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# Cu(II) Complexes with Heterocyclic Substituted Thiosemicarbazones: The Case of 5-Formyluracil. Synthesis, Characterization, X-ray Structures, DNA Interaction Studies, and Biological Activity

**Monica Baldini,[†] Marisa Belicchi-Ferrari,*[,†] Franco Bisceglie,[†] Giorgio Pelosi,[†] Silvana Pinelli,[‡] and Pieralberto Tarasconi[†]**

*Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Parco Area delle Scienze 17/A, and Dipartimento di Scienze Cliniche, via Gramsci 14, Università degli Studi di Parma, 43100 Parma, Italy*

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Two new 5-formyluracil thiosemicarbazone ($H_3$ut) derivatives, Me-$H_3$ut (**1**) and $Me_2$-$H_3$ut (**2**), were synthesized by reacting thiosemicarbazides, mono- and dimethylated on the aminic nitrogen, with 5-formyluracil and were subsequently characterized. These ligands, treated with copper chloride and nitrate, afforded three complexes: [Cu(Me-$H_3$ut)$Cl_2$]·$H_2O$ (**3**), [Cu($Me_2$-$H_3$ut)$Cl_2$]·$H_2O$ (**4**), and [Cu(Me-$H_3$ut)($NO_3$)($OH_2$)$_2$]$NO_3$ (**5**). The crystal structures of these complexes have been determined by single-crystal X-ray diffraction. In **3** and **4**, a similar pentacoordination is present; the copper atom is surrounded by the ligand SNO donor atoms and by two chloride ions. The structure of **5** consists of [Cu(Me-$H_3$ut)($NO_3$)($OH_2$)$_2$]$^+$ cations and nitrate anions. The copper coordination (4 + 2) involves the SNO ligand atoms and a water oxygen in the basal plane; the apical positions are occupied by a second water oxygen and by an oxygen of a monodentate nitrate group. Two biochemical techniques, namely DNA titration in the UV–vis region and thermal denaturation, have been employed to probe the details of DNA binding of these compounds. Analysis of the results suggests that our compounds are able to interact with DNA by electrostatic and groove binding but not by intercalation. The compounds have been also tested in vitro on human leukemic cell line U937, but they are not able to inhibit significantly cell proliferation.

## Introduction

Thiosemicarbazones show a wide range of biological properties depending on the parent aldehyde or ketone;[1] in particular, if these are heterocyclic aromatic systems, their nature seems to enhance their activity.[2−17]

Among these heterocyclic compounds, fluorouracil shows a remarkable antitumor activity and is already in use in the medical practice;[18] the study of prodrugs of 5-fluorouracil shows that also its derivatives have a high potential antitumor effect,[19] and their study is envisaged in order to overcome

* To whom correspondence should be addressed. E-mail: marisa.ferrari@unipr.it. Phone: +39-0521-90-5420. Fax: +39-0521-90-5557.

[†] Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica.

[‡] Dipartimento di Scienze Cliniche.

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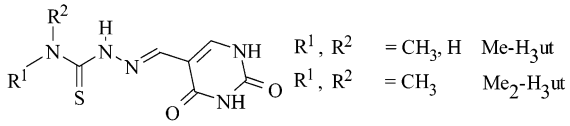
**Chart 1**




the limits of the parent drug. On the basis of these results, the parent derivatives of 5-formyluracil are molecules with potential biological properties.

These molecules possess three deprotonable NH groups as a function of pH (see Chart 1), and they can thus behave as anionic chelates toward a central metal. The resulting charge delocalization can stabilize the overall geometry and allow specific interactions with target molecules. In recent years, we already worked on 5-formyluracil thiosemicarbazone, and we found that its metal complexes showed an appreciably enhanced biological activity with respect to the free ligand. In particular, copper complexes were more active than their nickel or cobalt analogues, and one of these copper derivatives was also able to induce apoptosis.[20,21]

It has been observed that substitutions on the terminal N position can affect coordination and biological properties.[6−8,12,13,16,22−27] Moreover, as small molecules, these compounds can react at specific sites along a DNA strand through a series of weak interactions[28] allowing the metal center to approach the nucleic acid and to interfere with it. The present paper deals with the synthesis, characterization, and biological activity of two 5-formyluracil thiosemicarbazones with methyl substituents on the aminic nitrogen of the thiosemicarbazide chain, Me-$H_3$ut (**1**) and $Me_2$-$H_3$ut (**2**) (where $H_3$ut = 5-formyluracil thiosemicarbazone); moreover, three copper complexes, [Cu(Me-$H_3$ut)$Cl_2$]·$H_2O$ (**3**), [Cu($Me_2$-$H_3$ut)$Cl_2$]·$H_2O$ (**4**), and [Cu(Me-$H_3$ut)($NO_3$)($OH_2$)$_2$]$NO_3$ (**5**), containing the mentioned ligands are synthesized and also characterized structurally. The introduction of the methyl groups is made in order to vary the hydrophilic−lipophilic character of the compounds and in order to study if this alteration affects the uptake of the drugs by cells. To quantify the DNA binding capability in aqueous solution, a spectroscopic investigation with calf thymus DNA has been carried out. The ligands and their complexes have been also tested in vitro on human leukemic cell line U937 to find those compounds that are able to inhibit cell proliferation at least at the 50% level and to evaluate their ability to induce apoptosis.

