Synthesis, Characterization, and Comparative in Vitro Cytotoxicity Studies of Platinum(II), Palladium(II), and Gold(III) Methylsarcosinedithiocarbamate Complexes

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This work reports on the synthesis, characterization, and in vitro cytotoxic activity of some new platinum(II), palladium(II), and gold(III) derivatives of methylsarcosinedithiocarbamate and its *S*-methyl ester, to study their behavior as potential antitumor agents. The biological activity of these compounds, as determined by growth inhibition and apoptosis induction, has been investigated in both human leukemic promyelocites HL60 and human squamous cervical adenocarcinoma HeLa cell lines, and their activity has been compared to the well-known platinum-based anticancer agent cisplatin. On the basis of these experimental results, $[Pd(MSDT)X]_n$ (MSDT = methylsarcosinedithiocarbamate; X = Cl, Br) complexes show a strong dose-dependent growth inhibition of both HL60 and HeLa cells, with IC_{50} values slightly higher than those recorded for cisplatin; moreover, $[Au(MSDT)X_2]$ activity appears significantly higher or, at least, comparable to that of the reference drug. Exposure of both cell lines to $[Pd(MSDT)X]_n$ and $[Au(MSDT)X_2]$ complexes induces apoptosis, as determined by an Apo2.7 assay.

Introduction

In the last 3 decades, the interest toward platinum(II) and palladium(II) complexes containing *N*- and *S*-donor ligands has increased, to obtain metal-based drugs exhibiting high anticancer activity together with reduced toxicity, compared to cisplatin and analogous compounds.[1] Sulfur-containing molecules in particular are currently under study as chemoprotectants in platinum-based chemotherapy; in this regard, thiocarbonyl and thiol derivatives have shown promising properties for chemical use in modulating cisplatin nephrotoxicity.[2,3] Undoubtedly, a number of complexes sufficiently interesting for clinical trials have been produced, but none of these has overcome the parent drug in efficacy.[4]

We have recently synthesized mixed dithiocarbamate/aminoplatinum(II) and -palladium(II) complexes with dithiocarbamate derivatives of α-amino acids and aliphatic and aromatic amines, such as [M(ESDT)(Am)Cl] (M = Pt(II), Pd(II); ESDT = ethylsarcosinedithiocarbamate anion; Am = *n*-propylamine, cyclobutylamine, pyridine) in order to obtain potential anticancer drugs able to conjugate cytotoxic activity with a lack of nephrotoxicity. Indeed, these species contain (i) an amino ligand, that is, a ligand frequently present in bioactive platinum(II) complexes; (ii) a good leaving group (halide) able to promote, in the target cell cytosol, the formation of a coordinatively unsaturated active species potentially able to be "hooked" by DNA and to produce the desired selected structural lesion to the DNA itself, which is considered the key point in the cisplatin mechanism of action;[5] and (iii) a sufur-containing chelating molecule potentially able to protect the metal center from interactions with sulfur-containing enzymes, which are believed to be at the basis of the nephrotoxicity of platinum(II)-based drugs. In fact, the interaction of the metabolites of platinum(II) flowing through the kidney tubules, in which cysteine-containing kidney enzymes are located, is strongly believed to be the key reason for the occurrence of nephrotoxicity.[6] The dithiocarbamate moiety coordinates platinum through two sulfur atoms preventing or, at least, limiting the reaction with other sulfur-containing renal proteins.[7,8] In particular, the complex [Pt(ESDT)(Py)Cl] has been proved to be an antiproliferative agent more effective and extremely less nephrotoxic than cisplatin.[9,10]

Starting from these encouraging preliminary results, and in order to obtain compounds with a superior chemotherapeutic index in terms of increased bioavailability, higher cytotoxicity, and lower side effects than cisplatin, we report here on new platinum(II), palladium(II), and gold(III) derivatives of methylsarcosinedithiocarbamate (MSDT) and its *S*-methyl ester (MSDTM), which have been synthesized, purified, and characterized by means of elemental analyses, conductivity measurements, $^1H/^{13}C$ NMR and FT-IR spectroscopy, mass spectrometry, and thermal analyses. In particular, the choice of gold(III) was determined by the fact that this ion gives rise to complexes isoelectronic and isostructural to those of platinum(II),[11] and its coordination compounds sometimes exhibit interesting in vitro cytotoxic and antitumor properties.[12] Surpris-

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Table 1. Selected IR Frequencies (cm^{-1})

