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Synthesis, characterization, and biological activity of some complex combinations of nickel with α-ketoglutaric acid and 1-(*o*-tolyl)biguanide

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ABSTRACT

The four divalent nickel complexes having α-ketoglutaric acid (H_2A) and 1-(*o*-tolyl) biguanide (TB) ligands have been synthesized, characterized, and tested for antibacterial and antitumor activity.

The proposed formulas for these complexes are $[Ni(TB)(HA)(H_2O)_2]Cl$ (**C1**), $[Ni(TB)(HA)(H_2O)_2]Br$ (**C2**), $[Ni(TB)(HA)]NO_3 \cdot H_2O$ (**C3**), and $[Ni(TB)(HA)]CH_3COO$ (**C4**), where HA represents deprotonated H_2A.

For the four complexes and for the ligands used in the synthesis, the antibacterial activity against *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 and antitumor activity in HeLa tumor cells were tested. A moderate cytotoxic effect of **C3** and **C4** complexes has been observed on the development and metabolic activity of HeLa cells, whereas **C1** and **C2** ligands have a very low effect on them.

The synthesized complexes (obtained) inhibit adherence to the inert substrate of bacterial strains *S. aureus* and *P. aeruginosa*; therefore, they may be candidates for (potential) therapeutic applications.

1. Introduction

Biguanides are compounds of importance due to their biological properties, of which worth to be mentioned are the antimicrobial, antifungal, antibacterial, hypoglycemic, antimalarial, and antitumor activities [1–8]. The special interest in studying the coordination complexes in which the ligand is of biguanide type is given by the fact that many of them possess biological properties.

Thus, complexes of Fe(III), Ni(II), and Cu(II) with *N*,*N*-dimethylbiguanide ligands and their derivatives $[Fe(DMBG)_2]Cl \cdot 0.5H_2O$, $[FeO(DMBG)_2]$, $[Ni(DMBG)_2]$, $[Cu(DMBG)_2] \cdot H_2O$, where HDMBG = N,N-dimethylbiguanide [9–11], $[Cu(HTBG)_2]Cl_2$ and $[Cu(TBG)_2] \cdot 3H_2O$, where HTBG is 2-tolylbiguanide [12], are known.

Biological, spectral, and molecular docking studies of 1-(*o*-tolyl)biguanide demonstrated its antimicrobial activity [13].

α-Ketoglutaric acid plays an essential role in the Krebs cycle; α-ketoacids are very important agents in the synthesis and degradation of amino acids and proteins in the metabolism of lipids and carbohydrates [14]. Studies on α-ketoacids with biological activity have shown that they can produce different effects on the biological tissue, interacting at the cellular level at different pH values, temperatures, and enzyme-controlled medium [15–17].

Abbreviations: H_2A, α-ketoglutaric acid; HA, deprotonated α-ketoglutaric acid; TB, 1-(*o*-tolyl)biguanide; NAD(P)H, nicotinamide-adenine-dinucleotide phosphate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-dipheny ltetrazolium bromide; DMSO, dimethyl sulfoxide; MIC, minimum inhibitory concentration; MBEC, minimal biofilm eradication concentration.

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α-Ketoglutaric acid antagonizes toxic effects against both HCN and NaCN [18,19]. It has also a neuroprotective effect, helps in the healing and regeneration of tissues, in the development of the gastrointestinal tract, in lung disorders, and some types of cancer [14].

Complexes with lanthanides containing α-ketoglutaric acid as ligand have been synthesized [20].

Complexes with formula $[M_2(III)M(II)L_6(NO_3)_6(OH_2)_6](NO_3)$, where M(III) = Ce and M(II) = Cu, Co, Ni, and L is α-ketoglutaric acid, have antibacterial properties and antioxidant activity [21].

Taking into account the biological properties of α-ketoglutaric acid and 1-(*o*-tolyl)biguanide, we aimed to synthesize and afterward study the properties of new complex combinations of Ni(II) with these ligands.

2. Experimental section

2.1. Materials and methods

The substances used to obtain the four complexes were of high purity and came from Alfa Aesar (α-ketoglutaric acid—$C_5H_6O_5$) and Sigma–Aldrich (1-(*o*-tolyl)biguanide—$C_9H_{13}N_5$, $NiCl_2 \cdot 6H_2O$, $NiBr_2$, $Ni(NO_3)_2 \cdot 6H_2O$, $Ni(CH_3COO)_2 \cdot 4H_2O$, and ethanol).