## Experimental Section

**Physical Measurements.** Elemental analyses (C, H, N, S) were performed with a Carlo Erba model EA 1108 automatic analyzer. IR spectra (4000−400 $cm^{-1}$) for KBr disks were recorded on a Nicolet 5PC FT. Mass spectra were run on a Finnigan 1020 spectrometer (CI). Melting points were determined with a Gallenkamp instrument. UV measurements were performed on a Perkin-Elmer UV−vis Lambda 25 spectrometer with quartz cuvettes.

**General Procedures.** 5-Formyluracil (Aldrich, 98%), 4-methylthiosemicarbazide (Janssen), 4,4-dimethylthiosemicarbazide (Janssen), $CuCl_2$·$2H_2O$ (Carlo Erba), and $Cu(NO_3)_2$·$3H_2O$ (Carlo Erba) were commercially available and used without further purification. Calf thymus (CT) DNA was obtained from Serva and used as received. DNA samples were dissolved in 50 mM NaCl/5 mM Tris, pH 7.2. A solution of CT-DNA (ca. $10^{-5}$ M in base-pair, [bp]) in this buffer gave ratios of UV absorbance at 260 and 280 nm, $A_{260}/A_{280}$, of ca. 1.8, indicating that the CT-DNA was sufficiently free of protein. The concentration of the nucleic acid solutions was determined by UV absorbance at 260 nm after 1:100 dilution. The extinction coefficient $\epsilon_{260}$ was taken as 13100 $M^{-1}$ $cm^{-1}$.[29] Stock solutions were stored at 4 °C and used after no more than 4 days.

**Synthesis of Me-$H_3$ut·$2H_2O$ (1).** 4-Methylthiosemicarbazide (0.81 mmol) was solubilized in 30 mL of $H_2O$ by stirring and slight heating. An equimolar amount of 5-formyluracil was dissolved in 30 mL of $H_2O$, by stirring and gentle heating, and HCl was added until pH ca. 4 was reached. The solution of the aldehyde was added dropwise to the thiosemicarbazide. In 1 h of stirring and heating, a yellow powder was formed. The solution, cooled to room temperature, was filtered, and the isolated product was analyzed. Anal. Calcd for $C_7H_{13}N_5O_4S$: C, 31.94; H, 4.98; N, 26.60; S, 12.18%. Found: C, 32.01; H, 4.17; N, 26.31; S, 12.43. IR (KBr disks, $cm^{-1}$): 3465(m) and 3380(m) $\nu$(NH), 3261(m, br) $\nu$(OH), 1700(s) $\nu$(C=O), 1663(s) $\nu$(C=N) + $\nu$(C=C), 1533 (ms) $\nu$(CSN), 871(m) $\nu$(C=S). Mass spectrum *m*/*z*: 228 ($MH^+$), 197 ($MH^+$ − $CH_3NH$). Mp: 186 °C. Yield = 74%.

**Synthesis of $Me_2$-$H_3$ut·$2H_2O$ (2).** 5-Formyluracil (0.93 mmol) was dissolved in 25 mL of $H_2O$ by stirring and heating and added to 25 mL of $H_2O$ in which an equimolar amount of 4,4-dimethylthiosemicarbazide was previously solubilized under stirring at reflux temperature. After 1 h of stirring and heating, a yellow powder was formed, isolated by filtration when the solution was cooled to room temperature. The product was recrystallized in a mixture of $H_2O$/EtOH = 1/2, obtained as microcrystals, and then analyzed. Anal. Calcd for $C_8H_{15}N_5O_4S$: C, 34.65; H, 5.45; N, 25.26; S, 11.56%. Found: C, 35.43; H, 4.64; N, 25.26; S, 11.98. IR (KBr disks, $cm^{-1}$): 3482(ms) $\nu$(NH), 3264(m) $\nu$(OH), 1706(s) $\nu$(C=O), 1666(s) $\nu$(C=N) + $\nu$(C=C), 1561 (ms) $\nu$(CSN), 872(m) $\nu$(C=S). Mass spectrum *m*/*z*: 242 ($MH^+$). Mp: 195 °C. Yield = 80%.

**Synthesis of [Cu(Me-$H_3$ut)$Cl_2$]·$H_2O$ (3).** Me-$H_3$ut·$2H_2O$ (0.50 mmol) was dissolved in 80 mL of a mixture of $H_2O$/EtOH = 1/2 by stirring and heating. The solution was yellowish. An equimolar amount of $CuCl_2$·$2H_2O$ was dissolved in 20 mL of the same solvent mixture. The latter was added to the ligand solution that became green. The resulting mixture was stirred at 50 °C for 30 min. By

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slow evaporation of the solvent, green rhombic crystals were obtained. Anal. Calcd for $C_7H_{11}N_5Cl_2CuO_3S$: C, 22.14; H, 2.92; N, 18.44; S, 8.44%. Found: C, 22.32; H, 2.64; N, 17.18; S, 8.13. IR (KBr disks, $cm^{-1}$): 3377(m) and 3335(m) $\nu$(NH), 3244(m) $\nu$(OH), 1729(ms) $\nu$(C═O), 1642(ms) $\nu$(C═N) + $\nu$(C═C), 1500 (ms) $\nu$(CSN), 859(m) $\nu$(C═S). Mp: 245 °C.

