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Chemical and Biological Profiles of Novel Copper(II) Complexes Containing S-Donor Ligands for the Treatment of Cancer

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In the last years, we have synthesized some new platinum(II), palladium(II), gold(I/III) complexes with dithiocarbamato derivatives as potential anticancer drugs, to obtain compounds with superior chemotherapeutic index in terms of increased bioavailability, higher cytotoxicity, and lower side effects than cisplatin. On the basis of the obtained encouraging results, we have been studying the interaction of $CuCl_2$ with methyl-/ethyl-/*tert*-butylsarcosine-dithiocarbamato moieties in a 1:2 molar ratio; we also synthesized and studied the *N*,*N*-dimethyl- and pyrrolidine-dithiocarbamato copper complexes for comparison purposes. The reported compounds have been successfully isolated, purified, and fully characterized by means of several spectroscopic techniques. Moreover, the electrochemical properties of the designed compounds have been studied through cyclic voltammetry. In addition, the behavior in solution was followed by means of UV−vis technique to check the stability with time in physiological conditions. To evaluate their in vitro cytotoxic properties, preliminary biological assays (MTT test) have been carried out on a panel of human tumor cell lines. The results show that cytotoxicity levels of all of the tested complexes are comparable or even greater than that of the reference drug (cisplatin).

Introduction

Currently, a large variety of chemotherapeutic drugs are being used to treat cancer. Unfortunately, many compounds demonstrate restricted efficacy due to problems of delivery and penetration and a modest degree of selectivity for the tumor cells, causing severe damage to healthy tissues. However, the activity of these compounds is mainly limited by the development of drug resistance. Tumor cells are a rapidly changing target because of their genetic instability, heterogeneity, and high rate of mutation, leading to selection and overgrowth of a drug-resistant tumor-cell population.[1,2] In 1971, J. Folkman hypothesized that tumors are angiogenesis-dependent and that antiangiogenic therapy might represent a good alternative for the treatment of solid tumors.[3] A promising way of cancer research is the study of a group of compounds called angiogenesis inhibitors. These are drugs that block the development of new blood vessels, which supply tissues with oxygen and nutrients necessary for survival and growth.[4,5] In cancerous tissues, tumors cannot grow or metastasize without the development of new blood vessels. Solid tumors cannot grow beyond the size of 1 to 2 mm^3 without inducing the formation of new blood vessels to supply the nutritional and other needs of the tumor.[6]

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Endothelial cells are the source of new blood vessels and have a remarkable ability to divide and migrate. In an attempt to isolate a peptide, endothelial stimulating growth factor, McAuslan and Reilly[7] noted a high concentration of copper salts. They postulated that copper was the active principle in angiogenesis. Copper, but not other trace metals, stimulated the directional migration of endothelial cells and, more recently, copper was found to directly stimulates the in vitro proliferation of endothelial cells.[8] Copper metabolism is profoundly altered in neoplastic development in human cancer and in tumor-bearing animals.[9,10] Serum copper levels correlate with tumor incidence, tumor weight, malignant progression, and recurrence in a variety of human cancers: Hodgkin's lymphoma, sarcoma, leukemia, and cancer of the cervix, breast, liver, and lung[11–14] as well as brain tumors.[15,16] Consistently, the high serum and tissue levels of copper, found in many types of human cancers, support the idea that copper could be used as a potential tumor-specific target.

Recent studies show that some organic prodrugs, used to treat Wilson's disease (characterized by copper accumulation in different organs), present also an antiangiogenic effect toward some murine tumors.[17] The activity of these drugs is related not only to their ability to remove copper but also to the cytotoxic properties of the complexes formed between the organic prodrugs and the copper ion. In fact, we have recently demonstrated a correlation between the antiangiogenic and the antitumor properties of some copper proteasome inhibitors;[18,19] moreover, the inhibition of the proteasomal activity can be achieved by targeting tumor cellular copper with the nontoxic compound DSF (disulfiram, a member of the dithiocarbamate family, capable of binding copper), resulting in selective apoptosis induction of tumor cells.[20]

In our previous works, we demonstrated the high effectiveness of different metal-dithiocarbamato complexes toward a number of tumor cell lines sensible and resistant to cisplatin, which is a widely used drug in chemotherapy toward different types of tumors.[21–28]

In addition, these complexes were proved to induce very low toxicity levels, in particular regarding its nephrotoxic effects.[29] Considering the potential advantages in terms of noticeable in vitro and in vivo antitumor activity, lack of cross-resistance with cisplatin, and reduced adverse side effects, further studies on biochemical features of the dithiocarbamato complexes are warranted.

Dithiocarbamates are a class of metal-chelating compounds that have previously been used in the treatment of bacterial and fungal infections, and in the treatment of AIDS.[30,31] For example, pyrrolidine dithiocarbamate (PyDT) is a synthetic antioxidant and inhibitor of NF-κB, a transcription factor capable of binding copper.[32,33] PyDT and other dithiocarbamates have been found to induce apoptosis in conjunction with copper in different types of cancer cells.[34] Previously, a synthetic PyDT containing copper was found to be a potent proteasome inhibitor and apoptosis inducer.[35] Moreover, PyDT is capable of binding copper spontaneously, forming new complexes that have proteasome-inhibitory and apoptosis-inducing activities to tumor but not to normal/nontransformed breast cells. It was also found that premalignant or cancer breast cells cultured to contain elevated copper are sensitive to treatment with PyDT.[36]

Most recently, we reported that a gold(III) dimethyldithiocarbamato complex could inhibit the activity of a purified 20S and 26S proteasome in human breast cancer cell cultures and xenografts.[37]

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These findings altogether prompted us to study, as chemotherapeutics, new copper(II) dithiocarbamato derivatives of the esters of sarcosine; we have also synthesized, following a different reaction with respect to literature data,[38,39] and studied the *N*,*N*-dimethyl-/pyrrolidine dithiocarbamate (DMDT/PyDT)) complexes for comparison purposes.

