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Synthesis, physico-chemical properties and biological analysis of newly obtained copper(II) complexes with pyrazole derivatives

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ABSTRACT

Three new copper(II) complexes containing two different pyrazole bound ligands (**1**, **2**) have been synthesized and characterized by IR, LSI-MS (liquid secondary ion mass spectrometry) and elemental analysis. ^{1}H NMR spectra of the organic ligands have been recorded. We describe the influence of these complexes on particular cancer cell lines and DNA structure by MTT-assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], APA (acid phosphatase activity)-assay or CD-spectroscopy and agarose gel electrophoresis methods, together with their physico-chemical properties such as lipophilicity and stability in aqueous solution. The cytotoxic effect on HUVEC (endothelial cells) for the most active complex **4** has been also investigated. Moreover, the ability of these complexes to induce apoptosis in cancer cells has been assessed by using fluorescence microscopy. Our results indicate that dichloridobis{1-[amino(thioxo)methyl]-5-hydroxy-3-phenyl-1*H*-pyrazole-κN2}copper(II) is the most potent complex among the tested complexes.

1. Introduction

Almost every second person diagnosed with cancer dies due to non-effective therapy [1]. Chemotherapy, surgery and radiotherapy are general directions in cancer treatment. For many years, laboratories all over the world have been conducting research on new and more effective drugs that show fewer side effects. Despite substantial progress in the field, no compound has been developed that satisfies the expectations of both, patients and doctors. This problem relates to the resistance of many types of cancer to drugs, to undesirable side effects caused by currently prescribed drugs, to their low specificity, or to the necessity of using many different cytostatic drugs in long-term combination treatment [2]. Therefore, the search of new compounds with anticancer activity is one of the fundamental tasks of medicinal chemistry [3].

Many metal complexes that are produced by our organism or ingested through food are essential for our well being. Some of them act as anticonvulsants, others as neuronal messengers and again others are involved in the catalysis of enzymatic pathways of some metabolic processes [4].

Transition metal complexes are an interesting group of compounds, from which several anticancer drugs are derived. The variety of their chemical structure and properties gives a wide spectrum of possibilities in synthesis of compounds with different specificity or mode of action. It is stated that the addition of metal ion to an organic ligand with biological activity can lead to an increase of its properties [5,6]. Moreover, pharmacologic properties of complexes can depend on the properties of either metal or ligand or both [7–10].

It is known that many metal complexes with different organic ligands possess a distinct cytotoxicity against various cancer cell lines [6,11]. Copper complexes, as non-platinum drugs, with thiosemicarbazides were tested as a potential antitumor drug in early 1960s and the first related study had been presented by Padhye and Kauffman [12]. In the following years, some copper(II) complexes with thiosemicarbazone derivatives demonstrated anticancer activity in vitro against human leukemia U973 cells [13] and the ability to induce apoptosis is their main mechanism of action [14]. More recently, many Cu(II)–thiosemicarbazide complexes have been investigated as potential antibacterial or antifungal compounds [15].

In 2001, Easmon et al. [16] showed that modified thiosemicarbazide derivatives possess higher cytotoxic activity and lower toxicity when compared to normal cells. Since then, thiosemicarbazones as well as their complexes have been known for their anticancer properties. They act by inducing apoptosis in cancerous cell lines. Some of these anti-cancer drugs act as apoptosis-inducing agents by activation of

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DNA fragmentation [17]. This work describes the biological activity of pyridoxal thiosemicarbazone [18].

Also, many pyrazole complexes coordinated with metal ions are effective against cancer cells, e.g. pyrazole–rhodium(III) complexes indicate cytostatic activity against HCV29T tumor cells, while their Pd(II) complexes are active against solid-tumor cell lines and in some cases exhibit remarkable activity. It has been suggested that their biological activity depends on the nature of the ligand, the type of the counter ion used and the configuration of the complex [19,20].

Identification of the mode of action of newly obtained compounds is essential for designing an effective new drug. It is stated that the application of compounds with cytotoxic activity to cells can lead to cell cycle stop in the G1 (cell cycle phase G1) phase until all induced damages are fixed or until apoptosis is induced. Necrosis is usually a result of the exposition of cells to compounds in high concentrations [21]. Moreover, these types of compounds can also change the structure of the DNA or destroy the cytoskeleton [22].

