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Synthesis, characterization, toxicity, cytogenetic and in vivo antitumor studies of 1,1-dithiolate Cu(II) complexes with di-, tri-, tetra- amines and 1,3-thiazoles. Structure–activity correlation

C. A. Bolos [a,*], A. T. Chaviara [a], D. Mourelatos [b], Z. Iakovidou [b], E. Mioglou [b], E. Chrysogelou [c], A. Papageorgiou [c]

[a] Laboratory of Inorganic Chemistry, Chemistry Department, Aristotle University of Thessaloniki, University Campus, 541 24 Thessaloniki, Greece
[b] Laboratory of Biology and Genetics, Medical School, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece
[c] Department of Experimental Chemotherapy, Symeonidion Research Center, Theagenion Cancer Hospital, Al. Simeonides 2 street, Thessaloniki 540 07, Greece

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ABSTRACT

A series of new mixed-ligand neutral copper(II) complexes of the general type [Cu(amine)(i-MNT)] and [Cu(tz)(i-MNT)] was prepared and characterized by elemental, spectroscopic methods, μ_{eff}, Λ_{μ} measurements and molecular modeling studies. The acute toxicity, the *cytogenetic* and the in vivo antitumor activity of the new complexes, is related to their chemical and physicochemical properties. Among the Cu(II) compounds tested the complex with 2-amino-5-methyl thiazole increases significantly the life span of leukemia P388 bearing mice in vivo.

1. Introduction

The design and synthesis of biologically active molecules is a strenuous task and the factors affecting biological activity are diverse. Small molecules[1] such as NO, NO_2 and CN taking part in the coordination sphere of the complexes is such a factor[2] since the small size of the molecules allows them to penetrate the cell membrane and diffuse rapidly in the cell cytoplasm enabling them to act specifically.[3] Lipophilicity[3,4] is also a factor that should be taken into consideration. However, the lipophilic character of a compound is not always the desired criterion in the design of bioactive compounds, since the membrane is highly organized with many functional groups that enable them to play its unique role. This means that a combination of the lipophilic and hydrophilic[5,6] moieties in the same molecule[7] may produce biologically potent molecules. Such molecules, considered as promising candidate, may be derived from neutral Cu coordination compounds with 1, 1-dithiolate ligands, the lipophilicity of which is controlled by various di-, tri-, or tetra- amines and S, N-thiazole derivatives. The bidentate 1,1-dithiolate ligands and their complexes with various metal ions have been extensively studied in the past as organic conductors, molecular magnets, fungicides, pesticides, vulcanization accelerators, flotation agents[8] etc. Special attention was paid to 1,1-dithiolate-dicyanato complexes[9,10], since they are neutral with low coordination number whose the geometry of which allows them to penetrate into cells. The main constituents groups of 1,1-dithiolates, namely sulfur and the cyanide, are very well known for their biological activity.[11] The S-derivatives can mimic the function of metalloenzymes, stabilize the protein structure and act as catalysts, while the CN derivatives can kill tumor cells selectively at prodrug doses that are completely non toxic with respect to non-targeted cells.[12] The planar geometry of i-MNT complexes enables them to increase their coordination numbers by binding to bioactive molecules such as thiazole derivatives[13]. The characteristics of 1,1-dithiolates prompted us to synthesize a new series of mixed-ligand Cu(II) dithiolato complexes with the aim of studying the influence of their controlled lipophilic properties by nitrogenous bases on their anti-proliferative activity in vitro and in vivo. We have also attempted to discuss in detail the biological properties in order to identify the lead compounds.

Abbreviations: Dien, diethylenetriamine; dpta, dipropylenetriamine; trien, triethylenetetramine; bipy, bipyridine; dpk, dipyridylketone; dpk$(OH)_2$, dipyridylketone-hydrate; 2a-tz, 2-amino-thiazole; 2a-5mtz, 2-amino-5-methyl-thiazole; 2a-2tzn, 2-amino-2-thiazoline; SCE, sister chromatid exchange; PRI, proliferation rate index.

* Corresponding author. Tel.: +30 2310 997705; fax: +30 2310 997738.
E-mail address: bolos@chem.auth.gr (C.A. Bolos).

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2. Results and discussion

The reaction of $[Cu(amine)Cl_2]$ and $[Cu(tz)_2Cl_2]$ with Na_2(i-MNT)·$3H_2O$ or Na_2(CNPD)·$2H_2O$ 1,1-dithiolate ligands, produced eleven brown mixed-ligand complexes corresponding to the general formulas [Cu(i-MNT)(amine)], [Cu(CNPD)(dien)] and [Cu(i-MNT)(tz)(H_2O)] [where i-MNT = dianion of 1,1-dicyano-2,2-ethylenedithiolate, CNPD = 1-cyano-1-*p*NO_2-phenyl-2,2-ethylenedithiolate, amine = NH_3, ethylenediamine(en), diethylenetriamine (dien), dipropylene-triamine(dpta), bipy = 2,2′-bipyridine, dpk = di-2-dipyridyl ketone and tz = 2-amino-thiazole(2a-tz), 2-amino-5methyl-thiazole (2a-5mtz) and 2-amino-2-thiazoline(2a-2tzn)]. The new chelates are neutral, insoluble in water, methanol and ethanol, as well as in most organic solvents and are very soluble in dimethylsulfoxide (DMSO) and dimethylformamide (DMF). The analytical data C, H, N and Cu confirm the composition of the complexes and the stoichiometry proposed. Evidence of the mode of bonding of the ligands was gathered from the IR spectra, while their geometry was assumed from the electronic spectra, molar conductivity measurements and theoretical studies.

2.1. Spectroscopic data

2.1.1. IR spectral studies

In the infrared spectra of the compounds the plethora of bands may be classified as those emerging from the functional group of i-MNT and as those from the amine and the thiazole derivatives. The most relevant bands of the complexes were given in the experimental part. The absorptions of the 1,1-dithiols due to C≡N, the C=CS_2, and the C–SS functional groups are easily distinguishable. The very strong bands at the 2181–2210 and 1356–1436 cm^{-1} regions are attributed to the stretching vibrations of the C≡N bond of the cyanide group and to the >C=C< of the >C=CS_2 group, respectively.[14] Upon i-MNT coordination to Cu these bands are shifted to higher wave numbers: the former by 6–35 cm^{-1} and the later by 14–94 cm^{-1} as compared to the bands of the free ligand. These shifts reveal an increase of the C≡N and >C=CS_2 bond order, since the planar extensive π-conjugated system of the i-MNT ligand facilitates a drift of electron density towards the sulfur atoms, which in turn results in a shortening of the C≡N and >C=C< bonds lengths.[15] The medium intensity band at 918–989 and 794–896 cm^{-1} bands in the IR spectra of the ligands are assigned to the $\nu_{as}(CS_2)$ and $\nu_{sym}(CS_2)$ respectively. These two bands are shifted to higher (2–35 cm^{-1}) or lower (4–36 cm^{-1}) wave numbers, as compared to the infrared spectra of the complexes. These shifts could be attributed to the different extent of electron donation ability of the amine or thiazole ligands. The ligands' coordination, through the sulfur and nitrogen is supported by the appearance of the new medium or weak intensity peaks at 418–493 and 341–400 cm^{-1}, which should be assigned to the ν(Cu–N) and ν(Cu–S), respectively.[15]

In the infrared spectra of the thiazole compounds **9–11**, the 1599–1616 cm^{-1} bands are assigned to the ν(C=N) of the thiazole or thiazoline ring. These bands appear at lower frequencies (23–29 cm^{-1}) as compared to those of the free ligands confirming the ligands coordination through the heterocyclic nitrogen of the thiazoles.[16] In the spectra of the complexes **1–5** and **9–11** the three bands at 3310–3413, 3235–3266 and 1583–1616 cm^{-1} assigned to ν_s(N–H), ν_{as}(N–H) and δ(N–H), respectively, are shifted to wavenumbers lower than those encountered in the free 1,2-diamines or triamines, confirming the coordination of the amine groups with copper.[17] As far as the complex **5** is concerned, there is evidence that the ligand (trien) is tridentate – and not the tetradentate. This coordination was supported by two strong bands appearing at 3310 and 3235 cm^{-1} and they correspond to the ν_s(N–H) and ν_{as}(N–H) uncoordinated of primary amino groups. This finding was supported by theoretical calculations (vide infra), which indicate that the length of Cu–N(3) bond is much longer (3.68 Å), than that of the three other Cu–N bond lengths(1.93–2.00 Å), thus ruling out a possible fourth Cu–N contact and CuS_2N_4 with 4-coordinated ligand (Fig. 1A). Thus, the experimental and theoretical data suggest a structure with one free primary amino group and 5-coordinated CuS_2N_3 moiety (Fig. 1B).