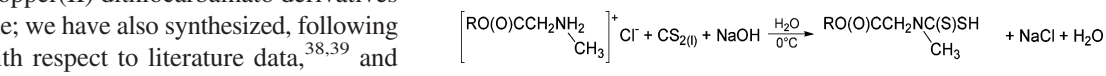
Experimental Section

Chemicals. *N*,*N*-dimethyl-/pyrrolidine dithiocarbamate, methyl/ethyl/*tert*-butylsarcosine hydrochloride (Fluka), carbon disulfide (Aldrich), copper(II) chloride (J. T. Baker-Chemicals) were used as supplied. All other reagents and solvents were of high purity and were used as purchased without any further purification.

Instrumentation. Electronic spectra were recorded at 298 K in the range of 190−750 nm with a PerkinElmer Lambda 15 double-beam spectrophotometer, using fresh 100 μM solutions of the samples; in both DMSO and PBS/DMSO (in the latter case, the complexes were previously dissolved in DMSO, to a final DMSO concentration of 0.5% (v/v)). FTIR spectra were recorded at room temperature in nujol between two polyethylene tablets on a Nicolet Magna 740 FTIR spectrophotometer for the range 600−50 cm^{-1}, and in solid KBr on a Nicolet-NEXUS 670 FTIR spectrophotometer for the range 4000−400 cm^{-1}. ^{1}H NMR spectra were recorded at 298 K in DMSO-d_6 on a Bruker Avance DRX400 spectrometer equipped with a Silicon Graphics workstation operating in Fourier transform, using tetramethylsilane (TMS, $\delta = 0.00$) as external standard. The thermogravimetric (TG) and differential scanning calorimetry (DSC) curves were recorded with a TA Instruments thermobalance equipped with a DSC 2929 calorimeter; the measurements were carried out in the range 25−1300 °C in alumina crucibles under air (flux rate 30 $cm^3 \cdot min^{-1}$) and at a heating rate of 10 °C·min^{-1}, using alumina as reference. Elemental analyses were performed in duplicate with a PerkinElmer 2400 CHN microanalyzer; S, was determined by Schöninger method and with a Carlo Erba 1108 CHNS-O microanalyser. The apparatus for electrochemistry and spectroelectrochemistry has been described elsewhere.[40] Electrochemical experiments were performed either in deaerated CH_2Cl_2 solution containing $[NBu_4][PF_6]$ as supporting electrolyte (0.2 mol dm^{-3}) or in deaerated H_2O-DMSO solution (1:1) using NaCl as supporting electrolyte (0.1 mol dm^{-3}). All potential values are referred to the saturated calomel electrode (SCE). In CH_2Cl_2 solution, the one-electron oxidation of ferrocene occurs at +0.41 V, whereas in H_2O−DMSO solution it occurs at +0.12 V. IC_{50} values were estimated by nonlinear regression analysis using *GraphPad Prism* software (San Diego, Ca. USA). Data are the means ±95% confidence intervals (CI) of quadruplicate determinations from three independent experiments; the absorbance at 570 nm was determined using a Victor2, multilabel counter (Wallac Instruments, UK).

Synthesis of [Cu(DMDT)$_2$] and [Cu(PyDT)$_2$]. The synthesis of these complexes was made following the literature.[41] The powder of $CuCl_2$ was added to a water solution of DMDT sodium salt $[(CH_3)_2NCS_2]Na$ or PyDT ammonium salt $[PyNCS_2]NH_4$ in molar ratio 1:2 metal-to-ligand under vigorous stirring, leading to the immediate precipitation of a brown solid that was filtered off and washed with water and dried in a desiccator with P_4O_{10}, the final yield being 90−95%.

Scheme 1. Reaction Leading to the Synthesis of methyl/ethyl/*tert*-butyl-Sarcosinedithiocarbamic Acid



bis(dimethylcarbamodithioato-*k*S,*k*S′)-copper(II), [Cu(DMDT)$_2$]. Anal. Calcd for $C_6H_{12}CuN_2S_4$ (M.W. = 303.98 g/mol), %: C, 23.24; H, 3.90; N, 8.89; S, 40.75. Found, %: C, 23.71; H, 3.98; N, 9.21; S, 41.12. FTIR (KBr, nujol, $\tilde{\nu}_{max}$): ν(N−CSS) = 1524; $\nu_{a,s}$(SCS)= 976, 563; ν(SCuS) = 352 cm^{-1}. ^{1}H NMR (DMSO-d_6, δ): 3.52 (12H, s, br, CH_3N).

bis(1-pyrrolidinecarbamodithioato-*k*S,*k*S′)-copper(II), [Cu(PyDT)$_2$]. Anal. Calcd for $C_{10}H_{16}CuN_2S_4$ (M.W. = 356.04 g/mol), %: C, 33.70; H, 4.53; N, 7.87; S, 36.02. Found, %: C, 33.66; H, 4.72; N, 8.10; S, 36.22. FTIR (KBr, nujol, $\tilde{\nu}_{max}$): ν(N−CSS) = 1500; $\nu_{a,s}$(SCS)= 948, 580; ν(SCuS) = 324 cm^{-1}. ^{1}H NMR (DMSO-d_6, δ): 2.01 (4H, m, CH_2−); 3.85 (4H, m, CH_2−N).

Synthesis of [Cu(RSDT)$_2$], R = CH_3, CH_3CH_2, $(CH_3)_3C$. The dithiocarbamato ligands were obtained by reacting in water at 0 °C R-sarcosinehydrochloride (RSHCl = $(RO(O)CCH_2N(CH_3)H_2]^+Cl^-$), CS_2, and NaOH in equimolar ratio. After 1 h, the pH value dropped from 10−11 to 6−7, according to the reaction shown in Scheme 1.

