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New copper complexes with *N*-(5,6-dihydro-4*H*-1,3-thiazin-2-yl)benzamide ligand

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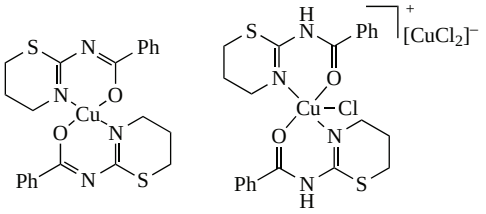
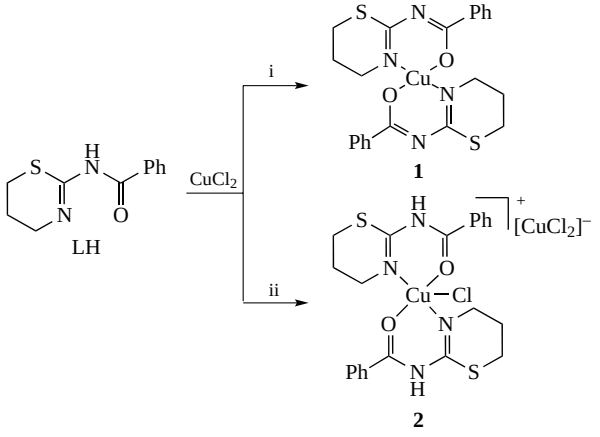
Crystals of two copper complexes with *N*-(5,6-dihydro-4*H*-1,3-thiazin-2-yl)benzamide were obtained and characterized by X-ray diffraction analysis. LETDI studies have shown that the composition of the complex is inhomogeneous, and the precipitated crystals can have different structures being of Cu^{II} or Cu^{I}–Cu^{II} types. The complexes are significantly more cytotoxic toward the leukemic Jurkat cell line than to healthy lymphocytes.



Keywords: copper complexes, *N*-(5,6-dihydro-4*H*-1,3-thiazin-2-yl)benzamide, X-ray crystallography, LETDI method, MTT-assay.

Among transition metal complexes, copper compounds are potentially attractive as antitumor agents.[1–5] In particular, this is due to the anoxic nature of cancer cells, which promotes the reduction of Cu^{II} to Cu^{I} and which is not possible in normal cells. In biological media, the properties of copper complexes strongly depend on the nature of the organic ligand and the presence of donor atoms.[6–11] Methods for the preparation of various copper complexes with organic ligands have been described earlier.[12–15] In particular, a bright green complex CuL_2Cl_2 [LH is *N*-(5,6-dihydro-4*H*-1,3-thiazin-2-yl)benzamide] was obtained,[3] its composition being confirmed by elemental analysis, LETDI (laser-induced electron transfer desorption/ionization) and ^{1}H NMR methods.[3] This ligand LH and its complexes are promising for medicinal[3] and radiopharmaceutical[16] applications.



Scheme 1 *Reagents and conditions*: i, EtOH/Pr^{i}OH (2 : 1), room temperature; ii, one week storage of the filtrate from step i.

In this work, we found that the reaction between $CuCl_2$ and compound LH in an EtOH/Pr^{i}OH medium (Scheme 1, path i)† gave violet crystalline Cu^{II} complex **1** which did not contain chlorine ions, namely, CuL_2. X-ray crystallography[17,18] showed that the crystals of this complex ($C_{22}H_{22}CuN_4O_2S_2$) belonged to the monoclinic system of the $P2_1/n$ group.‡ The molecular structure is outlined in Figure 1(*a*).

After separation of complex **1**, prolonged storage of the mother liquor at room temperature caused precipitation of

† *Bis{α-[(5,6-dihydro-4*H*-1,3-thiazin-2-yl)imino]benzyloxy}copper(II)* **1**. Ligand LH (0.24 g, 1.1 mmol) was dissolved in an EtOH/Pr^{i}OH mixture (2 : 1, 24 ml), and powdered $CuCl_2 \cdot 2H_2O$ (0.09 g, 0.5 mmol, Sigma, USA) was added with stirring. The formed precipitate was filtered off to afford violet substance **1** (0.09 g, 34%). Found (%): C, 53.02; H, 4.63; N, 11.28. Calc. for $C_{22}H_{22}CuN_4O_2S_2$ (%): C, 52.62; H, 4.42; N, 11.16.

