopy (ν(OH) = 3464, 3308 cm⁻¹). Powder X-ray diffraction analysis have been used to demonstrate the identity of single crystal and the synthesized polycrystalline phase of complex which proved the phase purity of the bulk sample (Fig. 1).

According to single-crystal X-ray diffraction data, the copper(II) complex [Cu(phendione)L₂]·C₂H₅OH reveals square pyramidal coordination environment of central atom. The coordination sphere of Cu(II) consists of two O atoms of the isothiazoles carbonyletes, one N of the isothiazole ligand and two N atoms of the phendione (Fig. 2). Additional contact (of 2.772 Å) is observed between the copper(II) and the O atom of the isothiazole moiety giving distorted octahedral 5 + 1 environment. Also there is outer-sphere ethanol molecule. Similar structure has been observed in our previous work, where bipicoline has been used instead of phendione [14].
important orbitals in a molecule. In case of copper(II) complex, the electron density of HOMO (Fig. 3) is concentrated on phendione mainly and energy of this orbital is equal to -5.978 eV. LUMO (-4.997 eV) is disposed on copper ion and donor atoms of its coordination sphere. The energy gap between HOMO and LUMO \((E_{\text{HOMO}} - E_{\text{LUMO}})\) is -0.981 eV. A molecule with large HOMO–LUMO gap is described as a hard molecule which is much less polarizable. In contrast, the soft systems have small HOMO–LUMO gap and are highly polarizable [23]. Thus, complex refers to soft molecules due to the small value of energy gap.

Vibrational spectrum has been calculated for the complex with optimized geometry. There are no imaginary frequencies in the obtained spectrum, consequently, the geometry corresponds to local minima. The main vibrational frequencies of calculated and experimental spectra are shown in Table 2. The vibrations of characteristic groups \((\text{COO}^-, \text{C–H}, \text{C–Cl}, \text{O=\text{C–C=O}})\) exhibit in both spectra. The slight shift and broadening of bands is observed in experimental IR-spectrum, which can be due to the presence of ethanol molecule in system. Despite these small differences spectra are in a good agreement.

The obtained IR-spectrum clearly indicates the formation of copper(II) complex. There is a disappearing of broad \(\text{ν(O–H)}\) band observed in the HL spectrum \((2915 \text{ cm}^{-1})\) in the result of ligand deprotonation during the synthesis. Moreover, the bands of asymmetrical stretching vibrations of the carboxylate group shift to the low-frequency region \((1651–1614 \text{ cm}^{-1})\) in contrast to the band in HL spectrum \((\nu(\text{COO})_{\text{as}} = 1724 \text{ cm}^{-1})\).

The cytotoxicity of copper(II) complex was tested by Hoechst/PI staining on HepG2 (human hepatocellular carcinoma) and MCF-7 (human breast adenocarcinoma) cell lines by exposing them for 48 h to the medium containing the compound in 0.1–25 \(\mu\text{M}\) concentration. The concentration-dependent cell viability graphs are given in Fig. 4. Calculated from these graphs IC\(_{50}\) values (concentration required to reduce survival in the cell lines to 50%) are summarized in Table 3.

Earlier, copper acetate and HL were shown to be nontoxic in the concentration range of 1–50 \(\mu\text{M}\) [14]. At the same time, 1,10-phenanthroline-5,6-dione is highly toxic to HepG2 and MCF-7 cells with IC\(_{50}\) values being in the nanomolar concentration range [24].

![Image 3. Electron density of [Cu(phendione)\(_2\)] (a), HOMO (b) and LUMO (c)]

Rис. 3. Электронная плотность [Cu(phendione)\(_2\)] (a), ВЗМО (b) и НСМО (c)

Table 2. Calculated and experimental vibrational frequencies (cm\(^{-1}\)) of [Cu(phendione)\(_2\)]

Таблица 2. Теоретически рассчитанные и экспериментальные колебательные частоты (см\(^{-1}\)) комплекса [Cu(phendione)\(_2\)]