The solution thus obtained was slowly added under stirring to an aqueous cold (0 °C) solution (2 mL) of $CuCl_2$ in a 1:4 metal-to-ligand molar ratio, leading to the immediate precipitation of a brown solid that was filtered off, washed with water, and dried in a desiccator with P_4O_{10}, the final yield being 82−85%.

bis[methyl *N*-(dithiocarboxy-*k*S,*k*S′)-*N*-methylglycinato]-copper(II), [Cu(MSDT)$_2$]. Anal. Calcd for $C_{10}H_{16}CuN_2O_4S_4$ (M.W. = 420.18 g/mol), %: C, 28.59; H, 3.84; N, 6.67; S, 30.53. Found, %: C, 28.42; H, 3.15; N, 6.46; S, 30.37. Mp = 199,0 °C (dec.). FTIR (KBr, nujol, $\tilde{\nu}_{max}$): ν(N−CSS) = 1506; ν(C═O) = 1752; ν(C−OMe) = 1206; $\nu_{a,s}$(SCS) = 970, 570; $\nu_{a,s}$(SCuS) = 366 cm^{-1}. ^{1}H NMR (DMSO-d_6, δ): 3.81 (6H, s,br, CH_3O); not detected (6H, CH_3N); not detected (4H, CH_2N).

bis[ethyl *N*-(dithiocarboxy-*k*S,*k*S′)-*N*-methylglycinato]-copper(II), [Cu(ESDT)$_2$]. Anal. Calcd for $C_{12}H_{20}CuN_2O_4S_4$ (M.W. = 448.11 g/mol), %: C, 32.16; H, 4.50; N, 6.25; S, 28.62. Found, %: C, 31.93; H, 4.47; N, 6.15; S, 28.05. Mp = 209,0 °C (dec.). FTIR (KBr, nujol, $\tilde{\nu}_{max}$): ν(N−CSS) = 1506; ν(C═O) = 1733; ν(C−OEt) = 1206; $\nu_{a,s}$(SCS)= 970, 572; $\nu_{a,s}$(SCuS) = 361 cm^{-1}. ^{1}H NMR (DMSO-d_6, δ): 1.22 (6H, br, CH_3CH_2); 4.27 (4H, br, CH_3CH_2); not detected (6H, CH_3N), not detected (4H, CH_2N).

bis[*tert*-butyl *N*-(dithiocarboxy-*k*S,*k*S′)-*N*-methylglycinato]-copper(II), [Cu(TSDT)$_2$]. Anal. Calcd for $C_{16}H_{28}CuN_2O_4S_4$ (M.W. = 504.22 g/mol), %: C, 38.11; H, 5.59; N, 5.55; S, 25.44. Found, %: C, 37.99; H, 5.51; N, 5.12; S, 25.32. Mp = 208,0 °C (dec.). FTIR (KBr, nujol, $\tilde{\nu}_{max}$): ν(N−CSS) = 1505; ν(C═O) = 1737; ν(C−O*t*-bu) = 1207; $\nu_{a,s}$(SCS) = 967, 566; $\nu_{a,s}$(SCuS) = 354 cm^{-1}. ^{1}H NMR (DMSO-d_6, δ): 1.46 (18H, br, $(CH_3)_3C$); not detected (6H, CH_3N), not detected (4H, CH_2N).

Cell Lines and Culture Conditions. Human ovarian carcinoma (2008 wild type and C13 cisplatin resistant subclone) and cervix squamous carcinoma (A431 wild type and A431Pt cisplatin resistant subclone) cell lines were grown incubated with RPMI 1640 medium, 10% fetal bovine serum, 2% glutamine, and 1% pen-strep in humidified condition at 5% CO_2 and 37 °C. All cell lines have a duplication time of about 24 h. Cells were collected every 2 days with minimum amount of 0.05% trypsin/0.02% EDTA and seeded 1×10^6 in 100 mm dish. All reagents were from Cambrex (NY, USA).

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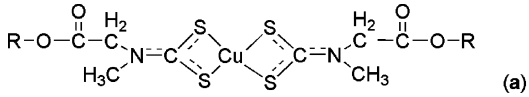
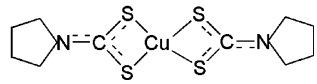
Scheme 2. Chemical Drawing of the Investigated Copper(II) Complexes: $[Cu(RSDT)_2]$ (**a**), $[Cu(PyDT)_2]$ (**b**), $[Cu(DMDT)_2]$ (**c**)

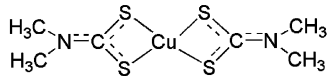


(**a**)

R = CH_3-; CH_3-CH_2-; $(CH_3)_3C$-;



(**b**)



(**c**)

In Vitro Cytotoxicity Studies. For the biological tests, the compounds were dissolved in DMSO, aliquoted, and stored at −80 °C. Compounds and cisplatin (Pharmacia & Upjohn, Milano, Italy) were dissolved in IMDM (Biochrome KG, Berlin, Germany) and filter sterilized (0.2 μM) immediately before use. The final DMSO concentration (0.1%) had no effect on cell killing. Cytotoxicity was measured by inhibition of cancer cell viability, determined using the 3-(4,5-dimethyl-thizol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[42] Cells were plated in 200 μl of medium in 96 well microtiter plates. Following overnight incubation, cells were exposed for 24 h to the different sulfured copper complexes. At the end of incubation, cells were washed and fresh medium was replaced. After 48 h, to each well was added 20 μl of 5 mg/ml MTT, and cells were incubated for 4 h at 37 °C. The cells were then lysed by adding 200 μl of acid isopropanol, and the absorbance at 570 nm was determined. Triplicate determinations and at least three separate experiments have been performed. IC_{50}, defined as the concentration causing 50% reduction of overall cell viability as compared to untreated cells, was extrapolated from dose−response curves of each copper complex.