*Chlorobis[*N*-(5,6-dihydro-4*H*-1,3-thiazin-2-yl)benzamide]copper(II) dichlorocuprate* **2**. The diaphanous filtrate from the previous synthesis was left at room temperature to crystallize slowly. About a week later, well-faceted dark green crystals precipitated from the solution. The crystals were filtered off, yield 0.06 g (52%). Found (%): C, 39.24; H, 3.64; N, 8.29. Calc. for $C_{22}H_{24}Cl_3Cu_2N_4O_2S_2$ (%): C, 39.20; H, 3.59; N, 8.31.

‡ *Crystal data for* **1**. $C_{22}H_{22}CuN_4O_2S_2$, M = 502.09, monoclinic, space group $P2_1/n$, 295(2) K, a = 6.8403(4), b = 13.5674(6) and c = 11.5670(6) Å, β = 97.162(4)°, Z = 2, V = 1065.10(10) Å^3, d_{calc} = 1.566 g cm^{-3}. $F(000)$ = 518, crystal size 21 × 16 × 12 mm. Total 7812 reflections were measured and 1963 independent reflections (R_{int} = 0.0410) were used in further refinement. The final R_1 = 0.0322 (ωR_2 = 0.0840) was calculated for 1512 observed reflections with $I > 2\sigma(I)$. R_{ind} for all data: R_1 = 0.0428 (ωR_2 = 0.0873). GOF = 0.977.

Crystal data for **2**. $C_{22}H_{24}Cl_3Cu_2N_4O_2S_2$, M = 674, triclinic, space group $P\bar{1}$, 295(2) K, a = 8.6885(7), b = 12.8230(9) and c = 12.8264(9) Å, α = 81.014(7)°, β = 87.864(6)°, γ = 70.607(7)°, Z = 2, V = 1331.24(18) Å^3, d_{calc} = 1.681 g cm^{-3}, $F(000)$ = 682. Total 9012 reflections were measured and 4392 independent reflections (R_{ind} = 0.0492) were used in further

green crystals of another complex **2** of the composition $[Cu(LH)_2Cl]^+[CuCl_2]^-$ (see Scheme, path ii).† X-ray study revealed that they belonged to the $P\bar{1}$ group ($C_{22}H_{24}Cl_3Cu_2N_4O_2S_2$).‡ From the molecular structure [Figure 1(*b*)] it can be seen that a partial reduction of Cu^{II} ions to Cu^{I} and the incorporation of chlorine ions occur. This causes an increase in the Cu···O bond lengths from 1.908(2) (**1**) to 2.055(3)–2.062(3) Å (**2**) and a decrease in the Cu···N bond lengths from 1.985(2) (**1**) to 1.956(3)–1.958(3) Å (**2**) with a corresponding change in the values of torsion angles. Besides, in complex **1**, the C=O···Cu distance [1.277(2) Å] is longer in comparison with that of complex **2** [1.234(5)–1.235(5) Å]. Studies of the first precipitate by the LETDI method[19] were carried out in a positive ionization mode (Figure 2). The mass spectrum shows two groups of peaks containing copper atoms with *m/z* 503–507 and 283–286, which correspond to $Cu(L–H)_2^+$ and its fragment ion $Cu(L–H)^+$. The theoretical isotope distribution completely coincides with the experimental one. The mass spectrum also contains two groups of peaks with *m/z* 221 and 105, corresponding to the protonated molecules of the ligand and its fragment ion. The intensity of these peaks exceeds the intensity of those for copper-containing ions, which is due to the fact that the ionization efficiency of highly basic compounds, to which the ligand belongs, is at least two orders of magnitude higher than that for metal-organic compounds.[20] Therefore, the presence of even a small amount of ligand in a sample leads to the appearance of a significant signal in the mass spectrum.

Thus, with an excess of ligand, copper complexes containing both only Cu^{II} and Cu^{II}–Cu^{I} pair were produced, which indicates a slow spontaneous copper reduction with a simultaneous change of the crystal structure from monoclinic (**1**) to triclinic (**2**).