Compound	ν(N−CSS)	ν(C=O)	ν(S−CH_3)	ν(SCS) asym	ν(SCS) sym	ν(SMS) asym	ν(SMS) sym	ν(M-X)
MSHCl	–	1749	–	–		–		–
MSDTM	1488	1737	731	1000/978[a]	465[b]	–		–
[Pt(MSDTM)Br_2]	1564	1728	735	1016/991[a]	575[b]	373	360	257/221[c]
[Pd(MSDTM)Br_2]	1561	1727	737	1012/990[a]	574[b]	384	368	252/223[c]
[Pt(MSDT)Cl]$_n$	1559	1747	–	962	573	385	355	326[d]
[Pd(MSDT)Cl]$_n$	1537	1749	–	960	565	384	352	328[d]
[Pt(MSDT)Br]$_n$	1553	1746	–	966	577	380	353	207[e]
[Pd(MSDT)Br]$_n$	1539	1745	–	963	568	378	352	202[e]
[Au(MSDT)Cl_2]	1560	1741	–	1016	578	409	382	359/339[f]
[Au(MSDT)Br_2]	1564	1742	–	1016	577	409	385	252/228[c]

[a] $\nu_{a,s}$(S=C−S). [b] ν(C−S). [c] $\nu_{a,s}$(Br−M−Br). [d] X = Cl. [e] X = Br. [f] $\nu_{a,s}$(Cl−Au−Cl).

ingly, despite the strict chemical similarity, little literature data exist concerning the use of gold(III) complexes as anticancer agents;[13−17] the paucity of data on these compounds probably derives from their high redox potential and relatively poor stability, which make their use rather problematic under physiological conditions.[18] Thus, opportune ligands should be selected to minimize these effects and make gold(III) complexes suitable for biomedical applications; we thought that methylsarcosinedithiocarbamate might be appropriate for this goal. In fact, the complexes of the type [Au(MSDT)X_2] (X = Cl, Br) reproduce the main features of cisplatin very closely: all of them exhibit an almost square-planar geometry and contain two *cis*-gold(III)−halogen bonds that may undergo easy hydrolysis, the remaining coordination positions being occupied by an anionic bidentate dithiocarbamate ligand that improves the complex stability.

Finally, we have investigated the in vitro cytotoxicity of these new complexes on human adenocarcinoma HeLa cells and human myeloid cell line HL60. Moreover, since one of the main purposes of cancer chemotherapy is to commit tumor cells to apoptosis following exposure to antitumor agents, we have examined the apoptotic activity of these new compounds.[19,20]

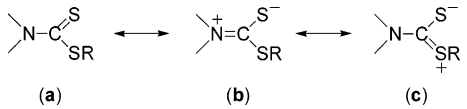
Chemical Characterization

By reaction of the MSDTM ligand with MBr_2 (M = Pt(II), Pd(II)) species in 1:1 molar ratio we have obtained the *S*-methylated complexes of the type [M(MSDTM)-Br_2] in which the dithiocarbamate ligand coordinates the metal center through the sulfur atoms. The pure demethylated [M(MSDT)Br]$_n$ species have been obtained by heating the corresponding precursors [M(MSDTM)Br_2] in the solid phase, thus leading to a mixture of polynuclear species with $n = 3-5$ as recently reported for analogous complexes.[21] However, we have not been able to obtain pure *S*-methylated [M(MSDTM)-Cl_2] species, the reaction product being always a mixture of methylated and demethylated species. Thus, the pure demethylated [M(MSDT)Cl]$_n$ species have been obtained by subsequent heating of the crude solid itself.

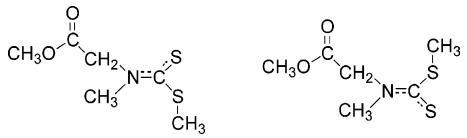
Gold(III) derivatives have been prepared by a template reaction between $KAuX_4$, MSHCl, CS_2, and NaOH in 1:2:2:2 molar ratio, leading to pure 1:1 metal-to-ligand species of the type [Au(MSDT)X_2] (X = Cl, Br).

All the synthesized complexes are nonconducting, as their Λ_M values recorded in nitromethane at (25.0 ± 0.1) °C do not occur in the range 75−95 $\Omega^{1-}\cdot cm^2\cdot mol^{-1}$, commonly attributed to 1:1 electrolytes.[22]

Scheme 1. Resonant Forms of the Dithiocarbamate Ester Moiety.



Scheme 2. Isomeric Forms of MSDTM Ligand.

