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Research paper

Synthesis, characterization, cytotoxicity and antiangiogenic activity of copper(II) complexes with 1-adamantoyl hydrazone bearing pyridine rings☆

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ABSTRACT

Three novel copper complexes with tridentate N_2O ligand di(2-pyridil) ketone 1-adamantoyl hydrazone (Addpy) of the formula $[Cu^{II}_2Cu^{I}_2(Addpy)_2Br_2(\mu\text{-}Br_4)]$ (**1**), *catena*-poly$[CuCl(\mu\text{-}Addpy)(\mu\text{-}Cl)CuCl_2]_n$ (**2**) and $[Cu(Addpy)(NCS)_2]$ (**3**) were synthesized. Complexes are characterized by X-ray crystallography, spectral (UV–Vis, FTIR), electrochemical (CV) analyses, and magnetochemical measurements. Investigation of anticancer potential of Cu(II) complexes, mode of cell death, apoptosis, and inhibition of angiogenesis were performed. All tested malignant cell lines (HeLa, LS174, A549, K562, and MDA-MB-231) showed high sensitivity to the examined Cu(II) complexes. It has been shown that the complexes induce apoptosis in the caspase 3-dependent manner, whereas the anti-angiogenic effects of **1**, **2**, and **3** have been confirmed in EA.hy926 cells using a tube formation assay.

1. Introduction

A number of Cu(II) complexes with acylhydrazone chelators have been synthesized and investigated for their possible therapeutic action as anticancer agents [1,2]. Salicylaldehyde benzoyl hydrazone complexed to copper have been generated as potential cancer therapeutics inhibiting growth of human adenocarcinoma cells [3], while salicylaldehyde pyrazole hydrazone was recognized as one of the most effective apoptosis inducers in H322 lung carcinoma cells [4] and inhibitors of angiogenesis on Matrigel and HUVEC migration *in vitro* [5]. Copper(II) complexes with 2-oxoquinoline-3-carbaldehyde hydrazones containing a pyrrole ring exhibited significant effectiveness against HL-60 leukemia cells (IC_{50} = 8 μM) [6]. The similar tridentate 2-oxoquinoline-3-carbaldehyde 4′-methylbenzoyl hydrazones coordinated to copper demonstrated good *in vitro* activity; the change of counterion from chloride to nitrate anion in the starting Cu(II) salt played here an important role in determination of enhanced cytotoxic potency in HeLa cells (IC_{50} = 19.3 μM) [7]. Cu(II) complexes with 2-acetylpyridine benzoyl hydrazone showed excellent cytotoxicity against HeLa and PANC1 pancreatic carcinoma cell lines [8]. Coordination of dipyridyl ketone benzoyl and isonicotinoyl hydrazine to copper(II) led to very active Cu(II) complexes in reducing SK-N-MC neuroblastoma cell survival [9].

Although many Cu(II) complexes with acylhydrazones show promising cytotoxic activity on the basis of *in vitro* experiments, only few data are available on their mechanism of action. As a continuation of our interest toward metal complexes with 1-adamantoyl hydrazone ligands [10,11], here we present synthesis, structural characterization and a possible mechanism of antiproliferative action of three novel Cu(II) complexes with di(2-pyridyl) ketone 1-adamantoyl hydrazone (Addpy).

☆ Transition Metal Complexes with 1-Adamantoyl Hydrazones, Part 3.