For many years our scientific attention has been focused on the investigation of chemical compounds with potential anticancer activity [23,24]. Therefore, in this paper, we present the synthesis as well as the physico-chemical and biological evaluation of newly obtained copper(II) complexes of pyrazole derivatives.

2. Experimental

2.1. General

The IR spectra were recorded with a Mattson Infinity MI-60 spectrophotometer in KBr. Melting points were determined using a Buchi Melting Point B540 apparatus and are uncorrected. Elemental analyses were obtained in the Microanalytical Laboratory of the Department of Bioorganic Chemistry (Medical University, Lodz) using a Perkin Elmer PE 2400 CHNS analyzer. LSI mass spectra (liquid secondary ion) were recorded on the Finnigan MAT 95 double focusing (BE geometry) mass spectrometer (Finnigan MAT, Bremen, Germany). Samples were dissolved in DMSO and 1 μL of 3-nitrobenzylalcohol (NBA) and mixed. For the ionization, the beam of cesium ions of energy of 13 keV was used. Spectra were recorded in positive and negative ion mode. Circular dichroism measurements were performed using a Jasco J-810 spectropolarimeter equipped with a Jasco PFD-4255 Peltier temperature controller. UV-visible (UV–vis) spectra were recorded on a Varian Bio 100 UV–vis spectrophotometer at room temperature.

In all experiments, each compound was dissolved in 10 μL of DMSO and diluted with bidistilled water or Tris–HCl NaCl buffer in order to calculate its concentration. The final percentage of DMSO was 0.1% at most.

2.2. Synthesis of compounds

2.2.1. Synthesis of 1-[amino(thioxo)methyl]-5-hydroxy-3-phenyl-1H-pyrazole (**1**)

The synthesis of ligand **1** was slightly modified from methods published previously [25,26]. Hydrazinecarbothioamide (2.37 g, 26 mmol) was dissolved in a mixture of ethanol (20 mL) and HCl (1 mL). Next ethyl benzoylacetate was added (4.5 mL, 26 mmol). The obtained mixture was refluxed for 1 h under an argon atmosphere. After cooling, the white precipitate was filtered off, washed with water and dried under reduced pressure. Yield: 3.85 g (67.5%), m.p: 159.1–160.3 °C. IR (KBr) ν (cm^{-1}): 3290, 3159, 3113 (NH_2), 3095 (CH aromat.), 1651 (C═O), 1584, 1558 (C═C, C═N), 1320 (C – NH_2), 1026 (N – N), 1102, 883, and 798 (C═S). ^{1}H NMR (270 MHz, DMSO-d6) δ (ppm): 6.18 (1H, s, C4-H), 7.40–8.04 (5H, m, arom.), 9.50–10.40 (2H, s, NH_2), and 11.75–12.65 (1H, s, OH/NH).

2.2.2. Synthesis of 1-[amino(thioxo)methyl]-3,5-dimethyl-1H-pyrazole (**2**)

Ligand **2** was synthesized according to a known procedure with slight modifications [27,28]. Hydrazinecarbothioamide (2.5 g, 27.4 mmol) was dissolved in HCl (100 mL, 0.05 M) and pentano-2,4-dione (2.5 mL, 24.3 mmol) was added dropwise to the stirred mixture. After stirring for 1.5 h and 3 h incubation at room temperature, a white precipitate was filtered off, washed with water and dried under reduced pressure. Yield: 3.320 g (88.0%), and m.p: 95.2–95.9 °C. IR (KBr) ν (cm^{-1}): 3389, 3241 (NH_2), 3133 (CH_3), 1604, 1574 (C═C, C═N), 1339 (C – NH_2), 1029 (N – N), 1099, 880, and 808 (C═S). ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 2.28 (6H, d, – CH_3), 6.29 (1H, s, C4 – H), and 7.00–7.46 (2H, d, NH_2).

