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Synthesis, structure and cytotoxicity of a zinc(II) bromide complex with caffeine

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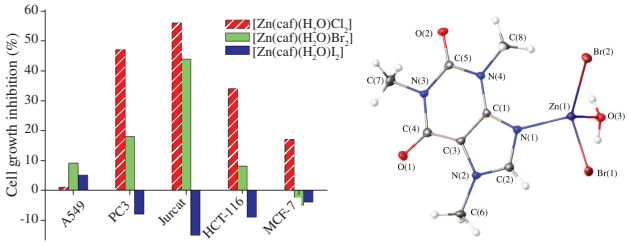
The synthesis and structure of a zinc(II) bromide complex with caffeine (caf), [Zn(caf)(H_2O)Br_2], are described in comparison with [Zn(caf)(H_2O)X_2] (X = Cl, I) complexes. Data on the cytotoxicity of [Zn(caf)(H_2O)X_2] (X = Cl, Br, I) towards human tumor cell lines are presented. The bromo complex has some advantages over chloro and iodo complexes for cytotoxicity on MCF-7 breast cancer cell line, and it is comparable to the chloro complex in cytotoxicity for Jurkat human T cell leukemia.



Since the discovery of the anticancer drug cisplatin, the metal-based complexes have attracted attention in searching for DNA intercalators or combinations of intercalators with minor-grove binders[1] and the ways to overcome platinum-containing compound toxicity and drug resistance.[2] A variety of transition metal properties and ligand combinations has produced an extremely broad spectrum of intercalating anticancer complexes with unique mechanisms of action[3] due to different structural peculiarities, central atom oxidation states, and the diversity of coordination numbers, configurations, and stereochemistry.[4] Metal-containing intercalators can overcome current metallo-drugs and provide more effective chemotherapy.[3] The bioactivity of transition metal complexes, including those of 4*f* elements, with different ligands (small molecules and their derivatives,[5–7] multidentant Schiff bases,[8] and macrocyclic ligands[9]) and mixed-ligand complexes,[10] was reported. Zinc plays an important role in the proliferation, differentiation and metabolic activity of cells.[11] Zinc(II) complexes with antipyrine (AP, 2,3-dimethyl-1-phenyl-3-pyrazolin-5-one, phenazone)[12] and its 4-acyl derivatives[13] demonstrate cytotoxic activity against the NCTC L929 cell line[12] and the DU145, LNCaP and PC-3 human prostate cancer cells,[13] respectively. Thus, zinc(II) ion is a promising alternative to platinum(II) one in the design of more effective antitumor agents,[13] and the simultaneous presence of flat chelating ligands around the zinc(II) ion is a promising way for endowing new non-platinum complexes with good antitumor activity.[13] The [M(ANA)$_2$Cl$_2$] complexes (M = Zn, Cd, Hg; ANA is 2-aminonicotinaldehyde) demonstrate[14] a synergistic effect on the cell growth suppressive action for breast cancer cell line (MCF-7), cervical carcinoma cell line (HeLa), alveolar carcinoma (A-549) and human embryonic kidney 293 (HEK-293) cell line in comparison with free ligand and non-active metal chlorides, zinc-containing complex exhibiting significant activity against three cancer cell lines with IC_{50} values of 19.02 ± 0.29 μM (HeLa), 21.72 ± 0.24 μM (MCF-7) and 17.25 ± 0.37 μM (A-549), as compared with the standard drug cisplatin (IC_{50} values of 2.24 ± 0.19, 1.82 ± 0.1, and 2.307 ± 0.32 μM, respectively). Similar results have been obtained for [ML_2(phen)]·H_2O (M = Mn, Co, Zn; HL is 2-phenyl-4-selenazole carboxylic acid; phen is 1,10-phenanthroline) against human pancreatic cancer cell line PANC-28 and human hepatocarcinoma cell line HuH7.[10] The anticancer tests revealed that the ligand showed no better anticancer activity against these cancer lines, but three complexes exhibited promising anticancer activity against PANC-28 cell line comparable with cisplatin, while the zinc-containing compound demonstrated much more stronger anticancer activity [IC_{50}, μg ml^{-1}: 0.32±0.02 (PANC-28), 21.05±0.039 (HuH7)] than cisplatin [IC_{50}, μg ml^{-1}: 10.24±0.002 (PANC-28), 19.22±0.001 (HuH7)].[10] Enhancing of antitumor activity upon coordination of a number of metal cations with (*E*)-2-hydroxy-*N*′-[(*Z*)-3-(hydroxyimino)-4-oxopentan-2-ylidene]benzohydrazide against human liver HepG2 cancer cells (HepG2 cell line) was demonstrated.[15] The zinc complex [Zn(bpbp)$_2$]$^{2+}$ of 2,6-bis(1-phenyl-1*H*-benzo[*d*]imidazol-2-yl)-pyridine (bpbp) manifested[3] low micromolar activity in a variety of cell lines with IC_{50} of 2.9 ± 0.3 μM against MCF-7 cells. The hypothesized mechanism of action was DNA damage