**Synthesis of [Cu(Me$_2$-H$_3$ut)Cl$_2$]·H$_2$O (4).** Me$_2$-H$_3$ut·2H$_2$O (0.55 mmol) was dissolved in 80 mL of a mixture of H$_2$O/EtOH = 1/2 by stirring and heating. The solution was yellowish. An equimolar amount of $CuCl_2 \cdot 2H_2O$ was dissolved in 10 mL of the same solvent mixture. The latter was added to the ligand solution that became red, and the formation of a powder was observed. The formed product was redissolved by heating. The resulting mixture was stirred at 60 °C for 30 min. By slow evaporation of the solvent, green crystals were obtained. Anal. Calcd for $C_8H_{13}N_5Cl_2CuO_3S$: C, 24.40; H, 3.33; N, 17.79; S, 8.14%. Found: C, 23.98; H, 2.74; N, 16.70; S, 7.26. IR (KBr disks, $cm^{-1}$): 3414 (m) $\nu$(NH), 3220 (br) $\nu$(OH), 1706 (s) $\nu$(C═O), 1667(ms) $\nu$(C═N) + $\nu$(C═C), 1525 (m) $\nu$(CSN), 860 (m) $\nu$(C═S). Mp: 255 °C.

**Synthesis of [Cu(Me-H$_3$ut)(NO$_3$)(OH$_2$)$_2$]NO$_3$ (5).** Me-H$_3$ut·2H$_2$O (0.45 mmol) was dissolved in 40 mL of a mixture of H$_2$O/EtOH = 1/2 by stirring and heating. The solution was yellowish. An equimolar amount of $Cu(NO_3)_2 \cdot 3H_2O$ was dissolved in 20 mL of the same solvent mixture. The latter was added to the ligand solution that became green. The resulting mixture was stirred at 50 °C for 10 min. By slow evaporation of the solvent, green rhombic crystals were obtained. Anal. Calcd for $C_7H_{13}N_7CuO_{10}S$: C, 18.64; H, 2.91; N, 21.75; S, 6.98%. Found: C, 18.25; H, 2.63; N, 20.85; S, 6.98. IR (KBr disks, $cm^{-1}$): 3400 (m, br) $\nu$(NH) and $\nu$(OH), 1729 (m) $\nu$(C═O), 1642(ms) $\nu$(C═N) + $\nu$(C═C), 1500 (m) $\nu$(CSN), 858 (m) $\nu$(C═S). Mp: 235 °C.

**Crystallography.** Relevant data concerning data collection and details of structure refinement are summarized in Table 1. All intensity data were collected on a SMART 1000 Bruker AXS diffractometer with Mo Kα radiation for compound **3** and on a Siemens AED single-crystal computer controlled by the $\theta-2\theta$ technique with Mo Kα radiation for **4** and Cu Kα radiation for **5**. Correction for absorption following the Walker Stuart method [30] was applied to the data of complex **5** while no correction was made for compounds **3** and **4** because of the small crystal dimensions. The structures were solved using direct methods (SIR-92[31] for **4** and **5** and SIR-97[32] for **3**). Refinements were carried out by full-matrix least-squares cycles SHELXL97[33] for all compounds. Anisotropic thermal motion was assumed for all non-hydrogen atoms. For complex **3**, the hydrogen atoms were located on a difference map and refined. For compound **4**, the hydrogen atoms were located on a difference map but not refined; those on the methyl groups were calculated with standard geometry and refined in riding position. In complex **5**, the hydrogen atoms were partly located on a difference map and not refined (water hydrogens, H2, H4, H5N) and partly calculated with standard geometry and refined in riding position. Atomic scattering factors were taken from ref 34. An ENCORE91 computer was employed with the program PARST[35] for the geometrical description of the structures and ORTEP[36] and PLUTO[37] for the structure drawings.

**Table 1.** Experimental Data for Crystallographic Analyses

| | **3** | **4** | **5** |
|---|---|---|---|
| formula | $C_7H_{11}Cl_2CuN_5O_3S$ | $C_8H_{13}Cl_2CuN_5O_3S$ | $C_7H_{13}CuN_7O_{10}S$ |
| mol wt | 379.71 | 393.73 | 450.83 |
| space group | $P2_1/c$ | $P2_1/c$ | $P2_1/c$ |
| $a$ (Å) | 9.077(1) | 9.035(3) | 15.696(6) |
| $b$ (Å) | 9.444(1) | 9.888(3) | 13.236(3) |
| $c$ (Å) | 15.499(2) | 15.830(6) | 7.515(2) |
| α (deg) | 90.0 | 90.0 | 90.0 |
| $\beta$ (deg) | 101.76(1) | 101.23(6) | 94.49(5) |
| $\gamma$ (deg) | 90.0 | 90.0 | 90.0 |
| $V$ (Å$^3$) | 1300.7(3) | 1387.1(9) | 1556.5(8) |
| $Z$ | 4 | 4 | 4 |
| $F$ (000) | 764 | 796 | 916 |
| $D_{calcd}$ (Mg/m$^3$) | 1.94 | 1.88 | 1.92 |
| $\mu$ ($mm^{-1}$) | 2.26 | 2.12 | 3.94 |
| $\lambda$ (Å) | 0.71069 | 0.71069 | 1.54184 |
| radiation | Mo Kα | Mo Kα | Cu Kα |
| $\theta$ range (deg) | 3−27 | 3−30 | 3−60 |
| *hkl* ranges | −11 to 7; −11 to 11; −18 to 19 | −11 to 12; 0 to 13; 0 to 15 | −17 to 16; 0 to 14; 0 to 8 |
| cryst size (mm$^3$) | 0.11 × 0.07 × 0.15 | 0.10 × 0.08 × 0.18 | 0.12 × 0.10 × 0.14 |
| no. measured reflns | 7440 | 4415 | 2399 |
| no. unique reflns | 2691 | 4071 | 1840 |
| no. refined params | 216 | 219 | 264 |
| max and min height in final $\Delta F$ map/e Å$^{-3}$ | 0.36;−0.30 | 1.03;−1.18 | 1.52;−1.22 |
| $R = \sum\|F_o\| - \|F_c\|/\sum\|F_o\|$ | 0.027 | 0.058 | 0.079 |
| wR2 | 0.073 | 0.175 | 0.249 |
| weights | $1/[\sigma^2(F_o^2) + (0.0469P)^2 + 0.20P]$[a] | unit weights are used | $1/[\sigma^2(F_o^2) + (0.2000P)^2 + 0.0P]$ |