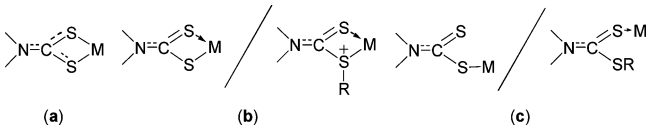


FT-IR Spectroscopy. The interpretation of FT-IR spectra of dithiocarbamate complexes of transition metals has aroused considerable interest both diagnostically to determine the mode of coordination and as a means of assessing the nature of bonding in these complexes. As concerns the dithiocarbamate moiety (Table 1), three main regions of the IR are of interest. First, the 1450−1580 cm^{-1} region, which is primarily associated with the "thioureide" band due to the ν(N−CSS) vibration; second, the 940−1060 cm^{-1} region, which is associated with ν(C−S) vibrations; and, third, the 250−420 cm^{-1} region associated with ν(M−S) vibrations.[23]

Dithiocarbamates are a large family of compounds showing a partial double bond character between the dithiocarbamic carbon and nitrogen atoms, producing an energetic rotational barrier of ~66 $kJ\cdot mol^{-1}$,[24] whose origin may be seen when considering of the three possible canonical resonance forms for the dithiocarbamate molecules (Scheme 1). Thus, the contribution of form b would induce a degree of double bond character in the carbon−nitrogen bond, preventing free rotation around the C−N bond itself and, at the same time, constrain the molecule into a near planar configuration. In the case of MSDTM ligand, the presence of two different substituents at the nitrogen atom leads to the possible existence of two isomeric forms (Scheme 2).

On passing from a free dithiocarbamate ligand to its corresponding complexes, the ν(N−CSS) mode is shifted to higher energies, due to an increase of the carbon−nitrogen double bond character.[1,8] When a dithioester molecule is bonded to a metal center in a monodentate way through the thiocarbonyl sulfur donor atom, the ν(N−CSS) absorption band is shifted toward higher energies of about 20−30 cm^{-1}; a larger shift (80−100 cm^{-1}), as observed in our case, is indicative of the

Scheme 3. Different Ways of Metal−Sulfur Binding in Dithiocarbamate Complexes: Symmetrical Bidentate (a), Asymmetrical Bidentate (b), and Monodentate (c).



presence of chelated k*S*,k*S*′ complexes.[25,26] Regarding MSDT derivatives, this behavior cannot be observed, as we were not able to isolate the free methylsarcosine-dithiocarbamate precursor, but the values of their ν(N−CSS) modes are in agreement with those reported in the literature for analogous metal derivatives of α-amino acid dithiocarbamates.[27−29]

To discern the bonding type of the dithiocarbamate ligands in their complexes, the Bonati−Ugo method[30] is, by far, the most popular one. It consists of tracing the 940−1060 cm^{-1} spectral region, where the ν(C−S) modes are thought to appear. In fact, the bands due to the −CSS moiety are usually coupled to other vibrations and are very sensitive to the environment around this group, but they are also useful to distinguish between mono- and bidentate coordination.[31] The presence of only one band in the investigated region, commonly attributed to the ν_a(SCS) mode, is assumed to indicate a completely symmetrically bonding of the dithiocarbamate ligand, acting in a bidentate mode (Scheme 3a), and this is the case for $[M(MSDT)X]_n$ palladium(II) and platinum(II) complexes (∼963 cm^{-1}) and both the gold(III) MSDT derivatives (1016 cm^{-1}). Conversely, a split band indicates an asymmetrically bonded bidentate ligand ($\Delta\tilde{\nu}$ < 20 cm^{-1}, Scheme 3b) or a monodentate bonded ligand ($\Delta\tilde{\nu}$ > 20 cm^{-1}, Scheme 3c). In both platinum(II) and palladium(II) methylated complexes $[M(MSDTM)X_2]$, the presence of two split bands of 22/25 cm^{-1} in the above-discussed range supports the hypothesis of an asymmetrical bidentate behavior for the dithioester ligand.[1,8]

New bands, absent in the spectra of the starting materials, are observed in the 350−410 cm^{-1} range, and they can be assigned to the metal−sulfur stretching modes according to the normal coordinate analysis of the dithiocarbamate complexes.[32]

In the 220−360 cm^{-1} range, other informative bands are detected, attributed to ν(M−X) modes. These bands are ascribed to the asymmetrical and symmetrical M−X stretching frequencies for terminal halides in MSDTM derivatives and all the gold(III) complexes, whereas the single band at lower energies recorded for $[M(MSDT)X]_n$ compounds is due to bridged halides.[33,34]

NMR Spectroscopy. The main features of the ^{1}H NMR spectra of the ligand and the synthesized complexes have been summarized in Table 2. As previously reported, MSDTM ligand may exist in two isomeric forms, and this is clearly underlined by the fact that two well-defined singlets have been recorded for each proton group. The largest signals (93%) have been detected at 2.53, 3.38, 3.64, and 4.82 ppm for SCH_3, NCH_3, OCH_3, and NCH_2 groups respectively, and have been attributed to the isomeric form *Z*, whereas the smallest remaining ones (7%) to the isomeric form *E*. In fact, it is known that when the NCH_3 group is cis with respect to the thiocarbonyl sulfur atom, its proton signal is recorded at lower fields than that of the corresponding group in the trans position.[35] This behavior may be observed also for the derivatives of the type $[M(MSDTM)Br_2]$, whose ^{1}H NMR spectra show a doubled signal for each proton group. On the contrary, only one species has been observed for MSDT platinum(II) and palladium(II) derivatives.