The bipy and dpk$(OH)_2$ diamines are known to be coordinated in a bidentate mode in complexes **6** and **7** through the pyridine nitrogens[18], the dpk adopting the diolate (dpk$(OH)_2$) form.[19] The bidentate coordination of the later through the two nitrogen atoms is confirmed by the lack of a C=O band and the presence of ν(O–H) at ca. 3500 cm^{-1}. The 3413–3450 cm^{-1} bands in the IR spectra of complexes **9–11,** are tentatively assigned to ν(O–H) and reveal the presence of water molecule coordinated to Cu(II).

2.1.2. UV–vis spectra

In the UV spectra of all 11 compounds there are only 2 bands (**A**, **B**) both in Nujol suspensions and in DMSO solutions. The bands span the following intervals

	Nujol	DMSO	Assignment[20]
Band **A**:	33.2–33.7 kK, Δ = 0.5 kK	29.5–30.3 kK, Δ = 0.8 kK	π→π*
Band **B**:	29.4–30.8 kK, Δ = 1.4 kK	27.4–28.6 kK, Δ = 1.2 kK	π→π*

The spread of the intervals Δ increases with decreasing electron transition energy in the order **B** > **A**. Upon dissolution in DMSO bands **A** and **B** are shifted to lower energies (band **A** by 3–4 kK and band **B** by 1–3.5 kK). The different sign of the shift provides strong evidence that either DMSO is coordinated to Cu(II) or it strongly perturbs the π-electron system of the i-MNT ligand. The same pattern emerges from the bands in the visible spectrum.

	Nujol	DMSO	Assignment[21]
Band **C**:	13.0–15.4 kK, Δ = 2.4 kK	14.0–15.8 kK, Δ = 1.8 kK	d→d
Band **D**:	_____	9.95–10.70 kK, Δ = 0.75 kK	d→d
Band **E**:	21.8–24.3 kK, Δ = 2.5 kK	22.2–25.7 kK, Δ = 3.5 kK	n→π* or LMCT

The spread of the intervals Δ is almost the same (Nujol). It is the lowest for band **E** as expected for an n→π* transition. With the exception of compound **6**, there is just one band in the 13.0–15.4 kK interval of the Nujol spectra. Although the coordination number of Cu(II) and the chromophore composition of its compounds studied here varies throughout the series (**4** to **6**), the appearance of a single band is an indication of the high symmetry of the chromophore (pseudo Td or Oh) group giving rise to one d→d band as expected for an d^9 (Cu(II). Upon dissolution in DMSO this single band (13.8–15.4 kK) is split into two components a band at slightly higher energy (14.0–15.8 kK) and a band at lower energies (9.95–10.7 kK). The very short Δ for the d–d bands spreads should be noted, indicating similar chromophore with different N-coordinated ligands. There may be additional splitting of the d levels and new d→d bands may be overlapped. This is particularly expected for compounds **9**, **10** and **11** for which intermolecular interactions are possible. The i-MNT ligand is known to be non-innocent; it shifts its electron density to Cu(II) and this is evidenced

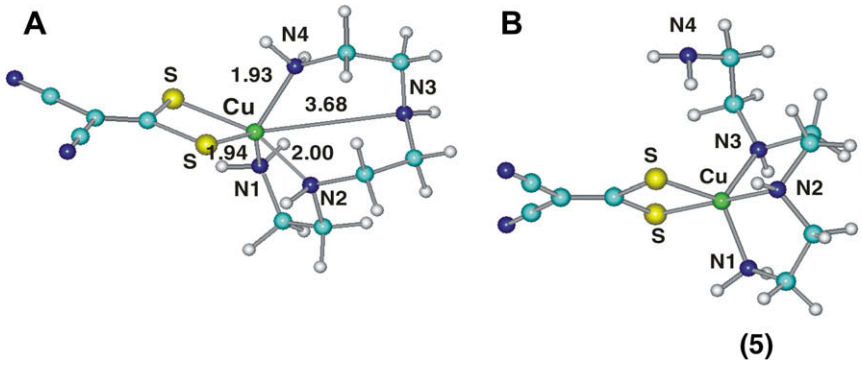


Figure 1. The tetradentate (**A**) and the tridentate (**B**) coordination mode of trien in [Cu(trien)(i-MNT)] complex obtained by MM calculations.

by the low magnetic moment values which are indicative of a spin-spin interaction between two Cu centers of type[21] Cu–S–Cu (see Scheme 4). According to the bands position in the visible spectra of the 5-coordinate **3**, **4**, **5** and **8** in DMSO, the band **C** is typical of the distorted square pyramidal (SPy) Cu(II) complexes, while the very weak band **D** ($\log\varepsilon = 1–1.5$), is typical of trigonal bipyramidal (TBP) in Cu(II) complexes.[22] The co-existence of the two geometries is unlikely so that a structure of a distorted SPy (towards TBP) CuS_2N_3 may be deemed as possible. The electronic spectra of the 4-coordinate **1**, **2**, **6**, **7**, **9**, **10** and **11** compounds indicate pseudo-tetrahedral for **1**, **2**, **9**, **10**, **11** and highly distorted square planar geometry[23] for **6** and **7**, CuN_2S_2 chromophore. Interaction of a sulfur atom from the neighboring dithiolate molecules with Cu, cannot be excluded, increasing the Cu coordination number to five for compounds **9**, **10** and **11.** This assignment is in accordance with the low μ_{eff} values which indicate of a spin-spin interaction between two Cu centers of type[22] Cu–S–Cu.

The new band **E** at the regions 21.8–24.3 kK (Nujol) and 22.2–25.7 kK (DMSO) can be assigned to S→Cu charge transfer (LMCT) or to intra ligand n→π* and π→π* transitions.[23] The position of these bands is determined by the different π-dative ability of the nitrogenous ligands coordinated with Cu(II) atom.

2.1.3. Molar conductivity measurements

The molar conductivity values of the complexes in 10^{-3} M DMSO solutions vary from 6 to 11 μS cm^{-1}, thus revealing[24] their non-electrolytic character.

2.1.4. Magnetic measurements

The magnetic measurements on the dithiolate complexes categorize them into two different classes: the first one encompasses the complexes **1–8**, with values in the 1.74–1.89 BM region, while the second one consists of **9**, **10** and **11** with values of 0.88–0.90 BM. The first class of complexes are obviously monomer Cu(II) complexes, with one spin-unpaired electron. The lower magnetic moment (~0.9 BM) of the second class of complexes may be attributed to the spin pairing between Cu(II) atoms of two neighboring molecules via Cu–S–Cu in the respective dimmer or polymer complexes or the presence of antiferromagnetism.[22] These complexes appear as five- or six-coordinated in a tentative distorted square pyramidal or pseudo-octahedral geometry, respectively (Scheme 4).