[a] $P = (\max(F_o^2, 0) + 2F_c^2)/3$.

**DNA Interaction Studies.** Binding constants for the interaction of the studied compounds with nucleic acid were determined as already described.[38] The intrinsic binding constant $K_b$ for the interaction of the studied compounds with CT-DNA was calculated by absorption spectral titration data using the following equation: $1/\Delta\epsilon_{ap} = 1/(\Delta\epsilon K_b D) + 1/\Delta\epsilon$ where $\Delta\epsilon_{ap} = |\epsilon_A - \epsilon_f|$, $\Delta\epsilon = |\epsilon_B - \epsilon_f|$, $D$ = [DNA], and $\epsilon_A$, $\epsilon_B$, and $\epsilon_f$ are the apparent, bound, and free extinction coefficients of the compound, respectively. $K_b$ is given by the ratio of the slope to intercept when it is reported in plot [DNA]/($\epsilon_A - \epsilon_f$) versus [DNA] and is expressed as $M^{-1}$. The preceding equation, originally used to calculate the binding constants for hydrophobic derivatives, is now broadly used to investigate a wide variety of metal complexes containing phenanthroline and its derivatives and, very recently, has been adopted to obtain binding constant values from metal complexes with different ligands.[39] Fixed amounts of the ligands and of the complexes were dissolved in DMSO because the high solubility of the compounds in this solvent allows us to prepare concentrated solutions and therefore to utilize

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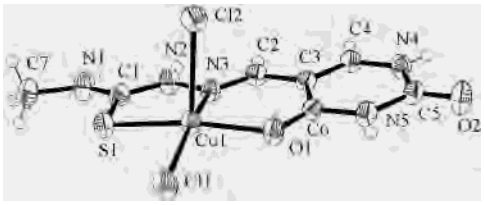


**Figure 1.** ORTEP drawing of complex [Cu(Me-$H_3$ut)$Cl_2$] (**3**) showing thermal ellipsoids at the 50% probability level.

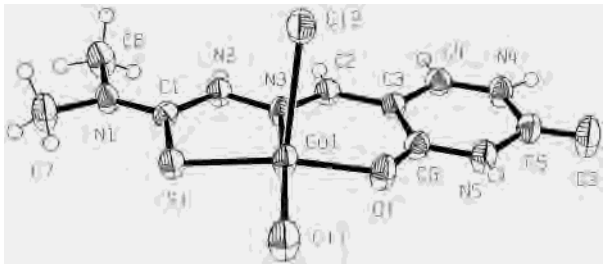


**Figure 2.** ORTEP drawing of complex [Cu($Me_2$-$H_3$ut)$Cl_2$] (**4**) showing thermal ellipsoids at the 50% probability level.

reduced volumes (5 $\mu$L) for titrations. It was also verified that the DMSO percentage (0.7%) added to the DNA solution did not interfere with the nucleic acid; in fact, the 260 nm absorption band is not subjected to modifications in intensity and position. Concentrated solutions of NaCl, Tris-HCl (pH 7.2) buffer, and DNA were prepared. Calculated amounts of the described stock solutions were taken to final concentration values of 50 mM NaCl, 5 mM Tris-HCl, and increasing amounts of DNA over a range of DNA concentrations from $10^{-5}$ to $10^{-3}$ M. These solutions were then added to the 5 $\mu$L solution of the considered compound in order to maintain the final volume of the solutions fixed to 700 $\mu$L. The compounds were titrated at room temperature. The changes in absorbance of DNA of an intraligand (IL) band upon each addition were monitored at the maximum wavelengths 331, 377, 336, 336, and 356 nm for **1**, **2**, **3**, **4**, and **5**, respectively. All melting measurements were carried out in the described solutions. DNA (45 $\mu$M) was then treated with our compounds at different mol/bp ratios ($r = 0.01$ and 0.1), and each sample was incubated for 24 h at room temperature. Samples were continuously heated with 1° $min^{-1}$ rate of temperature increase, while the absorbance change at 260 nm was monitored. The investigated interval of temperature ranged from 50 to 90 °C. Upon reaching 90 °C, samples were cooled back to 50 °C in order to follow the renaturation process. Values for melting temperature ($T_m$) and for the melting interval ($\Delta T$) were determined according to the reported procedures.[40] Differential melting curves were obtained by numerical differentiation of experimental melting curves.