Table 2. ^{1}H NMR Spectral Data (DMSO-d_6, TMS, 25.0 °C, ppm)

compound	^{1}H chemical shifts, δ			
	SCH_3	NCH_3	OCH_3	NCH_2
MSHCl	−	2.53	3.72	3.93
MSDTM	2.53/2.54[a]	3.38/3.45[a]	3.64/3.69[a]	4.70/4.82[a]
$[Pt(MSDTM)Br_2]$	2.53/2.54[a]	3.38/3.46[a]	3.69/3.71[a]	4.69/4.84[a]
$[Pd(MSDTM)Br_2]$	2.54/2.56[a]	3.39/3.48[a]	3.65/3.70[a]	4.62/4.72[a]
$[Pt(MSDT)Cl]_n$	−	3.27	3.72	4.55
$[Pd(MSDT)Cl]_n$	−	3.31	3.71	4.64
$[Pt(MSDT)Br]_n$	−	3.25	3.72	4.57
$[Pd(MSDT)Br]_n$	−	3.31	3.72	4.65
$[Au(MSDT)Cl_2]$	−	3.48/3.43[a]	3.74	4.76/4.81[a]
$[Au(MSDT)Br_2]$	−	3.39/3.42[a]	3.75	4.76/4.79[a]

[a] Isomers.

A different case is that of gold(III) derivatives; in fact, it is worth noting that two signals are recorded for both *N*-methyl and *N*-methylene protons in 1:1 ratio. The existence of two isomers in solution is also confirmed by the presence of two signals for NCH_3, NCH_2, and CSS carbon atoms in the ^{13}C NMR spectra (Table 3). These complexes do not possess evident stereocenters able to give rise to two different isomers. Nevertheless, a possible explanation of such apparently unaccountable behavior may be because these gold(III) dithiocarbamate derivatives have symmetrical dithiocarbamic fragments in the solid state (Scheme 4a), as previously explained in the FT-IR spectroscopy discussion, whereas in solution dithiocarbamic sulfur−metal bonding becomes asymmetric (Scheme 4b,c), and this occurrence would be promoted by the solvent. Similar behavior has been already reported in the literature for some gold dithiocarbamate derivatives which have asymmetrically bonded dithiocarbamate fragments in the solid state, whereas ^{1}H NMR results indicate symmetric bonding in solution.[36,37] Remarkably, this behavior has also been observed in $(CD_3)_2CO$ solutions, but in this case, the initial relative abundance of the two isomeric species (1:1) changes in time. In fact, the lower field peaks, referred to both isomers, progressively decrease in intensity until their almost complete disappearance within 24 h, confirming the crucial role of the solvent in these isomerization reactions.

The ^{1}H NMR spectra in DMSO-d_6 of all the here reported complexes have been periodically recorded to check their stability, showing that they are stable over 48 h with the exception of the $[M(MSDTM)Br_2]$-type (M = Pt(II), Pd(II)) complexes. In fact, their spectra collected at different times have shown a demethylation in solution within 4 h, confirmed by the disappearance of the SCH_3 signal previously recorded for the methylated species and the appearance of one singlet for both NCH_3 and NCH_2 groups, in agreement with the formation of the corresponding $[M(MSDT)Br]_n$ species. Furthermore, a new signal at 2.36 ppm, well ascribed to the CH_3Br volatile species, is observable.[8]

Thermal Analyses. The thermal behavior of the synthesized compounds has been studied to establish

Table 3. ^{13}C NMR Spectral Data for [Au(MSDT)X_2] (X = Cl, Br) Complexes (DMSO-d_6, TMS, 25.0 °C, ppm)

	^{13}C chemical shifts, δ				
compound	OCH_3	NCH_3	NCH2	COO	CSS
[Au(MSDT)Cl_2]	53.38	39.42/40.20[a]	53.22/53.61[a]	166.10	195.45/200.73[a]
[Au(MSDT)Br_2]	53.86	39.89/40.86[a]	53.12/53.40[a]	166.80	197.68/201.53[a]

[a] Isomers.

Scheme 4. Hypothesized Isomerization in Solution for [Au(MSDT)X_2] (X = Cl, Br) Complexes.