2.2. Molecular modelling

Since no single crystals suitable for X-ray determination could be isolated, structural information for these complexes were obtained through molecular modelling techniques. The molecular mechanics (MM) calculations have been carried out using the HyperChem program (release 7.1) at the PM3 level that allows for structural building, geometry optimization and for physicochemical properties.[25,26]

2.2.1. Description of structures

The molecular structures of Cu(II) compounds which were proposed by spectroscopic, conductivity and magnetic studies were confirmed by the calculation of structural data by theoretical studies. These are given in Figures 1–5, Figure S1, while selected bond lengths and angles are summarized in Table S1. According to these data, in the distorted tetrahedral structure for complexes **1**, **2** (Fig. 2) the valence angles S–Cu–N and N–Cu–N were ~120° and 98.65°, respectively. These significant deviations from the tetrahedral (109.28°) could be attributed to the considerable strain in the four-membered chelate rings of dithiolate and amine ligands.

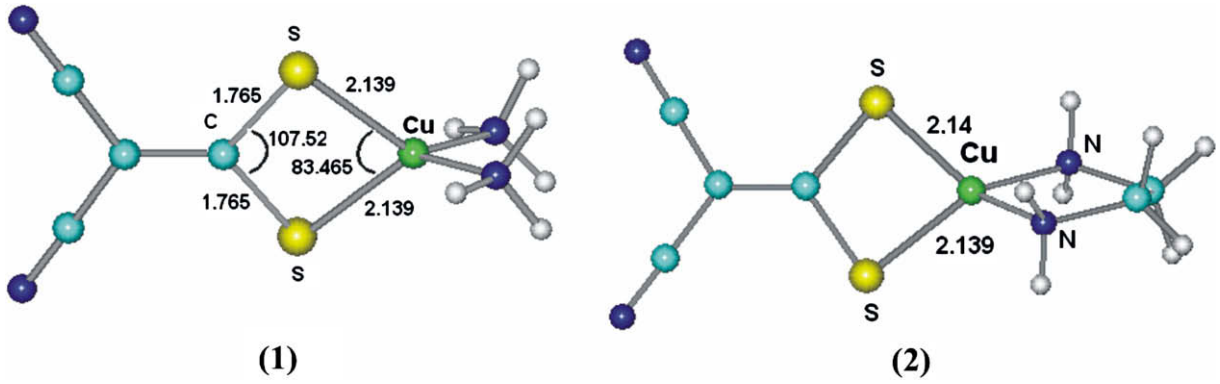
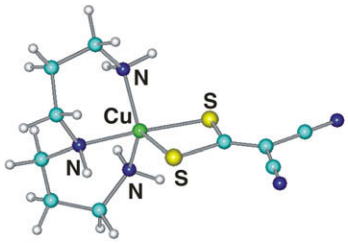


Figure 2. The molecular structure of complexes [$Cu(NH_3)_2$(i-MNT)] (**1**) and [Cu(en)(i-MNT)] (**2**) as obtained by MM.



(4)

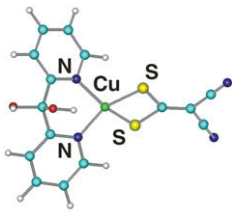
Figure 3. Geometry adopted by Cu(II) dithiolate complexes [Cu(dpta)(i-MNT)] **(4)** as discussed from MM calculations.

As far as the geometry of the complexes **3** (Fig. S1), **4** (Fig. 3), **5** (Fig. 1) and **8** (Fig. S1) is concerned, this can be clarified with the help of the method proposed by Addison et al.[27] This method uses the parameter τ, defined as $\tau = [(\theta - \sigma)/30] \times 100$ (θ and σ are the basal angles, with $\theta > \sigma$), as an index of the geometry of a five-coordinated complex. Parameter τ actually represents the percent distortion of a square pyramid (SP) towards a trigonal bi-pyramid (TBP), namely $\tau = 0\%$ for an ideal SP, whereas $\tau = 100\%$ for an ideal TBP. Therefore, complexes **3** and **8** seem to be of distorted square pyramidal geometry, since their τ values are 29% and 28%, respectively, while the τ values of 85% and 86% for complexes **4** and **5**, respectively, strongly imply a distorted trigonal bipyramidal geometry for the latter.

The square planar geometry of complexes **6** (Fig. S1) and **7** (Fig. 4) has been proposed by the characteristic bond angles of N–Cu–S and S–Cu–N (bisectors), and the rest of them are close to the ideal ones. Specifically, the N–Cu–S and S–Cu–N angles of 175.8° and 176.1° for **6** are very close to 180°, while N–Cu–N and the rest of N–Cu–S lie in 82–94° range, very close to 90°. Regarding the geometry of **7**, the deviations of N–Cu–S, S–Cu–N and N–Cu–N angles from the ideal, lead to the distorted square planar geometry due to the flexibility and conformation of dpk$(OH)_2$ ligand.

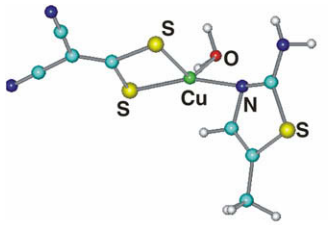
Tetrahedral or square planar geometry can be proposed for the compounds **9** (Fig. S1), **10** and **11** (Fig. 5). Each Cu atom is 4-coordinate two S atoms from the i-MNT ligand, one O atom from the coordinated water and one nitrogen atom from 1,3-*S*,*N*-thiazoles.

The observed distortions, with respect to the idealized tetrahedron or square-planar geometry, are visible in the coordination angles. The small S–Cu–S chelating angle (81.1–82.0°) for the three 1,3-*S*,*N*-thiazoles complexes is determined by the geometry of the rigid i-MNT ligand. The angles O10–Cu1–N13 and S2–Cu1–N13 of 91.7–102.3° and 102.5–103.62°, respectively, could be attributed to distorted tetrahedral or square planar geometry as mononuclear, and to square pyramidal or pseudo octahedral complexes in binuclear or polymeric configurations. The last proposal may be due to the tendency of both Cu and S to form weak inter-molecular interactions (vide supra Scheme 4).

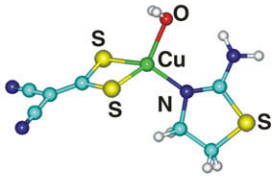


(7)

Figure 4. Geometry adopted by Cu(II) dithiolate [Cu(dpk$(OH)_2$)(i-MNT)] **(7)** complexes.



(10)



(11)

Figure 5. Optimized structures adopted by Cu(II) dithiolate [Cu(2a-5mtz)(i-MNT)(H_2O)] **(10)** and [Cu(2a-2tzn)(i-MNT)(H_2O)] **(11)** complexes, as deduced by MM calculations.

2.3. Biological studies

The biological studies performed for the dithiolate complexes include the estimation of acute toxicity, in vivo antitumor and in vitro cytogenetic activity. The *C*log*P* and the dipole moment (μ) of ligands and their compounds were calculated theoretically at the PM3 level of theory. From this point of view the Na_2(i-MNT) and Na_2(CNPD) ligands, the amines and the thiazole derivatives do not seem to have any biological role.

2.3.1. Acute toxicity

The results of the acute toxicity studies for the dithiolate complexes are given in Table 1. Although both parameters (LD_{10} and LD_{50}) were estimated for chemotherapy testing, the highest dose used for a single treatment was equal to the LD_{10} value.

The toxicity of the compounds tested increased according to the following order:

6 < **8** < **4** < **1** < **3** < **7** < **2** < **9** < **11** < **10** = **5**.

2.3.2. Antileukemic activity in vivo

The antitumor study of the complexes was performed after the evaluation of their toxicity, in order to allow the selection of the best compound with high antitumor activity and low toxicity. The antitumor activity results of the complexes under study, expressed as mean survival time (MST) of the animals used, are summarized in Table 2. It can be deduced from all the compounds tested Leukemia P388 responded better when compound **10** was administrated, in comparison with the others used in this study, yielding a treated/control (*T*/*C*) value of 164%, increasing the life span of treated animals by 64%. The activity of compounds **8** and **9** was almost the same, yielding *T*/*C* values of 128% and 130%, respectively. The activity of compounds **2** and **5** was close to the marginal values, whereas the rest of the compounds were inactive.