**Biological Data: Materials and Methods.** The cells U937 were seeded at $2 \times 10^5$/mL in the presence of RPMI 1640 supplemented with L-glutamine 200 mM, 10% foetal bovine serum (FBS), and antibiotics (penicillin 100 U/mL and streptomycin 100 $\mu$g/mL) in a humidified atmosphere of 5% $CO_2$ at 37 °C and then treated with the compounds previously stored dry at room temperature and dissolved in DMSO just before their use. The ranges of used concentrations were 20−40 $\mu$g/mL for compound **1**, 10−30 $\mu$g/mL for **2**, 5−25 $\mu$g/mL for **3** and **4**, and 10−40 $\mu$g/mL for **5**. Cell mortality, evaluated on the fourth day by the trypan blue exclusion method and determined by using a hemocytometer, never exceeded 5%.

## Results and Discussion

**Crystal and Molecular Structure.** In Figure 1 is reported the ORTEP view of neutral complex **3**, [Cu(Me-$H_3$ut)$Cl_2$]. The copper atom is surrounded by five donor atoms in a square pyramidal fashion (4 + 1). The basal plane is made up from the S, N, and O atoms of the ligand in its neutral form and one chloride ion, while the second chloride occupies the apical position. The overall geometry is very similar to that found in the corresponding complex with the unsubstituted ligand.[20] The copper atom lies 0.23 Å from the average basal plane toward the Cl2 atom.

The pyramid is fairly regular with the angle between the Cu−Cl bond and the normal to the coordination plane of 4.09(3)°. A very long contact (3.57 Å) with the N3 atom in position $-x, -y, -z + 1$ completes a copper elongated bipyramidal coordination. The five member chelation ring presents a $\phi_2$ of 166° corresponding to a twist conformation, and the six member one is characterized by a $\phi_2$ of −171° and a $\theta$ of 117°, i.e., an intermediate conformation between envelope and boat.[41] The dihedral angle between the mean planes of the chelation rings is 8.8°. The complexes are joined through hydrogen bonds involving N4 and N5 nitrogen atoms, the uracil oxygen atom O2, and the apical chlorine Cl2 [N5···Cl2($-x, y + {}^1/_2, {}^1/_2 - z$) = 3.162(2) Å, N4···O2($1 - x, y - {}^1/_2, {}^1/_2 - z$) = 2.962(3) Å, N4···Cl2($x + 1, y, z$) = 3.489(2) Å] in the $xy$ plane. Crystallization water molecules join these planes [O1W···Cl2 = 3.084(3) Å, O1W···Cl1($-x, y - {}^1/_2, {}^1/_2 - z$) = 3.127(3) Å, N1(amine)···O1W = 3.211(3) Å, N2(hydrazine)···O1W ($-x, y - {}^1/_2, {}^1/_2 - z$) = 2.770(3) Å] giving a complex three-dimensional lattice.

In [Cu($Me_2$−$H_3$ut)$Cl_2$] (**4**), the copper ion has an approximate square planar environment determined by the sulfur atom, the imine nitrogen N3, the uracil O1 oxygen, and the chlorine atom Cl1. The coordination completes an elongated square pyramid by a long interaction (2.814 Å) between Cu and the second chlorine atom Cl2 (Figure 2).

The large difference between the two Cu−Cl distances (2.231(1) Å in the basal plane and 2.814(2) Å in the apical position) can be ascribed to a Jahn−Teller distortion. By comparing complex **3** (monomethylderivative) with **4** (dimethylderivative), it can be noticed that the greater inductive effect in **4** decreases all bond distances in the equatorial plane, while the apical Cu−Cl distance increases (Table 2).

The copper center is displaced 0.21 Å toward the pyramid apex. The angle between the Cu−Cl2 bond and the normal to the mean coordination plane is 1.11(6)°. The five member chelation ring shows an intermediate conformation between envelope and twist ($\phi_2$ of −99°, $q_2 = 0.107(3)$ Å); the six membered one is characterized by a boat conformation ($\phi_2$ of −180°, $\theta_2 = 119°$).[41] The dihedral angle between these rings is 8.5(7)°. Packing is characterized by molecule chains linked by the crystallization water molecules O1W (O1W−H···Cl1 = 3.180(4) Å and N−H···O1W($x, y - 1, z$) =

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**Table 2.** Selected Bond Distances (Å) and Angles (deg) for Compounds **3**, **4**, and **5**