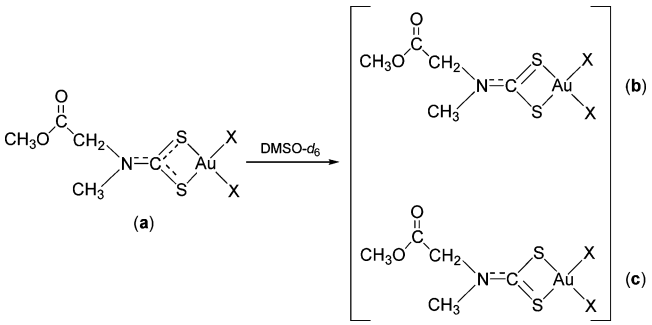


Table 4. Thermogravimetric (TG) and Differential Thermal Analysis (DTA) Data

		% weight loss		DTA peak temp, °C
compound	step	found	calcd	(process[a])
[Pt(MSDTM)Br_2]	− CH_3Br	17.02	17.32	159 (endo)/236 (endo)
	to Pt	63.92	64.41	547 (exo)
[Pd(MSDTM)Br_2]	− CH_3Br	19.31	20.66	157 (endo)/249 (endo)
	to PdO	74.41	73.35	428 (exo)
	to Pd	75.22	76.84	833 (endo)
[Pt(MSDT)Cl]$_n$	to Pt	51.56	52.27	381 (exo)/416 (exo)
[Pd(MSDT)Cl]$_n$	to PdO	62.74	61.76	246 (endo)/411 (exo)
	to Pd	66.80	66.76	817 (endo)
[Pt(MSDT)Br]$_n$	to Pt	57.69	56.96	558 (exo)
[Pd(MSDT)Br]$_n$	to PdO	65.58	66.42	292 (exo)/432 (exo)
	to Pd	69.26	70.81	821 (endo)
[Au(MSDT)Cl_2]	to Au(SCN)	41.27	42.83	187 (endo)
	to Au	56.30	55.84	462 (exo)
[Au(MSDT)Br_2]	to Au(SCN)	53.07	52.33	218 (endo)
	to Au	62.65	63.10	453 (exo)

[a] Endo/exo = endothermic/exothermic process.

the different decomposition processes and to confirm the proposed stoichiometry. The results of such analyses have been summarized in Table 4, indicating a good correlation between calculated and found weight loss values. The [Pd(MSDTM)Br_2] degradation occurs in three successive steps, the first deriving from evolution of a CH_3Br molecule to yield [Pd(MSDT)Br]$_n$ species. The shape of the thermogram above 400 °C depends on the formation of PdO in air flux, followed by the successive oxygen release to yield Pd(0) above 800 °C.

In Figure 1, the thermogram of [Pt(MSDTM)Br_2] complex is reported as an example. The first decomposition step derives, again, from the evolution of CH_3Br yielding [Pt(MSDT)Br]$_n$ species, whose complete decomposition to Pt(0) occurs at 547 °C.

The thermogravimetric curves of [M(MSDT)X]$_n$ (M = Pt, Pd; X = Cl, Br) complexes do not show any particular features, just complete degradation leading to the corresponding metal residue.[1,8]

Concerning gold(III) derivatives, the thermal degradation occurs in two successive, well-defined steps. The first TG step corresponds to pyrolysis, decarboxylation, and reductive elimination [Au(III) → Au(I)], thus leading to [Au(SCN)] as residue, a commonly discovered intermediate in the thermal decomposition of metal dithiocarbamates.[38,39] A very intense effect is recorded at higher temperature that corresponds to removal of the remaining ligand atoms and complete degradation, leading to metallic gold.[27−29] The formation of metallic gold as the residue is confirmed by the presence of an endothermic DTA peak at 1066 °C due to the metallic gold melting.