Table 1
Acute toxicity of the dithiolate complexes expressed as lethal dose for 50% (LD_{50}) and 10% (LD_{10}) of the animals used

No.	Complex	mg kg^{-1}	
		LD_{50}	LD_{10}
1	[Cu$(NH_3)_2$(i-MNT)]	77	48
2	[Cu(en)(i-MNT)]	45	17
3	[Cu(dien)(i-MNT)]	62	35
4	[Cu(dpta)(i-MNT)]	115	93
5	[Cu(trien)(i-MNT)]	26	10
6	[Cu(bipy)(i-MNT)]	175	153
7	[Cu(dpk$(OH)_2$)(i-MNT)]	64	32
8	[Cu(dien)(CNPD)]	165	145
9	[Cu(2a-tz)(i-MNT)(H_2O)]	30	12
10	[Cu(2a-5mtz)(i-MNT)(H_2O)]	40	10
11	[Cu(2a-2tzn)(i-MNT)(H_2O)]	>100	11
12	Na_2(i-MNT)·3H_2O	—[a]	—
13	Na_2(CNPD)·2H_2O	—[a]	—

[a] Not toxic.

Table 2
Calculated Lipophilicity (*Clog P*) of ligands and their complexes, dipole moment μ(D) and antitumor activity of the Cu(II) dithiolate complexes on P388 lymphocytic leukemia bearing animals

No.	Complex	*Clog P* of ligands	*Clog P* of complexes	μ (D)	Antitumor activity		
					MST (days)	Dose (mg/kg)	*T*/*C* (%)
	Control	–	–	–	11.5	Saline	100
1	[Cu(NH_3)$_2$(i-MNT)]	−1.20	−0.50	7.92	9.0	48	98
2	[Cu(en)(i-MNT)]	−2.02	0.53	18.91	10.8	17	118
3	[Cu(dien)(i-MNT)]	−2.28	1.10	18.91	8.8	35	96
4	[Cu(dpta)(i-MNT)]	−1.44	2.22	2.76	11.3	93	98
5	[Cu(trien)(i-MNT)]	−2.37	0.73	6.64	10.3	10	112
6	[Cu(bipy)(i-MNT)]	1.56	4.21	11.81	10.7	150	93
7	[Cu(dpk(OH)$_2$)(i-MNT)]	1.51	3.42	14.81	11.3	36	98
8	[Cu(dien)(CNPD)]	–	1.99	20.41	11.8	130	128
9	[Cu(2a-tz)(i-MNT)(H_2O)]	−0.24	0.27	12.51	15.0	12	130
10	[Cu(2a-5mtz)(i-MNT)(H_2O)]	0.73	0.79	11.90	18.8	10	164
11	[Cu(2a-2tzn)(i-MNT)(H_2O)]	0.53	−1.68	10.68	15	15	128
12	Na_2(i-MNT)·3H_2O	−0.594	–	0.000	–	–	–

MST = mean survival time of mice in days.
T/*C*% = mean survival of the drug-treated animals (*T*) versus corn-oil tested animals (*C*).

2.3.3. Cytogenetic activity

The *cytogenetic* activity was evaluated by estimating the SCE and PRI indices in normal human lymphocyte cultures. The results are given in Figures 6 and 7 and summarize in Table 3.

2.3.4. Evaluation of cytogenetic activity

The classification of the cytogenetic activity of the complexes according to the two parameters tested in each concentration has been presented as follows:

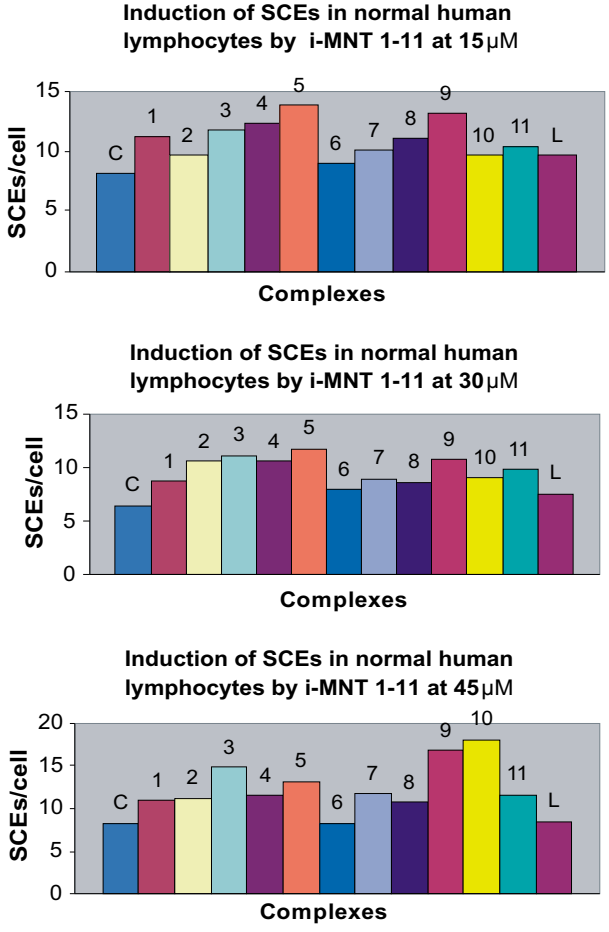


Figure 6. SCEs/cell for the i-MNT Cu(II) complexes (**1**–**11**), the ligand (**L**) and the control (**C**) at concentrations of 15 μM (A), 30 μM (B) and 45 μM. The values for the controls are 8.16, 6.46 and 8.27 SCEs/cell, respectively.

Concentration	Order of magnitude of the SCE frequency
(15 μM)	5 > 9 > 4 > 3 > 1 > 8 > 11 > 7 > 12 = 2 > 10 > 6
(30 μM)	5 > 3 > 9 > 4 > 2 > 11 > 10 > 7 > 1 > 8 > 6 > 12
(45 μM)	10 > 9 > 3 > 5 > 7 > 11 > 4 > 2 > 1 > 8 > 12 > 6

Concentration	Order of the PRI depression
(15 μM)	2 > 1 > 5 > 4 > 9 > 10 > 8 > 7 > 3 > 6 > 11 > 12
(30 μM)	1 > 10 = 7 > 11 > 8 > 4 > 2 > 9 > 5 > 3 > 6 > 12
(45 μM)	10 > 8 > 4 > 2 = 11 > 3 > 1 > 7 > 9 > 5 > 6 > 12

A statistically significant correlation ($P < 0.05$) was observed between the magnitude of the SCE response and the PRI depression ($r = -0.343$ and $t = -2.22$).

SCE frequency has been usually used as highly sensitive indicator of DNA damage and/or subsequent repair.[28,29] Non repaired damage expressed as SCEs in normal cells, caused by certain chemicals, may indicate inability to repair the damage induced by the same chemicals in the cancer cells, since both cell types have similar DNA repair mechanisms.[30] There are findings indicating that the effectiveness of SCE induction by potential antitumor agents in cancer cells in vitro[31] and in vivo[32] is positively correlated with the in vivo tumor response to these agents. This suggests that the SCE assay could be used to predict both the sensitivity of human tumor cells to chemotherapeutics and the heterogeneity of drug sensitivity of individual tumors.[30] Other studies investigating a relationship between SCEs induction and other expression of genotoxicity have also shown a positive relationship between SCEs, reduced cell survival and alteration in cell cycle kinetics.[33] In the present study a good correlation ($r = -0.343$, $t = -2.22$ and $P < 0.05$) between SCE enhancement and PRI suppression was observed. The results from the in vitro experiments indeed showed relevance with these from the in vivo studies, the greatest cytogenetic and antineoplastic activity being displayed by the complex **10** (Tables 2 and 3).