Results and Discussion

Synthesis of the Complexes. By the reaction of the DMDT sodium salt $[(CH_3)_2NCS_2]Na$ or PyDT ammonium salt $[PyNCS_2]NH_4$ with $CuCl_2$ in a 1:2 metal to ligand molar ratio, we have obtained the $[Cu(DMDT)_2]$ and $[Cu(PyDT)_2]$ complexes in which the dithiocarbamato ligands coordinate the metal center through the sulfured atoms. The sulfured ligands derived from the methyl-, ethyl-, and *tert*-butyl- esters of sarcosine were obtained by reacting RSHCl (R=CH_3, CH_3-CH_2, $C(CH_3)_3$), CS_2, and NaOH in equimolar ratio; the copper(II) complexes $[Cu(RSDT)_2]$ were obtained by adding $CuCl_2$ in 1:4 metal-to-ligand molar ratio (Scheme 2).

FTIR spectroscopy. In the FTIR spectra of all of the synthesized complexes, the ν(C−N) band is observable in the range 1493−1539 cm^{-1}; this band defines a C−N bond order intermediate between a single bond (1350−1250 cm^{-1}) and a double bond (1690−1640 cm^{-1});[43] in fact, moving from DMDTNa (ν(C−N) = 1486 cm^{-1}) or $PyDTNH_4$ (v(C−N) = 1410 cm^{-1}) to its metal derivatives, the ν(C−N) vibrational mode is shifted to higher energies, showing an increase of the C−N double-bond character. On the contrary, for the complexes obtained through the template reaction, this behavior cannot be observed as we were not able to isolate the free R-sarcosinedithiocarbamato precursor; anyway, the values recorded for ν(C−N) modes are in agreement with the ones observed for DMDT or PyDT complexes and with those reported in the literature for analogous metal derivatives of α-amino acid dithiocarbamates.[44–46] The presence of only one band in the region 1050−950 cm^{-1}, assigned to the ν(S−C−S) asymmetric mode, is assumed to indicate a symmetrical bonding of the dithiocarbamato moiety, acting in a bidentate mode.[47] The 420−240 cm^{-1} region is associated with ν(M−S) vibrations.[48] New bands, absent in the starting material, are observed in the 370−320 cm^{-1} region, and they can be attributed to the metal−sulfur stretching mode.[49]

Thermal Analysis. The thermal behavior of the synthesized compounds has been studied to confirm the proposed stoichiometry. The results have been summarized in Table 1; a good correlation exists between calculated and found values. The thermal behavior of the complexes was recorded up to 1200 °C in air flux. All of the copper complexes undergo the same degradation pathway, leading to the corresponding metal-oxide (CuO) as the final product. Moreover, $[Cu(DMDT)_2]$ and $[Cu(PyDT)_2]$ decompose gradually to CuO in the 180−1100 °C interval without melting.

Electrochemistry and Spectroelectrochemistry. The inherent electrochemical properties of these copper(II) dithiocarbamato derivatives have been preliminary studied through cyclic voltammetry soon after dissolution. Dithiocarbamates are redox-active ligands, the electron transfer activity of which is influenced by the electrode material as well as by the solvent. In fact, at platinum or glassy carbon electrodes they undergo oxidation to radical species, which rapidly dimerize in dimethylsulfoxide (DMSO) solution, whereas no process can be detected in CH_2Cl_2 solution.[50] Dithiocarbamato ligands have stabilizing ability toward copper ions, and their copper(II) complexes undergo reversibly oxidation and reduction processes to their monocations and monoanions, respectively.[50] Some characterization studies on dithiocarbamates of copper(I), copper(II), and copper(III) have been reported in the past.[51,52]

In CH_2Cl_2 solution, all of the copper(II) complexes exhibit both oxidation and reduction processes with a certain chemical reversibility in the cyclic voltammetric time scale.

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Table 1. Thermogravimetric (TG) and Differential Scanning Calorimetric (DSC) Data in Air Flux

		DSC	
compound	decomposition interval [°C]	TG weight loss [%] (calculated) to CuO	peak temperature [°C] (process[a])
$[Cu(DMDT)_2]$	200−1100	(−73.83)−74.71	314(exo)/361(exo)/1044(endo)
$[Cu(MSDT)_2]$	125−1200	(−81.06)−80.84	199(endo/melting)/294(exo)/502(exo)/1041(endo)/1127(exo)
$[Cu(ESDT)_2]$	170−1200	(−82.24)−82.71	178(endo/melting)/209(endo)/301(exo)/468(exo)/518(endo)/1040(endo)/1132(endo)
$[Cu(TSDT)_2]$	100−1100	(−84.22)−83.91	208(endo/melting)/303(exo)/384(exo)/1043(endo)
$[Cu(PyDT)_2]$	180−1100	(−77.66)−78.83	288(exo)/338(exo)/396(exo)/1025(endo)/1033(endo)

[a] endo/exo/melting = endothermic/exothermic/melting process.

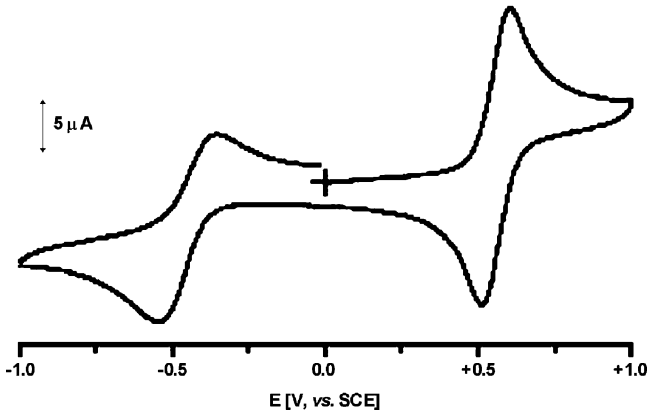

Figure 1. Cyclic voltammetric response recorded at a platinum electrode in a CH_2Cl_2 solution of $[Cu(ESDT)_2]$ ($0.4 \cdot 10^{-3}$ mol dm^{-3}); $[NBu_4][PF_6]$ (0.2 mol dm^{-3}) as supporting electrolyte; scan rate 0.2 V s^{-1}.