Biological Characterization

Inhibition of Cell Growth. Traditional antineoplastic therapy is based on the use of chemotherapeutic compounds that exert a cytotoxic effect on cell proliferation and promote the destruction of sensitive tumors.[19] The in vitro cytotoxic properties of the here reported platinum(II), palladium(II), and gold(III) dithiocarbamate derivatives have been evaluated, as a preliminary screening of their antiproliferative activity, on human leukemic promyelocites (HL60) and human squamous cervical adenocarcinoma (HeLa) cells. In vitro cytotoxicity has been evaluated after 72 h contact between the tumor cells and all the synthesized complexes at increasing concentrations (0.25−15 μM) by means of the trypan blue dye exclusion test. Trypan blue is one of the several stains recommended for use in the dye exclusion procedure for viable cell counting; this method is based on the principle that live cells do not take up the dye, whereas dead cells do.[40] In fact, trypan blue is a polar dye that cannot cross intact cell membranes but crosses the membranes of necrotic cells and apoptotic cells that are undergoing secondary necrosis. Results are expressed in terms of IC_{50} values, that is, the concentration of drug required to inhibit cell growth by 50% compared to control. For comparison purposes, the cytotoxic activity of cisplatin has been also evaluated under the same experimental conditions. As shown in Table 5, exposure of both HL60 and HeLa cells to increasing concentrations of [Pd(MSDT)X]$_n$ and [Au(MSDT)X_2] (X = Cl, Br) complexes results in a strong dose-dependent growth inhibition. Conversely, the *S*-methylated compound [Pd(MSDTM)Br_2] and all the platinum(II) dithiocarbamate derivatives do not significantly impair the proliferation of both tested cell lines, even at concentrations greater than 15 μM. To determine whether the cytotoxic effect of the most active complexes could be ascribed to the ligand itself, MSDTM has been also evaluated for its antiproliferative activity under the same experimental conditions, and this possibility was ruled out, as no cytotoxicity was observed at the assayed doses (data not shown). All in all, our results demonstrate that platinum(II) and *S*-methylated palladium(II) derivatives are essentially inactive, provoking only a slight cell growth inhibition. On the contrary, both [Pd(MSDT)Cl]$_n$ and [Pd(MSDT)Br]$_n$ complexes demonstrate a strong dose-dependent growth inhibition on both HL60 and HeLa cells, with IC_{50} values slightly higher than those of cisplatin. Moreover, the gold(III) derivatives [Au(MSDT)X_2] (X = Cl, Br) have

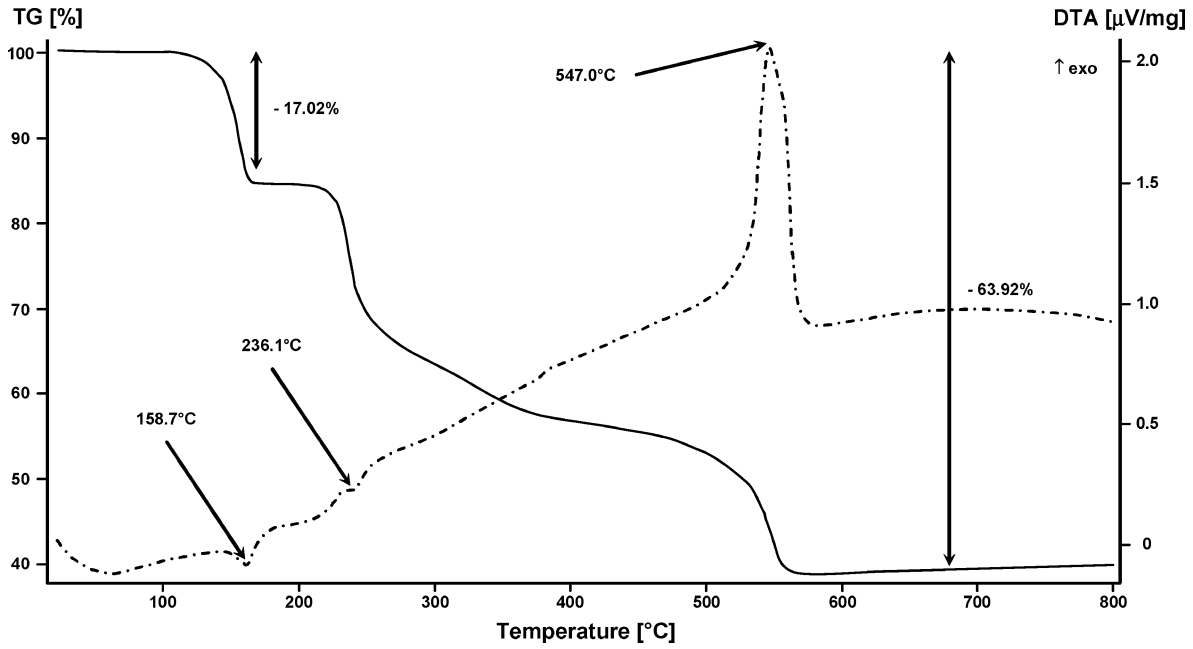


Figure 1. Thermogram of [Pt(MSDTM)Br_2] (—, TG; ---, DTA).