via intercalation and cleavage, resulting in apoptosis.[3] Another intercalating zinc complex of 5-bromo-8-hydroxyquinoline displayed higher cytotoxicity in BEL-7404 human hepatoma cells and T-24 human bladder carcinoma cells than cisplatin and induced cell cycle arrest in the G2 phase of the BEL-7404 cells.[3] Caffeine (1,3,7-trimethylpurine-2,6-dione, caf) and its derivatives can be used in drug design and in a combined therapy for brain tumors due to their ability to penetrate through the blood–brain boundary (BBB).[16–19]

Previously, we studied zinc and cadmium halide complexes with caffeine.[20] Here, we report on the synthesis, structure, identification, and cytotoxicity of the respective zinc bromide complex with caffeine. We synthesized $[Zn(caf)(H_2O)Br_2]$ **1** by the treatment of a zinc(II) bromide polyhydrate [obtained from zinc metal or zinc oxide (zinc carbonate) with hydrobromic acid] with caffeine taken in the molar ratio $ZnBr_2$: caf = (1–3) : 1. The reaction was carried out in aqueous medium at ambient temperature; the product yield was 50–70% (Online Supplementary Materials, Scheme S1).

Single crystals of complex **1** were obtained as colorless needles after isothermal (room temperature) solvent evaporation for one to two weeks.† The molecular and crystal structures of the complex were studied.‡ According to powder X-ray diffraction data,§ compound **1** was isolated in a pure form without impurities and the single-crystal structure is representative of the sample bulk (Figure S2). Dibromo(aqua)(caffeine)zinc **1** is a molecular compound (Figure 1) (the coordination polyhedron is a slightly distorted tetrahedron; crystal structure data and bond lengths for compound **1** are given in Tables 1, S1, S2).

Cytotoxicity was determined by a methylthiazole tetrazolium (MTT) assay[20] using the postnatal dental pulp stem cells (DPSC) and MCF-7 breast cancer cell line obtained from the Russian Collection of Cell Cultures (Institute of Cytology of the Russian Academy of Sciences) (Figure 2) and human tumor cell lines: Jurkat human T cell leukemia, PC-3 prostate cancer, A-549 lung carcinoma, HCT-116 colon cancer, MCF-7 breast cancer, and SH-SY5Y neuroblastoma (Figure 3).¶ All the test compounds

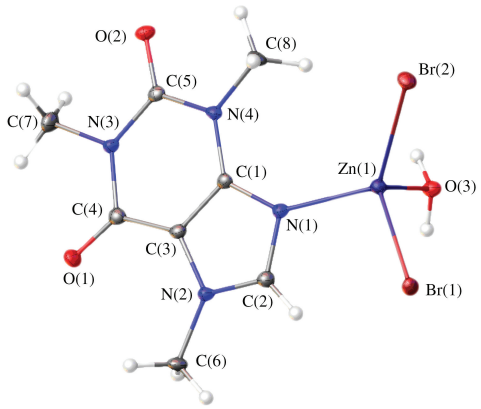


Figure 1 Molecular structure of $[Zn(caf)(H_2O)Br_2]$ **1** with thermal ellipsoids at a 50% probability level.