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2. Experimental

2.1. Synthesis of copper(II) complexes

[$Cu^{II}_2Cu^{I}_2$(Addpy)$_2Br_2$(μ-Br_4)] (**1**). Solution of di(2-pyridyl) ketone 1-adamantoyl hydrazone (Addpy) (36 mg, 0.1 mmol) in Me_2CO (5 mL), was mixed with solution of $CuBr_2$ (44 mg, 0.2 mmol) in EtOH (5 mL) at room temperature. In the beginning the thin needle-like crystals were formed, which were later transformed into block-shaped crystals. The obtained crystals were filtered after the solution was evaporated to a small volume. Yield: 45 mg (62%). Analysis, Calc. for $C_{44}H_{48}Br_6Cu_2N_8O_2$ (M = 1454.52 g mol^{-1}): C, 36.33; H, 3.33; N, 7.70%; found: C, 36.43; H, 3.40; N, 7.56%. FTIR (KBr, relevant bands $\tilde{\nu}$/cm^{-1}): 1602 [m, ν(C=O)], 643 [w, ρ(py)]. UV–Vis (DMF, λ_{max}/nm, (log (ε/M^{-1} cm^{-1})): 282sh (4.47), 392(4.44), 697(2.21). Conductivity (Λ_m/S cm^2 mol^{-1}): 188 in DMF.

catena-poly[CuCl(μ-Addpy)(μ-Cl)$CuCl_2$]$_n$ (**2**). Addpy (72 mg, 0.2 mmol) is dissolved in EtOH–Me_2CO (10 mL, 1:1 v/v) at room temperature, and the obtained solution was mixed with solution of $CuCl_2 \cdot 2H_2O$ (50 mg, 0.3 mmol) and 5 mL of the same solvent mixture. From the obtained brown solution green crystals were filtered after 24 h, and washed with Me_2CO. Yield: 44 mg (47%). Analysis, Calc. for $C_{22}H_{24}Cl_4Cu_2N_4O$ (M = 629.33 g mol^{-1}): C, 41.99; H, 3.84; N, 8.90%; Found: C, 42.03; H, 3.99; N, 8.76%. FTIR (KBr, relevant bands $\tilde{\nu}$/cm^{-1}): 1601 [m, ν(C=O)], 636 [w, ρ(py)]. UV–Vis (DMF, λ_{max}/nm, (log (ε/M^{-1} cm^{-1})): 290sh (4.17), 392(4.14), 739(1.69). Conductivity (Λ_m/S cm^2 mol^{-1}): 52 in DMF.

[Cu(Addpy)$(NCS)_2$] (**3**). To a solution of Addpy (40 mg, 0.1 mmol) in MeOH (5 mL) a solution of $Cu(ClO_4)_2 \cdot 6H_2O$ (37 mg, 0.1 mmol) in MeOH (3 mL) was added (room temperature). In the obtained solution the NH_4NCS (38 mg, 0.5 mmol) was added, and mixture was left to evaporate to a small volume. The formed red plate-like single crystals were filtered off. Yield: 48 mg (88%). Analysis, Calc. for $C_{24}H_{24}CuN_6OS_2$ (M = 540.15 g mol^{-1}): C, 53.36; H, 4.48; N, 15.56%; Found: C, 53.48; H, 4.60; N, 15.49%. FTIR (KBr, relevant bands $\tilde{\nu}$/cm^{-1}): 2079 [vs ν(CN) from NCS], 1625 [m, ν(C=O)], 644 [w, ρ(py)]. UV–Vis (DMF, λ_{max}/nm, (log (ε/M^{-1} cm^{-1})): 287sh (4.08), 390(4.13), 676(1.99). Conductivity (Λ_m/S cm^2 mol^{-1}): 62 in DMF.

2.2. Physical measurements

All chemicals for the synthesis were analytical reagent grade. Elemental analysis (C, H, N and S) of air-dried compounds was carried out by standard micro-methods. Molar conductivity of freshly prepared 1 mM solutions were performed on a Jenway 4010 conductivity meter. IR spectra were recorded on a Nicolet Nexus 670 FTIR (Thermo Scientific) spectrophotometer, in the range of 400–4000 cm^{-1} by KBr pellet technique. The electronic spectra of DMF solutions of the complexes were recorded with a T80+ UV/Vis Spectrometer (PG Instruments, Ltd.) in the spectral range 270–1000 nm.