2.3.5. Structure–activity relationship

The initial aim of this project to prepare compounds with different values of lipophilicity, which is an important physicochemical parameter in antitumor studies, was met by the synthesis of neutral Cu(II) dithiolate complexes with various amines. The results show that the anticancer activity of Cu(II) complexes is higher than that of the corresponding ligands. The correlation between two or

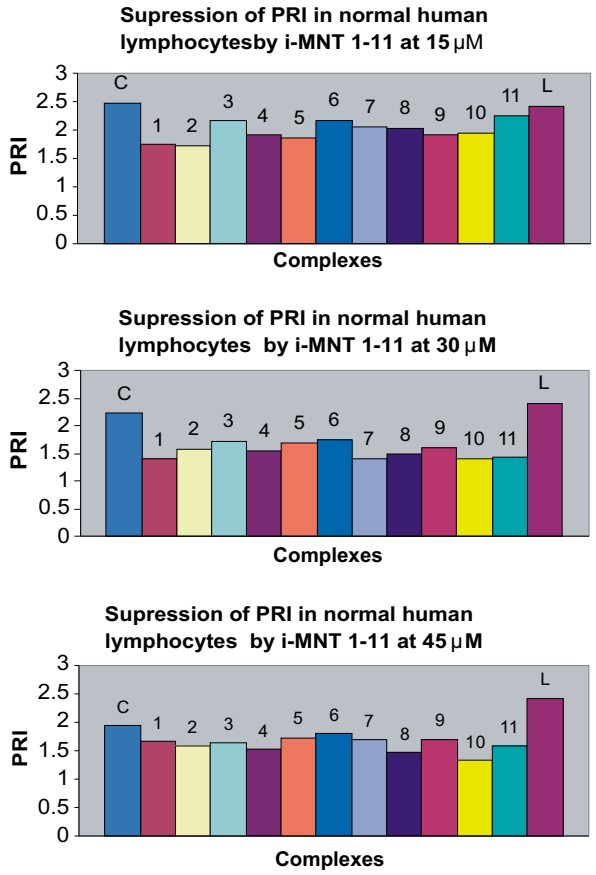


Figure 7. PRI indices for the i-MNT Cu(II) complexes (**1**–**11**), the ligand (**L**) and the control (**C**) at concentrations of 15 μM (A), 30 μM (B) and 45 μM (C). The values for the controls are 2.48, 2.22 and 1.94, respectively.

three complexes was based on their geometry (Table S1) and the nature of linear or heterocyclic amines, for example, configuration, length and the number of lipophilic methyl chain, (Scheme 2). Several findings emerging from Table 2 are discussed below.

- The comparison of the antitumor activity of the compounds **1** and **2** with distorted tetrahedral geometries reveals that the second is more potent, possibly due to higher lipophilicity and dipole moment introduced for both by the ethylenediamine ligand.
- The comparison of the antitumor activity of the compounds **2** and **3** with coordination number 4 and 5, respectively, shows that compound **2** is more active despite the higher lipophilicity of **3**. The better activity of **2** could be due to its lower steric hindrance.
- *Length of the triamines and the number of alkyl chains present:* Although the lipophilicity of **4** is higher than that of **5**, both having the same coordination number, the *T*/*C*% value of the later is higher. In this particular correlation the hydrophilicity of **5**, attributed to the existence of one primary free amino group and the three alkyl chains of the trien (Fig. 1), seems to play predominant role in antitumor activity. The same trend was also observed in SCE and PRI activities.
- *Dipyridine ligands:* The complexes **6** and **7**, having the same structure (distorted square planar) and although they exhibit the highest lipophilicity among all the new complexes, their in vivo anticancer activity and the SCE values are unexpectedly very low. These findings could be due to the bulky bipy and dpk hydrate ligands.
- *1-Cyano-1-p*NO_2-phenyl- 2,2- ethylenedithiolate ligand (CNPD): The replacement of a cyanide group from i-MNT ligand in complex **3** by a *p*-nitrophenyl group in **8**, doubles the lipophilicity and enhances the dipole moment. These two factors lead to the depression of PRI values in all concentrations and enhance the antitumor activity of **8** by 33.3%. The higher antitumor activity can be attributed to the polar and the specific bioactivity of *p*-NO_2 phenyl group. Taking also into account the low toxicity of this compound we may classified it as a promising lead compound.
- *Dithiolate complexes **9**, **10** and **11** with thiazole ligands:* Complexes **9**, **10** and **11** with 1,3-S, *N*-thiazole ligands, having the same distorted Th or Spl geometry (and active groups with S and N atoms), were found to be the most effective in both in vivo antitumor and in vitro SCE and PRI studies. Their activity can be correlated with the high polarity and possibly in other factors, such as electrostatic interactions and not with their lipophilicity. These complexes can be used as *lead compounds* since they meet the necessary criteria established by NCI.[34]

3. Conclusions

The synthesized mononuclear or dinuclear, paramagnetic and neutral Cu(II) complexes with dithiolate ligands (i-MNT or CNPD), amines and thiazole derivatives exhibit various structures, 4- and

Table 3
SCEs and PRI indices for the i-MNT Cu complexes(**1–11**) in normal human cultured lymphocytes

No.	Compound	SCEs/cell ± SE[c]			PRI[d]		
		15μM	30μM	45μM	15μM	30μM	45μM
	Control	8.16 ± 0.60	6.46 ± 0.38	8.27 ± 0.40	2.48	2.22	1.94
1	[Cu(NH3)2(i-MNT)]	11.19 ± 0.79	8.70 ± 0.42	10.96 ± 0.98	1.74	1.40[b]	1.66
2	[Cu(en)(i-MNT)]	9.69 ± 0.59	10.57 ± 0.73	11.15 ± 0.58	1.73	1.59[b]	1.58[b]
3	[Cu(dien)(i-MNT)]	11.70 ± 0.81	11.09 ± 0.66	14.85 ± 1.29[a]	2.16	1.73	1.64
4	[Cu(dpta)(i-MNT)]	12.30 ± 0.82	10.61 ± 0.62	11.48 ± 0.98	1.91	1.56[b]	1.53[b]
5	[Cu(trien)(i-MNT)]	13.8 ± 0.91[a]	11.73 ± 0.99	13.18 ± 0.96[a]	1.86	1.69	1.73
6	[Cu(bipy)(i-MNT)]	8.96 ± 0.60	7.95 ± 0.45	8.20 ± 0.58	2.18	1.75	1.80
7	[Cu(dpk)(i-MNT)]	10.07 ± 0.57	8.98 ± 0.72	11.65 ± 0.68	2.06	1.41[b]	1.69
8	[Cu(dien)(CNPD)]	11.05 ± 0.85	8.61 ± 0.63	10.73 ± 0.67	2.04	1.48[b]	1.47[b]
9	[Cu(2a-tz)(i-MNT)(H_2O)]	13.15 ± 0.80[a]	10.73 ± 0.67	16.80 ± 1.02[a]	1.93	1.60[b]	1.70
10	[Cu(2a-5mtz)(i-MNT)(H_2O)]	9.62 ± 0.61	9.03 ± 0.35	17.97 ± 1.12[a]	1.95	1.41[b]	1.34[b]
11	[Cu(2a-2tzn)(i-MNT)(H_2O)]	10.36 ± 0.64	9.78 ± 0.96	11.57 ± 0.98	2.26	1.42[b]	1.58[b]
12	Na2(i-MNT)·3H_2O (ligand)	9.74 ± 0.58	7.50 ± 0.40	8.50 ± 0.56	2.42	241	2.42

[a] Statistically significant increase ($P < 0.01$, by *t*-test) over the control.
[b] Statistically significant decrease ($P < 0,01$ by χ^2 test) compared to the corresponding controls.
[c] Sister chromatid exchanges per cell ± standard error of the mean.
[d] PRI = $(M_1 + 2M_2 + 3M_3 + \cdots)/100$ where M_1, M_2, M_3+ ·· are the percentage values of cells in the first, second, third and higher division respectively.