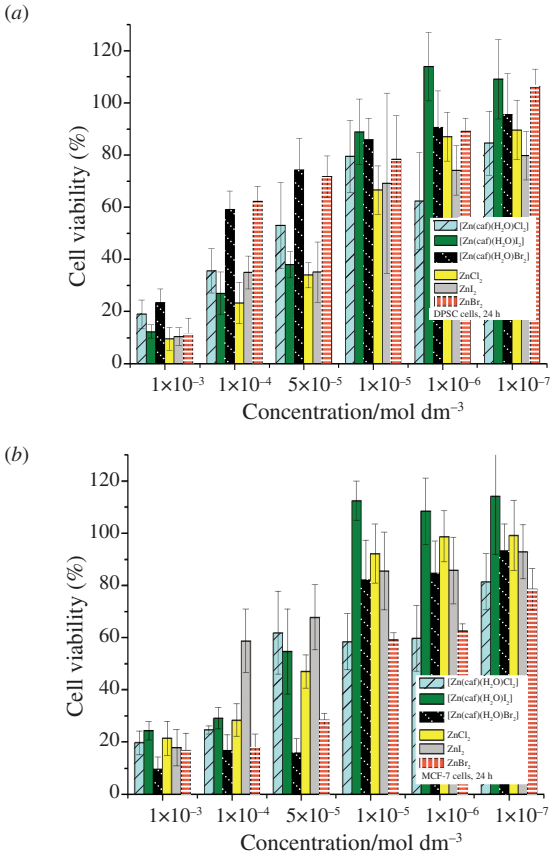


Figure 2 Effects of different compounds on the survivability of (*a*) DPSC line and (*b*) MCF-7 cells.

demonstrate dose-dependent behaviors and suppress cellular survivability of DPSC and MCF-7 cells at $c = 1\times10^{-4}$–1×10^{-3} mol dm^{-3} (see Figure 2, Tables S3, S4). Compound **1** is the most active in this concentration range towards MCF-7 cells (viability, <20%), while for stem cells it is 25–60% and comparable with that of the standard drug doxorubicin**.** The cytotoxicity varies in the sequence $[Zn(caf)(H_2O)Br_2]$ >

† See Online Supplementary Materials for the experimental procedures, IR, ^{1}H NMR and ESI-MS spectra of complex **1**.

‡ *Crystallographic data for* **1**. $[Zn(C_8H_{10}N_4O_2)(H_2O)Br_2]$ (M = 437.41), monoclinic, space group $P2_1/n$, a = 7.5774(15), b = 11.442(2) and c = = 15.383(3) Å, β = 91.259(3)°, V = 1333.4(5) Å^3, Z = 4, μ(MoKα) = = 7.840 mm^{-1}, F(000) = 848, d_{calc} = 2.179 g cm^{-3}. Reflections were measured at 150 K on a CCD SMART APEX-II diffractometer (graphite monochromatized MoK_α radiation, ω scan mode). The data reduction was performed by the SAINT program.[21] The absorption correction was applied by the SADABS program. The crystal structure was solved by direct methods and refined on F^2 by full-matrix least-squares in anisotropic approximation for non-hydrogen atoms. Positions of hydrogen atoms were calculated geometrically (the riding model). All calculations were performed using the Olex-2[22] and SHELXTL-Plus[23] program software. Intensities of 13670 reflections were measured and 3327 independent reflections (R_{int} = 0.046) were used in a further refinement. The refinement converged to $wR(F^2)$ = 0.058 and GOOF = = 1.026 for all independent reflections [R_1 = 0.0223 was calculated against F^2 for 3027 observed reflections with $I \geq 2\sigma(I)$]. The compound structures were visualized using the MERCURY program.[24]

CCDC 1870873 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

§ Powder X-ray diffraction patterns of the bulk samples (Bruker D8 Advance diffractometer, CuKα radiation, Ni-filter, LYNXEYE detector; reflection geometry; 2θ range, 5–80°; step, 0.01125°) were consistent with those calculated from the single crystal measurements.