2.2.3. Synthesis of dichlorido(1-[amino(thioxo)methyl]-5-hydroxy-3-phenyl-1H-pyrazole-κN^2)copper(II) (**3**)

A solution of 1-[amino(thioxo)methyl]-5-hydroxy-3-phenyl-1*H*-pyrazole (**1**) (197.4 mg, 0.9 mmol) in ethyl acetate (5 mL) was added dropwise to a solution of copper(II) chloride dihydrate (153.4 mg, 0.9 mmol) in ethyl acetate (4 mL) and methanol (1 mL) while stirring. The obtained red-orange precipitate was filtered off, washed with diethyl ether and dried under reduced pressure. Yield: 164.0 mg (49.6%), and m.p: 186.1–187.6 °C. IR (KBr) ν (cm^{-1}): 3361 (OH), 3243, 3126 (NH_2), 2987 (CH aromat.), 2363 (SH), 1573, 1520 (C═C, C═N), and 965 (N – N). Anal. Calc. $C_{10}H_9N_3SOCuCl_2 \cdot 3/4H_2O$ (M = 367.229 g/mol) Anal (%): C 32.71, H 2.88, and N 11.44. Found (%): C 32.79, H 2.76, and N 11.25. LSI-MS (*m/z*): 353($LCuCl_2^+$); and 281 (LCu^+).

2.2.4. Synthesis of dichloridobis{1-[amino(thioxo)methyl]-5-hydroxy-3-phenyl-1H-pyrazole-κN^2}copper(II) (**4**)

While stirring a solution of 1-[amino(thioxo)methyl]-5-hydroxy-3-phenyl-1*H*-pyrazole (**1**) (131.6 mg, 0.6 mmol) in ethyl acetate (5 mL), a solution of copper(II) chloride dihydrate (51.1 mg, 0.3 mmol) in ethyl acetate (4 mL) and methanol (1 mL) was added dropwise. Next, the mixture was refluxed and stirred for 2 h at 45 °C. The obtained black precipitate was filtered off, washed with ethyl acetate and diethyl ether and dried under reduced pressure. Yield: 87.9 mg (51.2%), and m.p: 160.2–160.9 °C. IR (KBr) ν (cm^{-1}): 3423 (OH), 3152, 3128 (NH_2), 3028 (CH, aromat.), 1606, 1523 (C═C, C═N), 1316 (C – NH_2), 1001 (N – N), 867, and 808 (C═S). Anal. Calc. $C_{20}H_{18}N_6S_2O_2CuCl_2$ (M = 572.984 g/mol) Anal (%): C 41.92, H 3.17, and N 14.67. Found (%): C 41.79, H 3.31, and N 14.27. LSI-MS (*m/z*): 574 ($L_2CuCl_2^+$ + 1H); 538 (L_2CuCl^+); and 219 (L).

2.2.5. Synthesis of dichlorido(1-[amino(thioxo)methyl]-3,5-dimethyl-1H-pyrazole-κN^2)copper(II) (**5**)

Compound **5** was synthesized according to the literature with minor modifications [29]. Copper(II) chloride dehydrate (85.2 mg, 0.5 mmol) in ethyl acetate (4 mL) and methanol (1 mL) was added dropwise to a solution of 1-[amino(thioxo)methyl]-3,5-dimethyl-1*H*-pyrazole (**2**) (77.6 mg, 0.5 mmol) in ethyl acetate (5 mL) while stirring. The reaction mixture was stirred for 24 h. Obtained gray-green precipitate was filtered off, washed with ethyl acetate and diethyl ether and dried under reduced pressure. Yield: 128.0 mg (88.4%), and m.p: 164.8–166.2 °C. IR (KBr) ν (cm^{-1}): 3296, 3106 (NH_2), 1621, 1588 (C═C, C═N), 1346 (C – NH_2), 999 (N – N), and 847 (C═S). Anal. Calc. $C_6H_9N_3SCuCl_2$ (M = 289.675 g/mol) Anal (%): C 24.88, H 3.13, and N 14.51. Found (%): C 24.72, H 3.14, and N 14.48. LSI-MS (*m/z*): 289 ($LCuCl_2^+$); 254 ($LCuCl^+$); and 155 (L).

2.3. The stability of compounds in aqueous solution

The stability of the metal(II) complexes in water/DMSO solution at concentration 10 μM was assessed by UV–vis spectrophotometric analysis in the range of 200–800 nm. Spectra were recorded over a period of 24 h using a Scanning Kinetics program. The timetable of all measurements is presented in Table S1. The obtained UV–vis spectra were compared to each other.