5-coordinate Cu(II) with square planar, tetrahedral or square pyramidal, trigonal bipyramid. Their antitumor in vivo and in vitro activity can be related to factors such as *C*log*P* and the geometry of the complexes. These factors are influenced in a different way by the length and the number of alkyl chains of the amines, the coordinated number and the free amino groups presence, the planarity of the ligand and their electronic charge distribution there in. The most noteworthy biological results were obtained for the complexes **9** (*T*/*C*% = 130) and especially **10** (*T*/*C*% = 164). These complexes can be used as lead compounds since they meet the necessary criteria established by National Cancer Institute. Although the initial aim of this project, which was to design and synthesis of Cu(II) mixed-ligands complexes with a variety of controlled lipophilicity values, was partly met, the interesting biological results obtained for the compounds have prompted us to continue work on this project. Further synthetic and synergistic contribution studies of new compounds are in progress in order to reveal their mechanism of action.

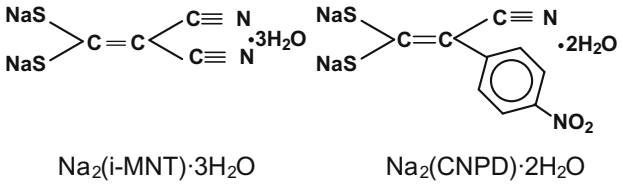
4. Experimental

4.1. Materials and instrumentation

All chemicals were purchased from Aldrich, EDH Chemicals and Merck were used as received. C, H and N were determined by microanalysis using a Perkin–Elmer 240B elemental analysis instrument, whereas copper analyses were performed with a Perkin–Elmer model 403 Atomic Absorption Spectrometer, equipped with the respective hollow cathode lamp. Molar conductivities were measured on a WTW conductivity bridge employing a calibrated dip type cell and 10^{-3} M solutions in MeOH. Magnetic susceptibility of powdered samples were measured at room temperature (25 °C) utilizing a Johnson-Matthey balance, calibrated with $Hg[Co(NCS)_4]$. Diamagnetic corrections were estimated based on Pascal's constants. The effective magnetic moment was calculated using the expression: $\mu_{eff} = 2.84(\chi_M T)^{1/2}$. Infrared spectra were recorded in the 4000–225 cm^{-1} region, with samples prepared as KBr or polyethylene discs, with a Perkin–Elmer FT-IR spectrophotometer, equipped with a CsI beam splitter. Far-infrared spectra (400–100 cm^{-1}) were recorded with polyethylene discs with a Bruker 113 V Spectrum Spectrometer. Electronic spectra of 10^{-3} M solutions in DMSO and in Nujol suspension were obtained with a Hitachi U-2001 spectrophotometer.

4.1.1. Synthesis of the dithiolate ligands and the starting compounds

The disodium salt of 1,1-dicyano-2,2-ethylenedithiolate trihydrate, Na_2(i-MNT)·$3H_2O$ and disodium salt of 1-cyano-1-pNO_2phenyl-2,2-ethylenodithiolate dihydrate, Na_2(CNPD)·$2H_2O$ (Scheme 1) were prepared by established procedures.[35–37]



Scheme 1. Structures of the ligands Na_2(i-MNT)·$3H_2O$ and Na_2(CNPD)·$2H_2O$.

The starting compounds used $[Cu(amine)_nCl_2]$ (n = 1,2 and $[Cu(tz)_2Cl_2]$ were prepared by the reaction of $CuCl_2·2H_2O$ with the amines (amine)[38] or thiazole (tz)[39] derivatives in various molar ratios. The formulae of the amines, thiazoles and their abbreviations are given in Scheme 2.

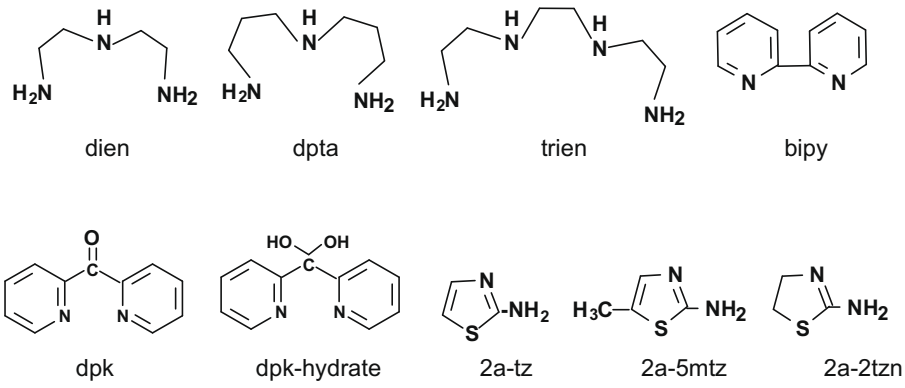
4.1.2. General procedure for the preparation of Cu(II) complexes 1–11

A solution of $[Cu(amine)_nCl_2]$ (n = 1,2,4) or $[Cu(tz)_2Cl_2]$ (10 mmol) in 20 mL MeOH was mixed slowly with a solution of Na_2(i-MNT)·$3H_2O$ or Na_2(CNPD)·$2H_2O$ (10 mmol) in 10 mL MeOH. After 1 h of stirring the resulting dark-brown solid was separated by filtration, washed several times with methanol and ether and dried in air. A representative reaction of the compound **3** is given in Scheme 3.

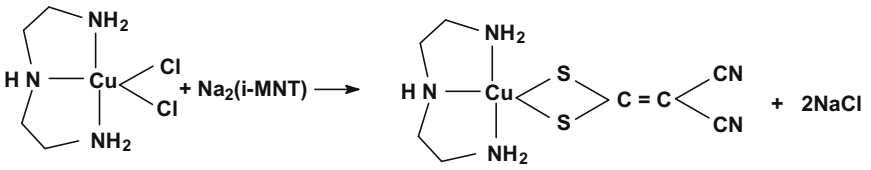
4.1.2.1. Compound $[Cu(NH_3)_2$(i-MNT)$]$ (1). Yield: 87%. Anal. Calcd for $C_4H_6N_4S_2Cu$: C, 20.20; H, 2.54; N, 23.56; Cu, 26.72. Found: C, 20.06; H, 2.47; N, 23.63; Cu, 26.59. IR (KBr, cm^{-1}): ν_{sec}(N–H) 3329s, ν_{as}(N–H) 3247s, ν(C≡N)2201vs, ν(C=CS_2) 1382vs, ν_{as}(C=CS_2) 949s, ν_s(CS_2) 890s, ν(Cu–Cu–N) 492 m, ν(Cu–S) 345w. UV–vis(kK, logε)(in DMSO): 15.77(2.21), 10.61(1,45), 29.85(3,08), 28.57(3,04), 24.94(2,74); (in Nujol): 14.68, 21.93, 33.33, 30.32. $\Lambda\mu$(μS cm^{-1}) (in DMSO): 8; μ_{eff}(BM): 1.74

4.1.2.2. Compound [Cu(en)(i-MNT)] (2). Yield: 80%. Anal. Calcd for $C_6H_8N_4S_2Cu$: C, 27.31; H, 3.05; N, 21.23; Cu, 24.08. Found: C, 27.37; H, 3.08; N, 21.45; Cu, 23.90. IR(KBr disk): ν_{sec}(N–H) 3326s, ν_{as}(N–H) 3259s, δ(N–H) 1583s, ν(C≡N) 2181vs, ν(C=CS_2) 1398vs, ν_{as}(C=CS_2) 956 m, ν_s(CS_2) 896 m, ν(Cu–N) 487w, ν(Cu–S) 343w. UV–vis(kK, logε)(in DMSO): 15.06 (2.31), 10.20(0,95), 30.12(3.31), 28.40sh, 22.57sh; (in Nujol): 13.81, 22.78, 33.22, 29.41. $\Lambda\mu$(μS cm^{-1}) (in DMSO): 9; μ_{eff}(BM): 1.79.