¶ Human tumor cell lines were obtained from the Cell Line Data Bank of the N. N. Blokhin Russian Cancer Research Center, Moscow, Russian Federation. Cell lines were selected as representatives from 60 cell lines recommended by National Cancer Institute (NCI, one dose experiment, http://www.dtp.nci.nih.gov/branches/btb/ivclsp.html): MCF-7, PC-3, A-549, HCT-116, and Jurcat (used in the USA for standard cytotoxic testing). These cell lines represent the spectrum of different nosologies for oncological diseases and their testing reflects reaction on tumor cells of various origins, evaluating cytotoxic reaction on malignant tumors as a whole. Standard MTT test includes the assessment of cell line growth inhibition at 1, 10, and 100 μM. The compound is cytotoxic if 50% cells die at 100 μM. Lower concentrations are required for the graphical determination of IC_{50}. Control is not required.[25]

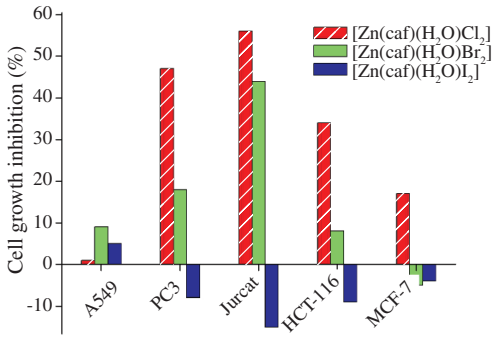


Figure 3 Cytotoxicity of $[Zn(caf)(H_2O)X_2]$ at $c = 1\times10^{-4}$ mol dm^{-3} (72 h).

Table 1 Bond lengths (Å) in complexes $[Zn(caf)(H_2O)X_2]$ (X = Cl, Br, I).

Bond	$[Zn(caf)(H_2O)Cl_2]$[7]	$[Zn(caf)(H_2O)Br_2]$	$[Zn(caf)(H_2O)I_2]$[7]
Zn–O	2.024(2)	2.025(3)	2.028(3)
Zn–N	2.068(2)	2.074(3)	2.053(4)
Zn–X	2.232(1)	2.370(1)	2.558(2)
	2.206(1)	2.344(1)	2.554(2)
N_1–O_3	3.09	3.124	3.09

$[Zn(caf)(H_2O)Cl_2]$ > $[Zn(caf)(H_2O)I_2]$ in accordance with the N(caf)–O (water) distance of 3.124 Å for $[Zn(caf)(H_2O)Br_2]$, which is close to the distance between the neighboring base pairs along the DNA helix (see Table 1). The cytotoxicity of compound **1** is intermediate between those of the chloro- and iodo-complexes, being the most pronounced towards Jurkat human T cell leukemia (see Figure 3), and at $c = 1\times10^{-5}$–1×10^{-3} mol dm^{-3}, it is higher than that of caffeine and comparable to the cytotoxicity of zinc bromide for DPSC and MCF-7 cell lines. It is probably related to electrostatic interaction of positively charged species with negatively charged phosphate groups in DNA[26] and DNA damage. The latter is confirmed by the DNA-comet assay[20] genotoxicity studies (Table S5) in the course of a 24-hour experiment with 31.83±2.54% tail DNA for MCF-7 cells and 8.37±1.47% tail DNA for DPSC. In other words, complex $[Zn(caf)(H_2O)Br_2]$ causes DNA damage similarly to the studied $[Zn(caf)(H_2O)X_2]$ (X = Cl, I) complexes.[20]

In conclusion, halide ions play an important role in the antitumor activity of complex compounds. The studied complexes $[Zn(caf)(H_2O)X_2]$ (X = Cl, Br, I) demonstrate antiproliferative activity in a low micromolar area. The cytotoxicity varies in the order $[Zn(caf)(H_2O)Br_2] > [Zn(caf)(H_2O)Cl_2] > [Zn(caf)(H_2O)I_2]$. Probably, $[Zn(caf)(H_2O)Br_2]$ possesses strong binding affinity towards ctDNA. In other words, similar cytotoxicity may be finely tuned by the partial varying of composition and dose of the compound.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.11.011.

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