<table>
<thead>
<tr>
<th>Assignment, cm(^{-1})</th>
<th>DFT-calculation</th>
<th>Experimental data</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\nu(\text{O–H})_{\text{sy}})</td>
<td>3130, 3115, 3106</td>
<td>3101, 3086, 3061</td>
</tr>
<tr>
<td>(\nu(\text{C–H}))</td>
<td>3192</td>
<td>2960, 2924, 2856</td>
</tr>
<tr>
<td>(\nu(\text{C–H})_{\text{sy}})</td>
<td>1664</td>
<td>1726</td>
</tr>
<tr>
<td>(\nu(\text{O=C–C=O})_{\text{as}})</td>
<td>1692</td>
<td>1710</td>
</tr>
<tr>
<td>(\nu(\text{O=C–C=O})_{\text{s}})</td>
<td>1642, 1628</td>
<td>1651, 1614</td>
</tr>
<tr>
<td>(\nu(\text{COO})_{\text{as}})</td>
<td>1261</td>
<td>1261</td>
</tr>
<tr>
<td>(\nu(\text{COO})_{\text{s}})</td>
<td>1055, 1044</td>
<td>1049, 1024</td>
</tr>
<tr>
<td>(R_{\text{avg}}, \delta(\text{C–H}))</td>
<td>1578, 1560, 1412, 1306, 1195, 1114, 979, 942</td>
<td>1576, 1408, 1300, 1205, 1128, 972</td>
</tr>
</tbody>
</table>
According to cytotoxicity study of [Cu(phen-dione)L]·C₂H₅OH, complex possesses dose-dependent cytotoxicity against both cell lines (IC₅₀ = 0.96±0.13 µM (MCF-7), 0.60±0.03 µM (HepG2)), while its toxicity is higher than that of oligopyridine on HepG2 cells and vice versa on MCF-7 cells (See Table 3). Thus, the activity of the complex is due to the presence of phendione ligand, which exhibits a similar cytotoxic activity. The activity of the obtained complex exceeds the activity of cisplatin by more than an order of magnitude. This compound is part of a series of mixed-ligand copper(II) complexes with HL and various oligopyridines, and turned out to be the most toxic in this series. So, for example, the IC₅₀ value against MCF-7 cells in the same conditions is 1.8±0.3 µM for [Cu(4,7-dimethyl-1,10-phenanthroline)L]_2 and 4.2±0.2 µM for [Cu(1,10-phenanthroline)(H₂O)L]_2 [14].

Table 3. IC₅₀ values of the compounds against MCF-7 and HepG2 cell lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ value, µM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCF-7 cells</td>
</tr>
<tr>
<td>Cu(OAc)_2</td>
<td>&gt;50</td>
</tr>
<tr>
<td>HL</td>
<td>&gt;50</td>
</tr>
<tr>
<td>1,10-phenanthroline-5,6-dione</td>
<td>0.73±0.06</td>
</tr>
<tr>
<td>[Cu(phen-dione)L]·C₂H₅OH</td>
<td>0.96±0.13</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>33.7±1.8</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Mononuclear copper(II) complex based on 4,5-dichloro-isothiazole-3-carboxylic acid and 1,10-phenanthroline-5,6-dione [Cu(phen-dione)L]_2 has been obtained and characterized by several physicochemical methods. The results of single crystal X-ray diffraction indicate that complex reveals square pyramidal coordination environment of central atom which consist of two O atoms of the isothiazoles carboxylates, one N of the isothiazole ligand and two N atoms of the phenidine. According to DFT-calculations, the value of energy gap between HOMO and LUMO of complex is small (-0.981 eV), thus, [Cu(phen-dione)L]_2 refers to soft molecules. This compound causes pronounced cytotoxic effect against HepG2 and MCF-7 cells in 0.1-25 concentration range which is an order of magnitude higher than cisplatin cytotoxicity. [Cu(phen-dione)L]_2 is part of a series of mixed-ligand copper(II) complexes with HL and various oligopyridines. Cytotoxicity of these complexes increases with the transition from 1,10-phenanthroline to 4,7-dimethyl-1,10-phenanthroline and is maximal in the 1,10-phenanthroline-5,6-dione based complex. Thus, this work helps to understand structure-cytotoxic activity relationships for the oligopyridine based isothiazole copper(II) complexes.

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