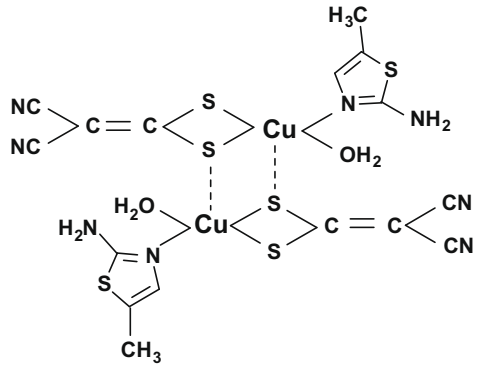
4.1.2.3. Compound [Cu(dien)(i-MNT)] (3). Yield: 56%. Anal. Calcd for $C_8H_{13}N_5S_2Cu$: C, 31.31; H, 4.27; N, 22.82; Cu, 20.70.



Scheme 2. Structural formulae of amine/imines bases used as ligands in this work: diethylenetriamine (dien), dipropylenetriamine (dpta), triethylenetetramine (trien), bipyridine (bipy), dipyridylketone (dpk), dipyridylketone- hydrate (dpk$(OH)_2$), 2-amino-thiazole (2a-tz), 2-amino-5- methyl thiazole (2a-5mtz), 2-amino-thiazoline (2a-2tzn).



Scheme 3. Representative reaction of $[Cu(dien)Cl_2]$ with i-MNT.



Scheme 4. Proposed layered polymer structure of complexes **9**, **10** and **11**.

Found: C, 31.20; H, 4.24; N, 22.80; Cu, 20.59. IR(KBr disk): ν_{sec}(N–H) 3341 m, ν_{as}(N–H) 3266s, δ(N–H) 1583w, ν(C≡N) 2198vs, ν(C=CS_2) 1392vs, ν_{as}(C=CS_2) 938s, ν_s(CS_2) 886s, ν(Cu–N) 489w, ν(Cu–S) 346w. UV–vis(kK, log ε)(in DMSO): 15.22(2.47), 10.19sh, 29.94(3.21), 28.57(3.29), 24.81(2,45); (in Nujol): 14.70, 23.53, 33.33, 29.50. $\Lambda\mu$(μS cm^{-1}) (in DMSO): 9; μ_{eff}(BM): 1.74.

4.1.2.4. Compound [Cu(dpta)(i-MNT)] (4). Yield: 97%. Anal. Calcd for $C_{10}H_{17}N_5S_2Cu$: C, 35.86; H, 5.11; N, 20.91; Cu, 18.97. Found: C, 35.79; H, 5.12; N, 20.84; Cu, 18.85. IR(KBr disk): ν_{sec}(N–H) 3315s, ν_{as}(N–H) 3240s, δ(N–H) 1589 m, ν(C≡N) 2197vs, ν(C=CS_2) 1363vs, ν_{as}(C=CS_2) 947s, ν_s(CS_2) 890 m, ν(Cu–N) 493w, ν(Cu–S) 341w. UV–vis(kK, log ε)(in DMSO):15.08(2.45), 10.66sh, 29.67(3.20), 28.49(3.33), 22.62(2.63); (in Nujol): 12.99, 25.12, 33.55, 30.30. $\Lambda\mu$(μS cm^{-1}) (in DMSO): 10; μ_{eff}(BM): 1.76.

4.1.2.5. Compound [Cu(trien)(i-MNT)] (5). Yield: 56%. Anal. Calcd for $C_{10}H_{18}N_6S_2Cu$: C, 34.32; H, 5.18; N, 24.01; Cu, 18.15. Found: C, 34.21; H, 5.13; N, 23.98; Cu, 18.10. IR(KBr disk): ν_{sec}(N–H) 3310s, ν_{as}(N–H) 3235s, δ(N–H) 1586 m, ν(C≡N) 2190vs, ν(C=CS_2) 1359vs, ν_{as}(C=CS_2) 943s, ν_s(CS_2) 886 m, ν(Cu–N) 485w, ν(Cu–S) 357w. UV–vis(kK, log ε)(in DMSO): 15.01(2.48), 10.52sh, 29.67(3.18), 28.49(3.33), 22.17(2.64); (in Nujol): 14.10, 22.27, 33.21, 29.76. $\Lambda\mu$(μS cm^{-1}) (in DMSO): 11; μ_{eff}(BM): 1.78.

4.1.2.6. Compound [Cu(bipy)(i-MNT)] (6). Yield: 47%. Anal. Calcd for $C_{14}H_8N_4S_2Cu$: C, 42.91; H, 2.40; N, 16.68; Cu, 17.65. Found: C, 42.79; H, 2.36; N, 16.87; Cu, 17.57. IR(KBr disk): ν(C≡N) 2206vs, ν(C=CS_2) 1433vs, ν_{as}(CS_2) 928w, ν_s(CS_2) 886 m, ν(Cu–N) 485w, ν(Cu–S) 357w. UV–vis(kK, log ε)(in DMSO): 14.64(2.34), 10.54sh, 30.30(3.31), 28.49(3.09), 25.70(2.80); (in Nujol): 14.83, 25.70, 33.33, 30.40. $\Lambda\mu$(μS cm^{-1}) (in DMSO): 9; μ_{eff}(BM): 1.93.

4.1.2.7. Compound [Cu(dpk(OH)$_2$)(i-MNT)] (7). Yield: 45%. Anal. Calcd for $C_{15}H_8N_4S_2Cu$: C, 42.91; H, 2.22; N, 15.39; Cu, 16.38. Found: C, 42.88; H, 2.27; N, 15.49; Cu, 16.15. IR(KBr disk): ν(O–H) 3500s, ν(C≡N) 2210vs, ν(C=CS_2) 1427vs, ν_{as}(CS_2) 931w, ν_s(CS_2) 794 m, ν(Cu–N) 482w, ν(Cu–S) 353w. UV–vis(kK, log ε)(in DMSO): 14.66(2.27), 10.0sh, 29.76(3.29), 28.40sh, 24.57(2.63); (in Nujol): 15.31, 22.70, 33.74, 30.77; $\Lambda\mu$(μS cm^{-1}) (in DMSO): 6; μ_{eff}(BM): 1.76.

4.1.2.8. Compound [Cu(dien)(CNPD)] (8). Yield: 84%. Anal. Calcd for $C_{13}H_{17}N_5S_2O_2Cu$: C, 38.75; H, 4.25; N, 17.38; Cu, 15.76. Found: C, 38.67; H, 4.17; N, 17.43; Cu, 15.65. IR(KBr disk): ν_s(N–H) 3328s, ν_{as}(N–H) 2854s, δ(N–H) 1587vs, ν(C≡N) 2181vs, ν(C=CS_2) 1436vs, ν_{as}(CS_2) 950s, ν_s(CS_2) 837s, ν(Cu–N) 481s, ν(Cu–S) 373s. UV–vis(kK, log ε)(in DMSO): 14.02sh, 9.95sh, 31.15(2.89), 29.32sh, 22.32(3.19); (in Nujol): 13.33, 24.33, 33.33, 29.32. $\Lambda\mu$(μS cm^{-1}) (in DMSO): 10; μ_{eff}(BM): 1.81.