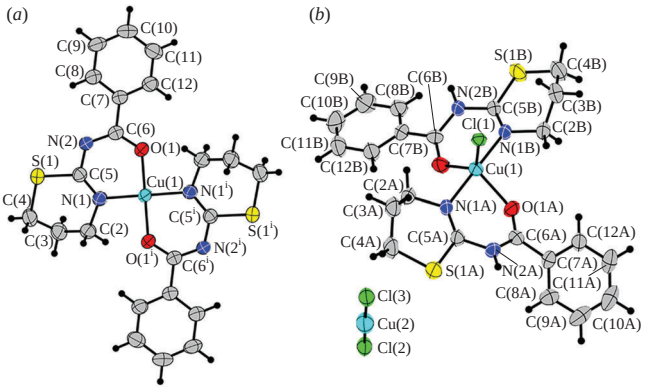


Figure 1 Molecular structure of (*a*) complex **1** and (*b*) complex **2** showing thermal ellipsoids at the 50% probability level.

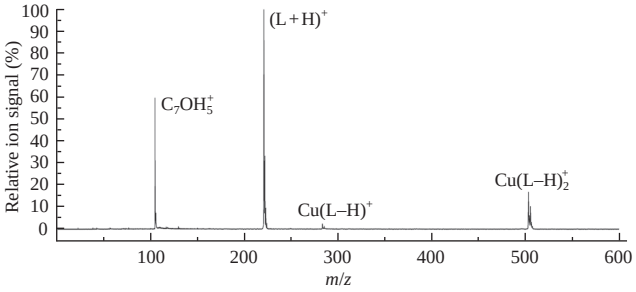


Figure 2 Mass spectrum of complex **1** recorded by the LETDI method in the positive ionization mode.

Table 1 Survival (LC_{50}/μmol ml^{-1}) of lymphocyte cells from healthy donors (HD) and T-lymphoblastic leukemia cells (Jurkat cell line and bone marrow cells – T-ALL) treated with complexes **1**, **2** and $(AP)_2CuCl_4$ (where AP is 2-aminopyrimidine).

Complex	HD	Jurkat	T-ALL	TW[a]
1	0.52 ± 0.08	(20 ± 0.5) × 10^{-3}	–	258
2	0.65 ± 0.05	0.057 ± 0.012	–	11.4
$(AP)_2CuCl_4$ (ref. 21)	0.38 ± 0.05	–	0.11 ± 0.02	3.5

[a] TW is therapeutic window, equal to LC_{50} (healthy donors)/LC_{50} (leukemic cells).

The cytotoxicity of the obtained complexes against the leukemic Jurkat cell line in comparison with normal lymphocytes is given in Table 1. The violet complex **1**, CuL_2 without Cl^- ions, exhibits greater cytotoxicity (determined by MTT-assay,[21,22] for details, see Online Supplementary Materials) in comparison with complex **2** demonstrating an order of magnitude higher therapeutic window TW = 258 [TW = LC_{50} (healthy donors)/LC_{50}]. Thus, complex **1** belongs to the promising anti-leukemic agents. A decrease in the TW value for complex **2** is associated with a decrease in cytotoxicity towards Jurkat cell line, while the effect on healthy cells is approximately similar to that of complex **1**. Earlier,[23] the cytotoxicity of $(AP)_2CuCl_4$-complex was found to be even lower with respect to leukemic cells (see Table 1) although cytotoxicity in relation to healthy cells was of the same order of magnitude as those for complexes **1** and **2**. Probably, T-lymphoblastic leukemic cells are more sensitive to structural changes in the acting agents.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.07.039.

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refinement. The final R_1 = 0.0466 (ωR_2 = 0.1044) was calculated for 5042 observed reflections with $I > 2\sigma(I)$, R_{ind} (all data): R_1 = 0.0820 (ωR_2 = 0.1134). GOF = 0.906.

The data were collected using STOE diffractometer Pilatus100K detector, focusing mirror collimation CuKα (1.54086 Å) radiation, rotation method mode. STOE X-Area software was used for cells refinement and data reduction. Data collection and image processing was performed with X-Area 1.67 (STOE & Cie GmbH, Darmstadt, Germany, 2013). Intensity data were scaled with LANA (part of X-Area) in order to minimize differences of intensities of symmetry-equivalent reflections (multi-scan method). The non-hydrogen atoms were refined using the anisotropic full matrix least-square procedure. Molecular geometry calculations were performed with the SHELX program,[17] and the molecular graphics were prepared using DIAMOND[18] software.

CCDC 2027415 and 2050373 contain 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.

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