For synthesis of complex combinations, the substances used (nickel salts and two ligands) were dissolved in ethanol. Reactions occurred at approximately 40 °C under agitation. The complexes obtained were vacuum filtered, washed with ethanol, and dried with ethyl ether. The molar ratio of metal salts/α-ketoglutaric acid/1-(*o*-tolyl) biguanide was 1:1:1 using 1 mmol of each substance (0.1461 g $C_5H_6O_5$, 0.1913 g $C_9H_{13}N_5$, 0.2377 g $NiCl_2 \cdot 6H_2O$, 0.2185 g $NiBr_2$, 0,2908 g $Ni(NO_3)_2 \cdot 6H_2O$, and 0.2488 g $Ni(CH_3COO)_2 \cdot 4H_2O$). Synthesis of complex combinations led to pure compounds, which did not require further purification.

The nitrogen, carbon, and hydrogen content of the synthesized complexes was determined by microcombustion using a Flash 2000 Organic Elemental Analyzer. The presence of chlorine in complex **C1** was revealed with $AgNO_3$, and the percentage of nickel was determined using a Perkin Elmer AAnalyst 400 atomic absorption spectrometer.

Electronic spectra were recorded using a Jasco V670 spectrometer by the diffuse reflection method at room temperature in the range 200–1500 nm, using MgO as a standard.

A Nicolet IS 50 FT-IR spectrophotometer was used for recording FT-IR spectra in the 4000–200 cm^{-1} range. Thermal analysis was performed with a simultaneous thermogravimetric analysis/differential scanning calorimetry (TGA/DSC) STA 449 F1 Jupiter working in a dynamic air atmosphere at a flow rate of 20 mL/min in the range 25–900 °C with a heating rate of 10 °C/min. The molar electrical conductivity was determined using a CyberScan PCD 6500 conductivity meter in 10^{-3} M of *N*,*N*-dimethylformamide solution at 25 °C. Magnetic susceptibility was measured with a Gouy balance using the Faraday method and $Hg[Co(SCN)_4]$ as a calibrant.

Antitumor activity was tested in HeLa tumor cells using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay at a concentration of 500 μg/mL for 24 h incubation at 37 °C in 5% CO_2 atmosphere. The method is based on the following: under certain conditions, NAD(P)H-dependent cellular oxidoreductases may reflect the number of viable cells in a culture. These enzymes may reduce the MTT tetrazolium reagent to violet insoluble formazan (Fig. 1). The color intensity of formazan was evaluated by measuring optical density at a wavelength of 570 nm using a Filter Max F5 Multi-Mode Microplate Reader spectrophotometer.

The tetrazolium dye reduction method is often used to measure the cytotoxicity of some compounds and cell proliferation. Because MTT reagent is sensitive to light, the whole procedure is done in the dark.

The antibacterial activity of ligands and complexes synthesized was determined in vitro against *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacterial strains. To determine the minimum inhibitory concentration (MIC), the broth microdilution method was used by successively diluting a 10 mg/mL complex combination solution using 96-well plates. According to the dilution scheme of complex compounds, the influence of a diluted dimethyl sulfoxide (DMSO) solvent was also quantified.

To determine the influence of the test compounds on the adhesion of a microbial biofilm to the inert substrate, the microbial cells were cultivated in the presence of compounds of interest into nutrient broth using 96-well plates. They were incubated at 37 °C for 24 h. The plates were emptied and washed twice with physiological sterile water, and the adhered cells were fixed with 0.1 mL of 80% methanol for 5 min. The methanol solution was removed by overturning, and the adhered cells were stained with 1% crystal violet alkaline solution (0.1 mL/well) for 15 min. The staining solution was removed and then the plates were washed under water stream. The microbial biofilms formed on the plastic plates were converted into suspension by bubbling in 33% acetic acid. The intensity of the suspension color was evaluated by measuring the absorbance at 492 nm using a Max F5 Multi-Mode Microplate Reader.

3. Results and discussion

3.1. Elemental analysis

To establish the formulas of coordination compounds that were synthesized, elemental analysis was performed. A good concordance between calculated and experimental percents of carbon, nitrogen, hydrogen, and nickel was found (Table 1).

3.2. Thermal analysis

On the basis of the interpretation of the results of the TGA, information has been obtained that has contributed to the proposed formulations for coordination complexes, namely, the presence of water molecules and the thermal effects associated with mass loss processes. Thermogravimetric (TG) and DSC curves of the four complexes are presented in Fig. 2(a–d).

From the analysis of the **C1** complex TG and DSC curves, the first decomposition step (<180 °C) indicates the loss of