A representative example is shown in Figure 1, which refers to $[Cu(ESDT)_2]$. Controlled potential coulometry in correspondence of the anodic process ($E_w = +0.8$ V) consumed one electron *per* molecule, and cyclic voltammetric tests on the resulting solution gave rise to profiles quite similar to the original ones, the chemical reversibility of the electron removal, not only in the short times of cyclic voltammetry (i_{pc}/i_{pa} constantly equal to the unity in the scan rate range from 0.02 to 2.0 Vs^{1-}) but also in the longer times of macroelectrolysis.

The reduction pattern appears more complicated: the current ratio i_{pa}/i_{pc} decreases with the increase of the scan rate, and the peak-to-peak separation notably swerves from the 60 mV expected for an electrochemically reversible process. An E_rC_r mechanism, in which the quasireversible copper(II)/copper(I) reduction is coupled to a slow, reversible chemical side-reaction, could account for this trend.[53] Controlled potential coulometry ($E_w = -0.8$ V) consumed approximately 0.6 electrons *per* molecule, leading to a cyclic voltammetric profile in which only marginal residues of the monoanion could be identified.

The formal electrode potentials of the copper(III)/copper(II)/copper(I) sequence for all of the complexes in CH_2Cl_2 solution are collected in Table 2 together with those recorded in H_2O−DMSO (1:1) solution, the electrochemical behavior of which will be discussed below.

As proved by the substantially constant HOMO−LUMO gap (∼1 eV), the location of the redox potentials is governed by the inductive effects of the dithiocarbamato substituents.[50,51]

Figure 2 illustrates the UV−vis spectral changes of $[Cu(ESDT)_2]$ recorded upon either one-electron removal or one-electron addition. The original neutral species show in CH_2Cl_2 two main bands at $\lambda_{max} = 284$ and 428 nm. In agreement with the color changes observed in the exhaustive oxidation (from yellow-brown to yellow), upon stepwise oxidation, the UV band tends to disappear, whereas the visible band progressively increases with a blue shift of 15 nm.

Upon reduction, the visible band tends to disappear and, as a possible consequence of the mentioned slow chemical side-reaction, a new band at 350 nm tends to appear (in accordance with the pale-yellow color observed upon exhaustive reduction).

Table 2. Formal Electrode Potentials (V vs SCE) and peak-to-peak separation (mV) for the redox changes exhibited by the Cu(II)-dithiocarbamate complexes in different solutions

compound	$E^{\circ\prime}$ [Cu(III)/Cu(II)]	ΔE_p[a]	$E^{\circ\prime}$ [Cu(II)/Cu(I)]	ΔE_p[a]	solvent
$[Cu(DMDT)_2]$	+0.41	74	−0.57	69	CH_2Cl_2
	+0.47	79	−0.29	88	H_2O-DMSO (1:1)
$[Cu(MSDT)_2]$	+0.55	96	−0.46	140	CH_2Cl_2
	+0.34	84	−0.35[b]		H_2O-DMSO (1:1)
$[Cu(ESDT)_2]$	+0.55	82	−0.46	180	CH_2Cl_2
	+0.52	75	−0.20	90	H_2O-DMSO (1:1)
$[Cu(TSDT)_2]$	+0.52	69	−0.48	134	CH_2Cl_2
	+0.50		−0.50		H_2O-DMSO (1:1)
$[Cu(PyDT)_2]$	+0.45	74	−0.52	88	CH_2Cl_2
	+0.54	108	−0.23[b]		H_2O-DMSO (1:1)

[a] Measured at 0.2 Vs^{-1}. [b] From Osteryoung square wave voltammetry.

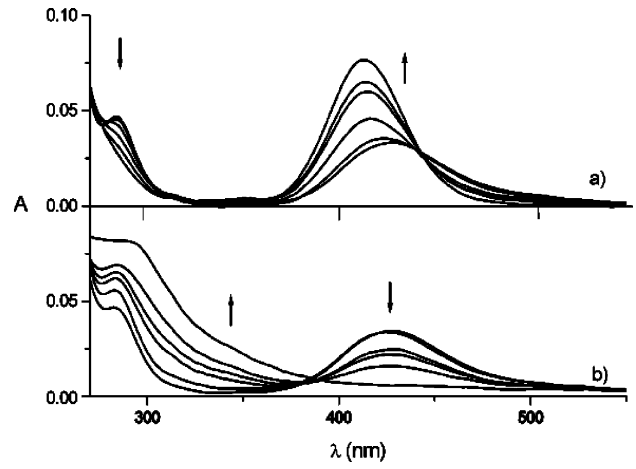

Figure 2. UV−vis spectral changes recorded in a OTTLE cell in CH_2Cl_2 solution of $[Cu(ESDT)_2]$ (0.4×10^{-3} mol dm^{-3}): (a) upon stepwise oxidation ($E_w = +0.7$ V, vs pseudoreference silver electrode); (b) upon stepwise reduction ($E_w = -0.7$ V).