4.1.2.9. Compound, [Cu(2a-tz)(i-MNT)(H_2O)]$_n$ (9). Yield: 56%. Anal. Calcd for $C_7H_6N_4S_3OCu$: C, 26.12; H, 1.88; N, 17.16; Cu, 19.74. Found: C, 26.05; H, 1.87; N, 16.98; Cu, 19.72. IR(KBr disk): ν(O–H) 3413vs, ν_s(N–H) 3413s-br, δ(N–H) 1616s, ν(C≡N) 2209vs, ν(C=N) 1603s, ν(C=CS_2) 1384s, ν_{as}(CS_2) 978w, ν_s(CS_2) 871w, ν(Cu–N) 478w, ν(Cu–S) 400w. UV–vis(kK, log ε)(in DMSO): 15.65(1.99), 10.45sh, 29.59(3.15), 27.93sh, 403sh; (in Nujol): 14.70, 22.12, 33.33, 30.30. $\Lambda\mu$(μS cm^{-1}) (in DMSO): 10; μ_{eff}(BM): 1.08.

4.1.2.10. Compound [Cu(2a-5mtz)(i-MNT)(H_2O)]$_n$ (10). Yield: 24%, Anal. Calcd for $C_8H_8N_4S_3OCu$: C, 28.60; H, 2.40; N, 16.68; Cu, 18.92. Found: C, 28.52; H, 2.30; N, 16.83; Cu, 18.85%. IR(KBr disk): ν(O–H) 3413vs, ν_s(N–H) 3351vs, ν_{as}(N–H) 3260s, δ(N–H) 1614 m, ν(C≡N) 2206s, ν(C=N) 1591s, ν(C=CS_2) 1356vs, ν_{as}(CS_2) 989w, ν_s(CS_2) 819 m, ν(Cu–N) 418 m, ν(Cu–S) 390w. UV–vis(kK, log ε)(in DMSO): 14.90(1.99), 10.30(1.41), 30.12(2.97), 27.40sh, 24.81sh; (in Nujol): 14.22, 23.86, 33.44, 30.86. $\Lambda\mu$(μS cm^{-1}) (in DMSO): 9; μ_{eff}(BM): 1.09.

4.1.2.11. Compound [Cu(2a-2tzn)(i-MNT)(H_2O)]$_n$ (11). Yield: 21%. Anal. Calcd for $C_7H_8N_4S_3OCu$: C, 25.96; H, 2.49; N, 17.30; Cu, 19.62. Found: C, 25.80; H, 2.48; N, 17.17; Cu, 19.50. IR(KBr disk): ν(O–H) 3450vs, ν_s(N–H) 3413vs, δ(N–H) 1558s, ν(C≡N) 2205s, ν(C=N) 1616s, ν(C=CS_2) 1385s, ν_{as}(CS_2) 918w, ν_s(CS_2) 856w, ν(Cu–N) 481 m, ν(Cu–S) 396w. UV–vis(kK, log ε)(in DMSO): 15.67(1.98), 10.43(1,50), 29.50(2,80), 26.9sh, 24.33sh; (in Nujol): 15.33, 21.83, 33.22, 29.24.$\Lambda\mu$(μS cm^{-1}) (in DMSO): 11; μ_{eff}(BM): 0.94.

4.2. In vitro SCEs and PRI assays

Healthy donors who were 25–35 years old, non-smokers, not receiving any drugs, not consuming considerable quantities of alcohol and not suffering from any kind of infection for the last 15 days, gave blood for the cytogenetic experiments. Human lymphocyte cultures were prepared by adding in 5 mL chromosome medium (RPMI-1640, Biochrome, Berlin), at the beginning of culture life:

i. 11–12 drops of normal human heparinized whole blood,
ii. 5 μg/mL 5-Bromodeoxyuridine (BrdU) and
iii. the dithiolate complex solutions (15, 30 and 45 μM final concentration).

The cultures were incubated at 37 °C for 72 h in the dark to minimize photolysis of BrdU and colchicine (0.3 μg/mL) was added

2 h before the collection of the cultures. The cells were then collected by centrifugation and exposed to 0.075 M KCl for 10 min. The hypotonic solution spreads the chromosomes and hemolyses the red blood cells. The pellet was fixed three times with a mixture of methanol:acetic acid (3:1). Drops of concentrated suspension of cells were placed on microslides and allowed to air dry in. For SCEs and PRI analysis, the slides were stained by a modification of the Fluorescence Plus Giemsa (FPG) procedure to obtain harlequin chromosomes.[40] For SCEs 20 suitably spread 2nd division cells from each culture were blindly scored. For PRI, 100 cells in the 1st, 2nd, 3rd and higher divisions from each culture were blindly scored. PRI = $M_1 + 2M_2 + 3M_3 + \cdots/100$, where M_1, M_2, and M_3, are the percent values of cells in the 1st, 2nd, 3rd and higher divisions respectively. For the statistical evaluation of the experimental data, Student's t-test was performed to determine whether any SCE values differed significantly from the controls and the χ^2 test was used for the cell kinetic comparisons (PRI). Simple linear correlation between the PRI and the SCE frequencies was also calculated using Pearson's product moment correlation coefficient.

4.3. In vivo experiments

4.3.1. Compounds for intra-peritoneal (ip) treatment

Stock solutions of the compounds used in this study were prepared immediately before use. They were suspended in corn oil in the desired concentration following initial dissolution in 10% DMSO, an efficient solvent for those Cu(II) complexes and ligands. The DMSO concentration by itself produced no observable toxic effects in our experiments.

4.3.2. Experimental animals

Male and female DBA/2 and BDF1(C57B1/6xDBA/2) mice 4–6 weeks old and weighing 20–25 g were used in the toxicity and antitumor evaluation experiments, respectively. Mice provided by the Experimental Animal production Laboratory at Theagenion Cancer Hospital, were kept under constant temperature and humidity, in sterile cages with water and food ad libitum.

4.3.3. Toxicity studies

The toxicity of all compounds was tested on DBA/2 mice both male and female in groups of 10 animals per cytotoxic dose. For each compound **4** different doses were administrated. The number of survived animals was determined 30 days after a single ip injection. The animals were observed for later deaths, but these were not included in the calculations because such death was not due to a general pharmacologic effect. The LD_{50} and LD_{10} doses (LD = lethal dose) were estimated graphically, where the percentage of deaths due to the toxicity of each dose is plotted on the ordinate, while the administered doses are plotted on the abscissa on semi-logarithmic paper. The point on the line that corresponds to 50% and 10% mortalities gives the LD_{50} and LD_{10}, respectively.[41]

4.3.4. Tumor transplantation

Transplantation of lymphocytic P388 leukemia maintained in on ascitic form was carried out by withdrawing peritoneal fluid from donor DBA/2 mice with 7-day tumor growth. The suspension was centrifuged for 2 min (2000 g). The supernatant of peritoneal fluid was decanted and the precipitate was diluted 10-fold with 0.9% NaCl. The cell number was determined. The resulting cell suspension of 0.1 mL containing 10^6 cells, was injected ip into each animal.

4.3.5. Chemotherapy evaluation

In all experiments, the copper compounds were administered by ip injection. They were dissolved in 10% DMSO and suspended in corn oil. Stock solutions of compounds were prepared immediately before administration. They were ip injected in a single dose into BDF1 mice 24 h after leukemia propagation.

For the survival experiments, the antitumor activity of the above mentioned compounds against P388 murine lymphocytic leukemia was assessed by the oncostatic parameter $T/C\%$: the mean of median survival time of the drug-treated animals (T), excluding long-term survivors, versus corn-oil treated controls (C), expressed as a percentage. The minimum criterion for activity is $T/C \geqslant 125\%$, according to the experimental evaluation of antitumor drugs in the National Cancer Institute (NCI), USA.[34]

All experiments were performed with 6 mice in each drug treatment group and eight mice in the tumor control group. Experiments were initiated by implanting mice with tumor cells. Drug treatments were given as single ip injections, using the LD_{10} as a therapeutic dose. Experiments were terminated when no mice remained alive.

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Supplementary data

Table S1 for selected bond lengths (Å) and angles (°) of i-MNT dithiolate Cu(II) complexes theoretically calculated and Figure S1 for optimized structures **3**, **6**, **8** and **9** complexes as deduced by MM calculations. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.02.059.

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