| | 3 | 4 | 5 |
|---|---|---|---|
| Cu−S1 | 2.258(1) | 2.226(2) | 2.252(2) |
| Cu−O1 | 1.998(2) | 1.964(4) | 1.969(4) |
| Cu−N3 | 2.005(2) | 1.999(3) | 1.971(4) |
| Cu−O1W | | | 1.965(4) |
| Cu−O2W | | | 2.516(4) |
| Cu−CL1 | 2.261(1) | 2.231(1) | |
| Cu−CL2 | 2.671(1) | 2.814(2) | |
| Cu−O3 | | | 2.549(5) |
| S1−C1 | 1.701(2) | 1.712(4) | 1.711(6) |
| O1−C6 | 1.242(3) | 1.242(6) | 1.251(7) |
| O2−C5 | 1.203(3) | 1.208(7) | 1.196(8) |
| N1−C1 | 1.329(3) | 1.331(6) | 1.307(8) |
| N2−N3 | 1.382(3) | 1.389(5) | 1.356(7) |
| N2−C1 | 1.348(3) | 1.348(6) | 1.344(7) |
| N3−C2 | 1.286(3) | 1.284(6) | 1.286(7) |
| N4−C4 | 1.346(3) | 1.338(6) | 1.332(8) |
| N4−C5 | 1.384(3) | 1.390(6) | 1.363(8) |
| N5−C5 | 1.384(3) | 1.374(6) | 1.398(7) |
| N5−C6 | 1.367(3) | 1.360(6) | 1.351(8) |
| C2−C3 | 1.439(3) | 1.428(6) | 1.409(8) |
| C3−C4 | 1.366(3) | 1.377(7) | 1.390(8) |
| C3−C6 | 1.444(3) | 1.443(6) | 1.437(8) |
| N1−C7 | 1.465(4) | 1.458(7) | 1.457(8) |
| N1−C8 | | 1.456(6) | |
| O1−Cu−N3 | 90.34(7) | 91.7(1) | 91.0(2) |
| S1−Cu−N3 | 85.96(5) | 86.7(1) | 87.2(1) |
| S1−Cu−O1 | 169.24(5) | 168.8(1) | 174.6(1) |
| CL1−Cu−N3 | 164.98(5) | 167.5(1) | |
| CL1−Cu−O1 | 91.05(5) | 89.7(1) | |
| CL1−Cu−S1 | 89.95(2) | 89.5(1) | |
| CL2−Cu−N3 | 89.91(5) | 84.3(1) | |
| CL2−Cu−O1 | 88.93(5) | 92.4(1) | |
| CL2−Cu−S1 | 101.15(2) | 98.5(1) | |
| CL1−Cu−CL2 | 105.06(2) | 108.0(1) | |
| S1−Cu−O1W | | | 91.7(1) |
| O1−Cu−O1W | | | 90.4(2) |
| N3−Cu−O1W | | | 176.3(2) |
| O2W−Cu−O1 | | | 85.4(2) |
| O2W−Cu−N3 | | | 92.0(2) |
| O2W−Cu−S1 | | | 99.8(1) |
| O2W−Cu−O1W | | | 84.7(1) |
| O3−Cu−O1 | | | 78.5(2) |
| O3−Cu−N3 | | | 103.6(2) |
| O3−Cu−S1 | | | 96.9(1) |
| O3−Cu−O1W | | | 80.0(2) |
| O3−Cu−O2W | | | 157.6(1) |

2.794(5) Å) extending along the *b* axis. These chains are connected through two hydrogen bonds O1W−H···Cl2 apical ($1 - x$, $y + {}^1/_2$, ${}^1/_2 - z$) = 3.092(5) Å and N5−H···Cl2($1 - x$, $y + {}^1/_2$, ${}^1/_2 - z$) = 3.232(4) Å to form strands that face one another with the apolar zones of the amino nitrogen methyl substituents.

The structure of **5** consists of $[Cu(Me\text{-}H_3ut)(NO_3)(OH_2)_2]^+$ cations (Figure 3) and nitrate anions. The coordination geometry is an elongated distorted square bipyramid (4 + 2) and involves the N, S, and O atoms of the terdentate neutral thiosemicarbazone and a water oxygen O1W in the basal plane, while the apical positions are occupied by a second water oxygen O2W and by an O3 atom of a monodentate nitrate group (Cu−O distances are 2.516(4) and 2.549(5) Å, respectively). The copper atom lies only 0.04 Å out of the mean basal plane toward the water oxygen. The angles between the Cu−O3 and Cu−O2W bonds and the normal to the basal plane are 13.6(1)° and 9.5(1)°, respectively. The five member chelation ring presents a $\phi_2$ of −65° corresponding to an intermediate conformation between twist and envelope, and the six membered one is characterized by a $\phi_2$ of 34° and a $\theta$ of 55°; i.e., it shows a half-chair conformation.[41] The corresponding nitrate complex with the unsubstituted 5-formyluracilthiosemicarbazone ($H_3ut$) was cationic with formal charge +2 because both nitrate groups were out of the coordination sphere.[21]

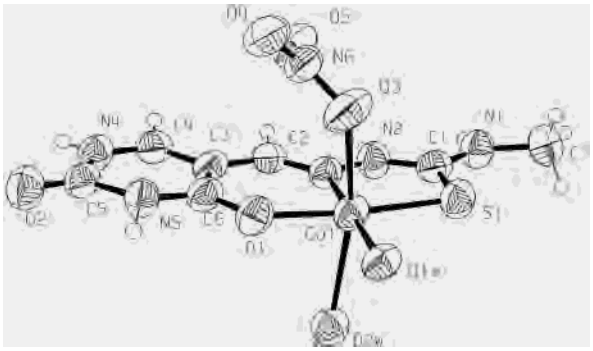

**Figure 3.** ORTEP drawing of cation $[Cu(Me\text{-}H_3ut)(NO_3)(OH_2)_2]^+$ showing thermal ellipsoids at the 50% probability level.