Table 5. Evaluation of in Vitro Growth Inhibitory Effects of the Investigated Platinum(II), Palladium(II), and Gold(III) Dithiocarbamate Derivatives toward HL60 and HeLa Human Tumor Cell Lines by the Trypan Blue Dye Exclusion Test after 72 h

compound	IC_{50},[a] μM	
	HL60	HeLa
cisplatin	1.7	1.2
[Pt(MSDTM)Br_2]	>15	>15
[Pd(MSDTM)Br_2]	>15	>15
[Pt(MSDT)Cl]$_n$	>15	>15
[Pd(MSDT)Cl]$_n$	3.8	6.0
[Pt(MSDT)Br]$_n$	>15	>15
[Pd(MSDT)Br]$_n$	3.7	5.0
[Au(MSDT)Cl_2]	0.9	1.7
[Au(MSDT)Br_2]	0.8	1.8

[a] IC_{50} is defined as the concentration of drug required to inhibit cell growth by 50% compared to the control. Cisplatin was used as reference.

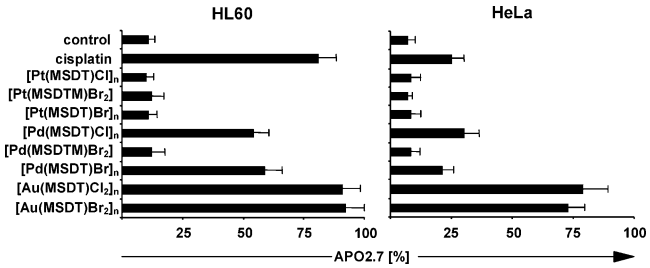


Figure 2. Effect of the platinum(II), palladium(II), and gold(III) dithiocarbamate derivatives on apoptosis of HL60 and HeLa human tumor cell lines after 72 h. The results are expressed in terms of APO2.7-positive cell expression percentage. Bars represent the corresponding standard deviations. Cisplatin was used as reference.

shown an antiproliferative activity higher or, at least, comparable to that of the reference drug. Interestingly, the myeloid HL60 cell line has shown to be more sensitive than the adenocarcinoma HeLa cells to the growth inhibition induced by our new complexes, but more resistant to cisplatin effects.

Apoptosis Studies. On the basis of the above-reported growth inhibition data, we have tried to determine the possible capability of our new complexes in inducing cell apoptosis, which represents the predominant mechanism by which tumor cells die in response to an immune attack or to cytotoxic drugs.[20] Due to our interest in comparing the activity of each synthesized complex, we have performed apoptosis experiments simultaneously using for such an assay a single dose (10 μM) of each compound. As shown in Figure 2, exposure of both HL60 and HeLa cell lines to *S*-methylated compound [Pd(MSDTM)Br_2] and all the platinum(II) dithiocarbamate derivatives does not significantly increase the APO2.7 expression. Conversely, exposure of HL60 cells to [Pd(MSDT)X]$_n$ and [Au(MSDT)X_2] (X = Cl, Br) complexes induces apoptosis, the effect being more marked for gold(III) dithiocarbamate derivatives with a percentage of APO2.7-positive cell expression even higher than that of cisplatin.

Concerning HeLa cells, only gold(III) complexes provoke a strong apoptosis effect, the percentage of APO2.7-positive cell expression increasing from 24% for the reference drug to 79% and 83% for bromo and chloro gold(III) derivatives, respectively.

Thus, our results demonstrate that apoptosis may be the mechanism involved in the cell growth inhibition observed for the most active among the tested compounds and, primarily, for gold(III) complexes. Moreover, a different mechanism of action for palladium(II) complexes may be suggested, compared to platinum(II) analogues.

Conclusions

Although none of the here reported complexes have been obtained in the crystalline state and, thus, their structure cannot be securely proposed, the spectroscopic results suggest that coordination takes place in a near square-planar geometry through the sulfur atoms, the −NCSS moiety coordinating the metal ion in a bidentate asymmetrical/symmetrical mode for *S*-methylated ([M(MSDTM)Br_2], M = Pt(II), Pd(II)) and demethylated [M(MSDT)X]$_n$, M = Pt(II), Pd(II); X = Cl, Br; n = 3−5)

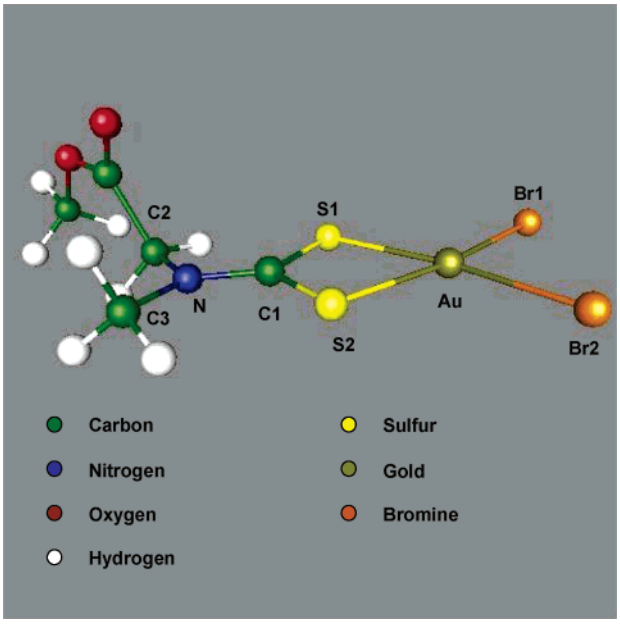


Figure 3. Optimized structure of [Au(MSDT)Br_2] obtained from GGA calculations.