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Table 3. UV−vis Spectral Parameters Recorded upon Reduction and Oxidation of the Copper(II)−Dithiocarbamate Complexes in CH_2Cl_2 Solution

compound	λ_{max}/nm	
$[Cu(DMDT)_2]$	281	428
$[Cu(DMDT)_2]^+$		413
$[Cu(DMDT)_2]^-$	281	
$[Cu(MSDT)_2]$	285	426
$[Cu(MSDT)_2]^+$		414
$[Cu(MSDT)_2]^-$	285	
$[Cu(ESDT)_2]$	284	428
$[Cu(ESDT)_2]^+$		413
$[Cu(ESDT)_2]^-$	289	
$[Cu(TSDT)_2]$	285	426
$[Cu(TSDT)_2]^+$		417
$[Cu(TSDT)_2]^-$	285	
$[Cu(PyDT)_2]$	280	427
$[Cu(PyDT)_2]^+$		412
$[Cu(PyDT)_2]^-$	280	

As shown, the appearance of isosbestic points in both processes confirms the relative stability of both $[Cu(ESDT)_2]^+$ and $[Cu(ESDT)_2]^-$ ions.

A similar spectroelectrochemical behavior is shown by the other complexes, and the spectral characteristics of the whole series in CH_2Cl_2 solution are reported in Table 3.

The redox behavior of the complexes has been examined also in H_2O/DMSO (1:1) solution. The complexes tend to precipitate, thus hampering detailed electrochemical studies. The most stable, and relatively most soluble, derivative resulted to be $[Cu(ESDT)_2]$ (Supporting Information for the voltammetric profile).

The copper(III)/copper(II)/copper(I) sequence is observed also in this medium, and both processes are complicated by concomitant chemical reactions, probably because the stabilizing planar and tetrahedral geometries of the electrogenerated copper(III) and copper(I) species are here distorted by the coordination of DMSO or water molecules.

Solution Properties Analyzed by UV−vis Absorption Spectroscopy. The evolution with time of all of the complexes was followed by UV−vis spectroscopy in different media for 24 h (Supporting Information). The compounds were initially dissolved in DMSO, giving a final concentration of 10^{-5} M.

For the UV−vis studies at pH 7.4, we have dissolved the compounds in DMSO (10^{-3} M), and then we have diluted the DMSO solution in phosphate buffer saline (PBS) concentration of 100 μM, consistent with the maximum concentration of the complexes used for the biological tests.

All the complexes behave similarly and are quite inert in DMSO at 298 K; in fact, the slow spectral changes with time, that is, a decrease in absorbance values, are caused by a slow formation of a brown solid derived from a diminished solubility of the complexes, and the nature of the solid obtained has been confirmed by IR spectra (data not shown). Figure 3 illustrates the $[Cu(TSDT)_2]$ complex spectrum with time as an example. Each complex is characterized by five absorption bands as shown in Table 4 (Supporting Information); for some complexes, bands 1 and 3 are only detectable as shoulders, only on concentrated (10^{-3} and 10^{-4} M) mother solutions (Supporting Information). For all the studied complexes, bands 1 and 2 are related to electronic transitions

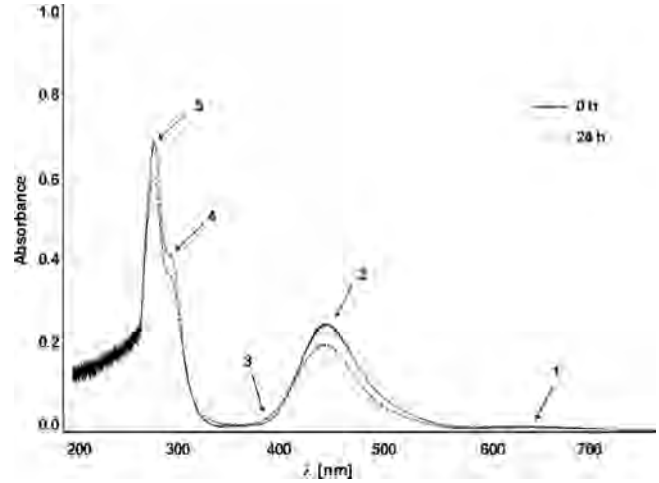

Figure 3. UV−vis spectral with time of $[Cu(TSDT)_2]$ complex in DMSO solution, 2.46×10^{-5} M.

located in the d orbitals of the metal center, whereas bands 3, 4, and 5 are typical of dithiocarbamato moieties. The two strong bands at around 287 and 270 nm (bands 4 and 5 respectively) can be attributed to intramolecular intraligand $\pi^* \leftarrow \pi$ transitions located in the S−C−S ed N−C−S moieties respectively.[49,54–56] Band 3, at about 360 nm, is ascribed to $\pi^* \leftarrow n$ transition in sulfur atoms; moreover, band 1 is related to a $d_{xy} \rightarrow d_{z^2}$ transition, and the broad band 2 could be an overlapping of $d_{xy} \rightarrow d_{xz}$ and $d_{xy} \rightarrow d_{yz}$ transitions,[57] and the presence of an asymmetry toward low energies is probably caused by the presence of the $d_{xy} \rightarrow d_{x^2-y^2}$ transition.[58,59]

On the other hand, when dissolved in DMSO/PBS, the samples give rise to a cloudy solution, but the spectra are very similar to those in DMSO, and the λ of the main absorptions remain unchanged with time (Supporting Information). The only difference is in the relative intensity of the absorptions: the overall effect is an approximately constant intensity for band 1 accompanied by a regular drop of the other absorptions that also present in a few cases a small red shift, probably caused by a major polarity of the aqueous solvent compared with the DMSO (Figure 4, also Supporting Information).[60] The drawdown of the baseline with time, is accompanied by the formation of an insoluble residue and by a dramatic lowering the bands 4 and 5 intensity when if compared with the spectra in DMSO ($\varepsilon 5/\varepsilon 2$(DMSO) = 2.63/3.03; $\varepsilon 5/\varepsilon 2$(DMSO/PBS) = 1.23/1.84). To clarify this behavior, we performed IR spectra (Supporting Information) of the solid species formed in PBS solution:

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Table 4. UV−vis Copper(II) Complex Absorptions in the 190−750 nm Range in DMSO Solvent (10^{-5} M)

	λ_{max}/nm (ϵ/cm^{-1}M^{-1})				
compound	band 1	band 2	band 3	band 4	band 5
[Cu(DMDT)$_2$]		436.4 (10 188)		286.4 (16 886)	269.2 (28 251)
[Cu(MSDT)$_2$]	634.0 (32)	438.8 (9038)	367.0 (636)	288.2 (15 657)	270.6 (27 368)
[Cu(ESDT)$_2$]	639.8 (37)	493.0 (9859)		287.2 (16 766)	270.4 (29 125)
[Cu(TSDT)$_2$]		438.4 (7528)	367.0 (315)	288.2 (13 166)	270.6 (22 830)
[Cu(PyDT)$_2$]	629 (178)	437.0 (11 750)	358.0 (475)	286.8 (18 189)	269.8 (30 948)

the only difference is a shoulder at about 1080 cm^{-1} for [Cu(TSDT)$_2$]; this band might be assigned to an S-coordinated DMSO molecule.[61] On the contrary, for the solid obtained from [Cu(MSDT)$_2$] complex, a new double band assignable to the ν(C−N), appears at 1460−1470 cm^{-1} at lower energies compared to the starting species (1508 cm^{-1}). This behavior can be explained, considering that ν(C−N) shifts toward higher energies on passing from the free ligand to the complexes, showing an increased double-bond character upon coordination.[43] In this case, the appearance of a new absorption at lower energies could be caused by a partial breakage of the metal−dithiocarbamate coordination assisted by a DMSO molecule.[62] Altogether, the IR spectra do not show big differences, suggesting that only a few changes on the coordination sphere after solubilization in DMSO/PBS occur.

Biological Assay. Human ovarian and cervix carcinoma cell lines (2008 and A431) and the cisplatin resistant subclones (C13 and A431Pt) were exposed for 24 h to increasing concentrations (0.01−100 μM) of the different copper−dithiocarbamato complexes or to cisplatin, as a reference antineoplastic drug. Cytotoxicity was analyzed by measuring cell viability by MTT assay (Figure 5). Dose−response curves clearly show a dose-related inhibition of cell viability caused by all the copper compounds, both in wild-type and in cisplatin-resistant cells. Some of these compounds were even more potent than cisplatin, mainly in cisplatin-resistant subclones. IC$_{50}$ values calculated from dose−response curves are reported in Table 5. As expected, cisplatin was more potent in wild-type cancer cells (0.19 μM and 0.78 μM in A431 and 2008 cells) than in resistant subclones (3.5 μM and 5.4 μM in A431Pt and C13). By contrast, especially [Cu(ESDT)$_2$], [Cu(MSDT)$_2$], [Cu(DMDT)$_2$] were almost equally active in wild type and cisplatin resistant cells, indicating lack of cross-resistance with cisplatin. [Cu(ESDT)$_2$] was the most active compound with IC$_{50}$ < 0.5 μM in all cell lines also in comparison with cisplatin. Furthermore, [Cu(ESDT)$_2$], [Cu(MSDT)$_2$], and [Cu(DMDT)$_2$] were all more active than cisplatin in ovarian carcinoma cells (2008-C13) and also in A431Pt cells.

The consistent evidence in the literature correlating copper, but not other metal, with the biochemistry of the tumor and the micromolar IC$_{50}$ concentration in the present cytotoxicity studies suggest that the molecular mechanism of copper might target mainly specific proteins of relevance in regulating the balance of proliferation/apoptosis in cancer cells. Data are encouraging to further investigate the potential copper pharmacological activity in in vitro biological models.

Final Remarks. Our previous findings stimulated us to study, as chemotherapeutics, new copper(II) dithiocarbamato complexes derived from the esters of sarcosine. The conclusions reached upon application of the spectroscopic techniques suggest that coordination in all the copper(II) derivatives takes place in a chelate manner, where the copper atom is coordinated to the sulfur-donating atoms. The presence of a paramagnetic copper(II) metal center in high-spin d^9 electronic configuration was confirmed by the particularly severe shifts and broadening of the NMR signals of the coordinated ligands. In the Supporting Information, we reported the ^{1}H NMR of the [Cu(ESDT)$_2$] complex, where the N(CH$_3$) and N(CH$_2$) signals are not recorded, whereas *CH$_3$*−CH$_2$− and CH$_3$−*CH$_2$*− signals are in the expected region of the NMR spectrum, although they have lost their multiplicity.

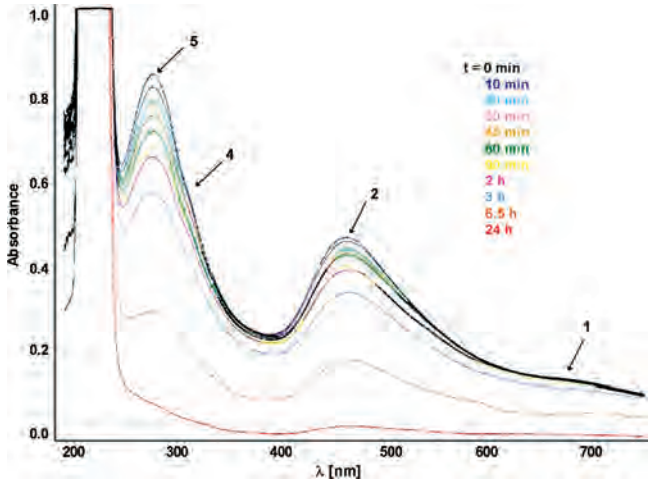

Figure 4. UV−vis spectral with time of [Cu(TSDT)$_2$] in DMSO/PBS solution, 101μM.