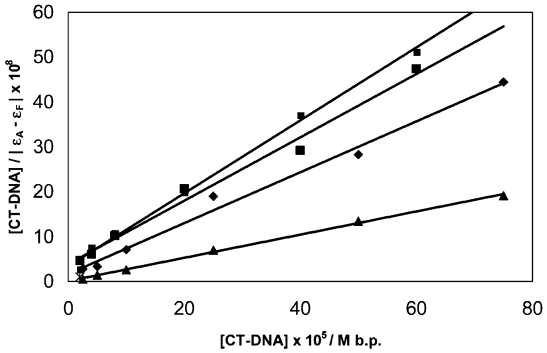


**Figure 4.** Typical plots of [CT-DNA]/($\epsilon_A - \epsilon_F$) vs [CT-DNA] for absorption titration with CT-DNA in 50 mM NaCl/5 mM Tris buffer, pH 7.2 at 25 °C: triangle = **1**, diamond = **3**, large square = **2**, small square = **4**

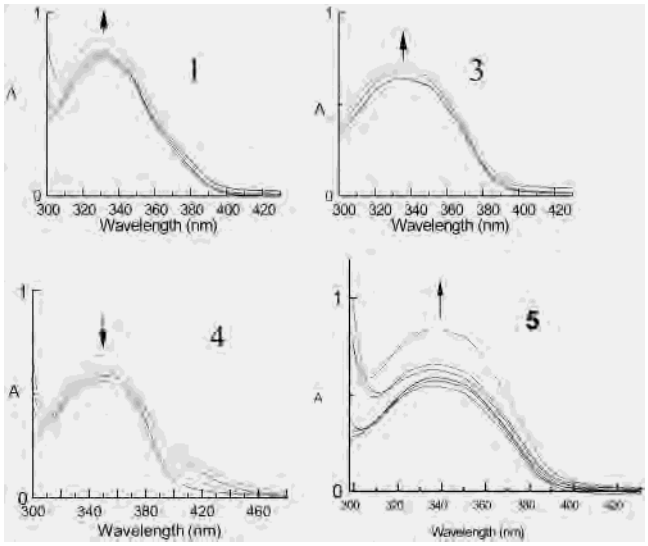


**Figure 5.** Variation in IL absorption spectra of compounds Me-$H_3$ut (**1**) (40 $\mu$M), $[Cu(Me\text{-}H_3ut)Cl_2]$ (**3**) (40 $\mu$M), $[Cu(Me_2\text{-}H_3ut)Cl_2]$ (**4**) (50 $\mu$M), and $[Cu(Me\text{-}H_3ut)(NO_3)(OH_2)_2]NO_3$ (**5**) (40 $\mu$M), with increasing amount of CT-DNA.

Packing is described by chains of complex molecules stretching along the *z* direction linked by hydrogen bonds

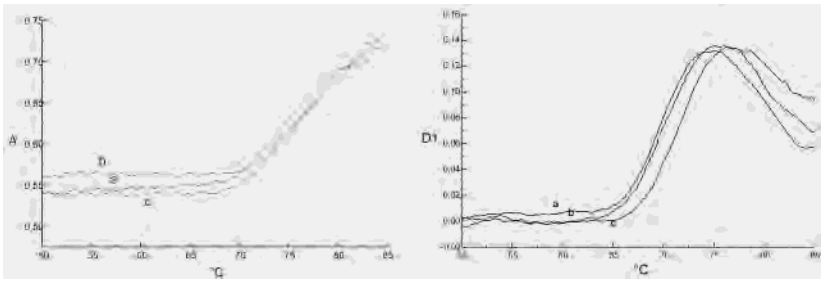

**Figure 6.** Thermal denaturation profiles (left) and differential melting curves (right) of calf thymus DNA before (a) and after addition of [Cu($Me_2$-$H_3$ut)$Cl_2$] (**4**) at $r = 0.01$ (b) and $r = 0.1$ (c). DNA concentration: $4.5 \times 10^{-5}$ M. Buffer: 50 mM NaCl, 5 mM Tris-HCl.

between the water molecules and the O4 and O5 oxygen atoms of the coordinated nitrate group (O1W−H···O4 ($x$, $y$, $z + 1$) = 2.751(5) Å, O2W−H···O5 ($x$, $y$, $z + 1$) = 2.761(6) Å). These chains are coupled by hydrogen bonds involving the protonated hydrazine N2 and aminic N1 atoms of the thiosemicarbazide moiety, the uracil N4 and N5 nitrogens, and the oxygen atoms of the nitrate groups. Between these double chains, only hydrophobic contacts involving the terminal methyl groups are present.

In all three complexes, the mono- and disubstituted ligand is neutral and in its thionic form (only a very limited charge delocalization is shown by the S−C1 and C2−N3 distances typical of a double bond (Table 2)). In octahedral complex **5**, a similar conjugated charge density on the whole ligand is moreover present (N1−C1 = 1.307 Å, N2−N3 = 1.356 Å, C2−C3 = 1.409 Å).

**Absorption Spectral Features of DNA Binding and Thermal Denaturation.** The binding constant obtained for ligand **1** is $3.4 \times 10^4$, while for compound **2** the changes in absorption spectra upon DNA addition were so small that the resultant difference in ($\epsilon_A - \epsilon_F$) was not significant if compared with the single values. The binding constants for complexes **3**, **4**, and **5** are $1.3 \times 10^4$, $2.7 \times 10^4$, and $4.9 \times 10^4$, respectively. In Figure 4 is reported the plot of [DNA]/($\epsilon_A - \epsilon_f$) versus [DNA] for absorption titration of the studied compounds, useful for obtaining $K_b$ by the ratio of the slope to intercept.