Details of magnetic, electrochemical, and crystallographic measurements are given in the Supplementary content.

2.3. Cell culture

Human cervical adenocarcinoma cell line (HeLa), human colon adenocarcinoma (LS174), non-small cell lung carcinoma (A549), human chronic myelogenous leukemia (K562) and human breast adenocarcinoma cell line (MDA-MB-231) were grown in RPMI-1640 medium (Sigma) at 37 °C. Medium was supplemented with 10% fetal bovine serum, L-glutamine, and penicillin-streptomycin (Sigma).

2.4. Treatment of cell lines

Target cells HeLa (2000 cells per well), LS174 (7000 cells per well), A549 (5000 cells per well), K562 (5000 cells per well) and MDA-MB-231 (5000 cells per well) were seeded into wells of a 96-well flat-bottomed microtiter plate. Twenty-four hours later, after the cell adherence, different concentrations of investigated compounds were added to the cells, except for the control cells to which only nutrient medium was added. Final concentrations reached in treated wells were 3.13 μM, 6.25 μM, 12.5 μM, 25 μM, and 50 μM. The final concentration of DMSO solvent never exceeded 2.5%, which is non-toxic to the cells. Finally, compounds were applied to the suspension of K562 cells 2 h after the cell seeding. All investigated concentrations were set up in triplicate. Nutrient medium with corresponding concentrations of investigated compounds, but without cells, was used as a blank, also in triplicate. The cultures were incubated for 72 h.

2.5. Determination of cell survival

The effect of the investigated compounds on survival of the specified cell lines was determined by the microculture tetrazolium test (MTT) 72 h after addition of the compounds according to Mosmann [12] with modification by Ohno and Abe [13]. Briefly, 10 μL of MTT solution (5 mg/mL phosphate-buffered saline) was added to each well. Samples were incubated for a further 4 h at 37 °C in a humidified atmosphere of 95% air/5% CO_2 (v/v). Then 100 μL of 100 g/L sodium dodecyl sulfate was added to dissolve the insoluble product formazan resulting from conversion of the MTT dye by viable cells. The absorbance (A) at 570 nm was measured 24 h later. The number of viable cells in each well was proportional to the intensity of the absorbance of light, which was read in an enzyme-linked immunosorbent assay (ELISA) plate reader. To determine the cell survival (%), the A of a sample with cells grown in the presence of various concentrations of the investigated compounds was divided by the control optical density (the A of control cells grown only in nutrient medium) and multiplied by 100. In each experiment, the A of the blank was always subtracted from the A of the corresponding sample with target cells. IC_{50} is defined as the concentration of an agent inhibiting cell survival by 50% compared with a vehicle-treated control. As positive control, *cis*-diamminedichloroplatinum was used. All experiments were done in triplicate.

2.6. Cell cycle analysis

Cervical adenocarcinoma (HeLa) cells, were seeded into six-well plates ($2.5 \cdot 10^5$ cells/well), and after 24 h treated with investigated compounds, except control cells, and incubated at 37 °C for the next 24 h. Concentrations used corresponded to IC_{50} or $2 \cdot IC_{50}$ values. After incubation, the cells were collected by trypsinization, and fixed in ice-cold 70% ethanol for 1 h on ice, then at −20 °C for at least a week. After fixation, the cells were washed in PBS and pellets obtained by centrifugation were treated with RNase (100 μg/mL) at 37 °C temperature for 30 min and then incubated with propidium iodide (PI) (40 μg/mL) for at least 30 min. DNA content and cell-cycle distribution were analyzed using a Becton Dickinson FACS-Calibur flow cytometer. Flow cytometry analysis was performed using a CellQuest software (Becton Dickinson, San Jose, CA, USA) on a minimum of 10,000 cells per sample [14].

2.7. Morphological analysis (AO/EB double staining)

Acridine orange is a vital green dye that can stain nuclear DNA across an intact cell membrane, whereas ethidium bromide can