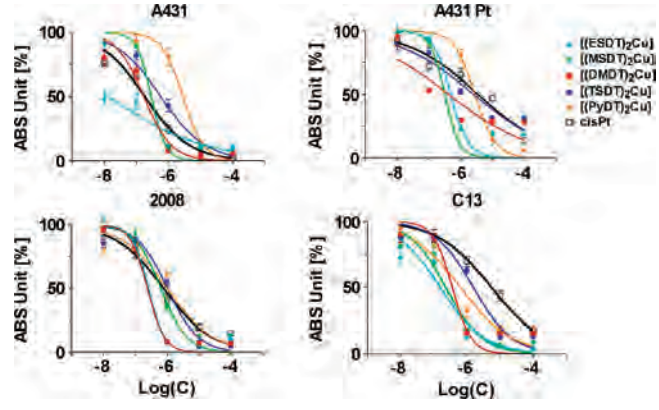

Figure 5. Cytotoxicity of sulfur−copper complexes in A431 and 2008 and in cisplatin resistant subclones (A431Pt and C13). Cell lines were treated for 24 h with increasing concentration (10^{-8} to 10^{-4} M) of copper complexes or cisplatin. The percent of live cells was quantified by the MTT assay as described in the Experimental Section. Cell viability has been expressed as a percent of absorbance of untreated cells.

Table 5. IC_{50} Values of Cytotoxicity by Different Sulfured Ccopper Complexes[a]

compound	A431 *mean* IC_{50} (μM) *95% CI*	A431Pt *mean* IC_{50} (μM) *95% CI*	2008 *mean* IC_{50} (μM) *95% CI*	C13 *mean* IC_{50} (μM) *95% CI*
Cisplatin	0.19	3.54	0.78	5.45
	0.06 to 0.54	0.74 to 17.04	0.26 to 0.60	3.35 to 8.85
$[Cu(DMDT)_2]$	0.72	0.32	0.24	0.36
	0.05 to 0.60	0.01 to 12.57	0.12 to 0.49	0.07 to 1.84
$[Cu(MSDT)_2]$	0.29	0.32	0.63	0.23
	0.10 to 0.80	0.10 to 0.98	0.22 to 1.80	0.06 to 0.92
$[Cu(ESDT)_2]$	0.01	0.49	0.26	0.16
	0.0006 to 0.03	0.79 to 3.01	0.09 to 0.76	0.03 to 0.87
$[Cu(TSDT)_2]$	0.59	2.60	1.03	1.56
	0.14 to 2.45	0.45 to 15.27	0.34 to 3.16	0.04 to 5.50
$[Cu(PyDT)_2]$	2.80	2.80	0.99	0.63
	0.77 to 10.41	1.63 to 4.79	0.15 to 6.68	0.09 to 4.17

[a] The data are expressed as μM concentration $\pm$95% confidence Intervals (CI). Each value represents the average of three to four sets of independent experiments.

Essential requirements for the pharmacological evaluation of new metal complexes as cytotoxic agents are an appreciable solubility in water and a sufficient chemical stability under physiologically relevant conditions. Unfortunately, the reported copper(II) complexes are not soluble in water; thus, before any further biological investigation, they had to be previously dissolved in DMSO and then added to PBS solution or to the growth medium containing cells. For this reason, we studied in different media their redox behavior through cyclic voltammetry, and the evolution with time of all the complexes was followed by UV−vis spectroscopy for 24 h. When dissolved in DMSO/PBS, the compounds react slowly, with a partial breakage of the metal−ligand coordination supported by a solvent molecule, suggesting that only a few changes on the coordination sphere after solubilization in PBS occur.

The citotoxic properties of the complexes were then studied on human ovarian and cervix carcinoma cell lines (A431 and 2008) and the cisplatin resistant subclones (A431Pt and C13). All the copper dithiocarbamato derivatives here reported show generally a good activity toward all the cancer cells. Nevertheless, among the compounds, the copper(II)/ESDT complex appeared to be the most stable and relatively most soluble. This finding well correlates with the results of the biological assays; in fact $[Cu(ESDT)_2]$ was proved to be much more cytotoxic in vitro, with $IC_{50} < 0.5$ μM, than cisplatin even toward human tumor cell lines resistant to cisplatin itself with activity levels comparable to those on the corresponding wild-type cancer cells, ruling out the occurrence of cross-resistance phenomena and supporting the initial hypothesis of a different antitumor activity mechanism of action compared with platinum(II) compounds.

In conclusion, the chemical characterization carried out in different media allowed us to establish that the five investigated copper(II) dithiocarbamato derivatives are sufficiently stable within a physiological-like environment, thus representing the essential prerequisite for any further pharmacological evaluation.

The encouraging chemical and biological properties of the copper(II) dithiocarbamato complexes warrant further studies to assess their pharmacological properties in vivo and to elucidate the actual mechanism of their biological activity.

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Supporting Information Available: ^{1}H NMR spectrum of $[Cu(ESDT)_2]$ complex in DMSO-d_6. Cyclic and Osteryoung square wave voltammetric responses; UV−vis spectral data with time of copper(II) dithiocarbamate complexes in DMSO solution; UV−vis spectra with time of $[Cu(TSDT)_2]$ in DMSO solution at 298 K, 1.03×10^{-3}M mother solutions; UV−vis spectral data with time of copper(II) dithiocarbamate complexes in DMSO/PBS solution; UV−vis spectra with time of $[Cu(PyDT)_2]$ in DMSO/PBS solution, 99 μM; FTIR spectra details of the $[Cu(TSDT)_2]$ (**a**) and $[Cu(MSDT)_2]$ (**b**) original complex and the brown precipitate from PBS solution. This material is available free of charge via the Internet at http://pubs.acs.org.

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