Our experimental $K_b$ values are lower than those observed for classical intercalators (ethidium-DNA, $1.4 \times 10^6$ in 25 mM Tris-HCl/40 mM NaCl buffer, pH = 7.9;[40] $3.0 \times 10^6$ in 5 mM Tris-HCl/50 mM NaCl buffer, pH = 7.2 [this work]), indicating that the compounds bind DNA with less affinity, as already noticed for copper(II) complexes with macrocyclic ligands.[39] Since upon complexation no significant increase of $K_b$ was observed, the good constant values can probably be related to the role played by the ligand in the binding to DNA. Hypochromism and red shift (bathochromism) in electronic absorption spectra of DNA bound to different compounds are generally attributed to intercalation, involving a strong stacking interaction between aromatic chromophores and the base pairs of DNA. In this work, these features were observed only for compound **4**, and the spectra are different from those for ligand **1** and its complexes **3** and **5** (see Figure 5) that instead are fairly similar.

The ratio $r$ ($r$ = [CT-DNA]/[complex]) varied between 0 and 15 in 5 mM Tris-HCl buffer (pH = 7.2), 50 mM NaCl at 25 °C. The arrows indicate the directions of absorbance changes as a function of $r$. For ligand **1**, this behavior can be explained as due to the dissociation of ligand aggregates as already seen for other aromatic ligands,[42] and the same hypothesis could be suggested also for its complexes. A similar hyperchromism was observed also for a Cu(II) complex with a ligand bearing NH and OH groups[39] and for the Soret bands of certain porphyrins showing interactions with DNA.[43] This feature has not been yet well explained. We think that the difference in the absorption spectral behavior is due to the possibility for **1**, **3**, and **5** to have more hydrogen bonding interactions, in agreement with ref. 39 while for compound **4**, the more classical behavior is probably due to its increased hydrophobicity; in addition, the electronic spectrum of the adduct of **4** with DNA shows the presence of a shoulder (at 410 nm) not visible in the spectra of the other compounds, which highlights the different behavior and suggests the presence of a new molecular species responsible for interactions.

Increase in hypochromicity is related to the increase in hydrophobicity.[28,44] Nevertheless, even for compounds **1**, **3**, and **5**, a partial intercalation cannot be excluded to explain the $K_b$ values, or an external mode of binding to DNA leading to modest electronic coupling with the host could be considered, as already noted for Cu(II) complexes.[42] To better verify and confirm these results, thermal denaturation profiles of DNA were carried out. Melting temperatures ($T_m$) of all compounds were compared with 75 °C for CT-DNA $T_m$ without drugs.

As an example, the thermal denaturation profiles and the differential melting curves for compound **4** are reported in Figure 6 (left and right, respectively).

At the used concentrations, compounds had only negligible effects on the melting temperatures, suggesting that they have no capability to stabilize the DNA duplex because they do not significantly increase $T_m$. We can therefore suppose that the binding of these molecules to DNA involves exclusively either the DNA grooves or the phosphates of the backbone; an intercalative behavior can be excluded.

**Effects on Cell Proliferation.** The effects of both ligands **1** and **2** and of three complexes **3**, **4**, and **5** on U937 cell

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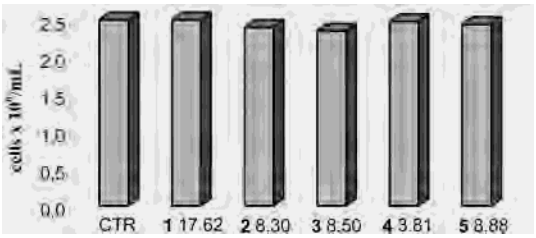


**Figure 7.** Effects on U937 proliferation on the fourth day following addition of the studied compounds; experiments were performed in quintuplicate, and the control contains the maximum concentration of DMSO used to dissolve tested compounds. Concentration values are micromolar.

line proliferation on the fourth day are reported in Figure 7. We wanted to compare the biological effect of one or two methyl substituents on aminic nitrogen N1 in the free ligands and investigate the influence of the metal also changing the counterion (chloride or nitrate). Unfortunately, in the concentration ranges used in the tests (see Experimental Section), not one of the ligands and complexes inhibits cell proliferation.

Higher concentrations of these compounds were used, but the cytotoxic effect of the solvent began to become relevant (for **1** 50 $\mu$g/mL, DMSO 1%; for **2** 30 $\mu$g/mL, DMSO 1.3%; for **3** and **4** 25 $\mu$g/mL, DMSO 1.25%) and masked the biological activity of our compounds. Consequently to their lacking in inhibitory effect, apoptosis assay could not be carried out.

## Conclusion

Our results show that the substituents on the aminic nitrogen of the thiosemicarbazide chain do not affect the coordinative environment of the molecules more than a change of the counterion. These kind of molecules bind quite strongly to DNA, probably for the presence of the uracil moiety. Analysis of the results suggests that the compounds are able to interact with DNA by electrostatic or groove binding but not by intercalation. This kind of binding is not able to induce significant damage to DNA. The in vitro tests show that neither ligands or metal complexes are able to interfere with cell proliferation of leukemic cells. This behavior confirms that the molecules have no effect on nuclear DNA and shows that the methyl groups have no positive effects on the uptake of the drugs by cells. It also shows that the presence of the aminic $NH_2$ free of substituents[20,21] is very important for the biological properties of this kind of heterocyclic thiosemicarbazone.

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**Supporting Information Available:** Crystallographic information in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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