Table 6. Structural Parameters of [Au(MSDT)Br_2] Obtained from GGA Calculations

bond length, Å		bond angle, deg	
Au−S1	2.40	S1−Au−S2	73.86
Au−S2	2.39	S1−C1−S2	112.10
C1−S1	1.74		
C1−S2	1.73		
C1−N	1.34		
C2−N	1.45		
C3−N	1.47		

complexes, respectively. Anyway, for demethylated dithiocarbamate derivatives, a correct structure cannot be assigned; they are probably constituted by a mixture of trimeric, tetrameric, and pentameric structures, as suggested by ESI-MS results and in agreement with previously reported results.[21]

Concerning MSDT gold(III) derivatives, even in this case a near square-planar geometry through the sulfur atoms is proposed, the −NCSS moiety coordinating the metal center in a bidentate symmetrical mode. To achieve further confirmations of the above exposed hypotheses, the molecular structure of [Au(MSDT)Br_2] complex has been investigated by means of density-functional calculations, choosing as a structural starting point the X-ray study of a palladium(II) analogue compound.[1] The obtained optimized structure (Figure 3 and Table 6) shows the characteristic square-planar coordination of the gold(III) metal center and also reveals that C3, C2, N, C1, S1, S2, Au, Br1, and Br2 atoms predominantly lie on the same coordination plane.

Regarding the biological activity of the here reported complexes, [Pd(MSDT)X]$_n$ (X = Cl, Br) exhibit a strong dose-dependent growth inhibition of both HL60 and HeLa cells, with IC_{50} values slightly higher than those recorded for cisplatin; moreover, [Au(MSDT)X_2] (X = Cl, Br) activity appears significantly higher or, at least, comparable to that of the reference drug. Exposure of both cell lines to [Pd(MSDT)X]$_n$ and [Au(MSDT)X_2] (X = Cl, Br) complexes induces apoptosis, this effect being more evident in the HL60 cell line and, in particular, for gold(III) derivatives.

Experimental Section

Chemicals. Methylsarcosine hydrochloride (Fluka), carbon disulfide (Aldrich), methyliodide (Carlo Erba), platinum(II) and palladium(II) chloride and bromide (Johnson Matthey), potassium tetrachloro- and tetrabromoaurate(III) (Alfa Aesar) were used as supplied. All the other reagents and solvents were of high purity and were used as purchased without any further purification.

HL60 (human leukemic promyelocytes) and HeLa (human squamous cervical adenocarcinoma) cell lines were obtained by the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cells were maintained in Iscove's modified Dulbecco's (IMD, Biochrom KG) medium supplemented with 10% heat-inactivated fetal calf serum (FCS, Biochrom KG), 0.2 mg·mL^{-1} penicillin and streptomycin, and 0.1% (w/v) L-glutamine (Biochrom KG) at 37 °C in a 5% CO_2 fully humidified atmosphere.

Instrumentation. Conductivity measurements were carried out with an Amel 134-type conductivity bridge for freshly prepared 10^{-3} M solutions in nitromethane at (25.0 ± 0.1) °C.

FT-IR spectra were recorded in Nujol between two polyethylene tablets on a Nicolet Vacuum Far FT-IR 20F spectrophotometer for the range 600−50 cm^{-1} and in solid KBr on a Nicolet FT-IR 55XC spectrophotometer for the range 4000−400 cm^{-1}.

NMR spectra were recorded in the appropriate deuterated solvent on a Bruker Avance DRX400 spectrophotometer equipped with a Silicon Graphics workstation operating in Fourier transform mode, using tetramethylsilane (TMS) as internal standard for $^1H/^{13}C$ measurements.

Elemental analyses were performed with a Carlo Erba 1108 CHNS-O microanalyzer.

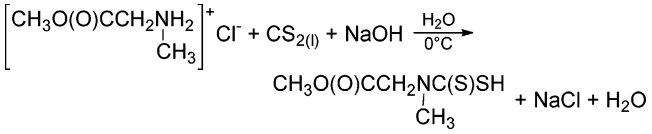
ESI-MS (electrospray mass spectra) were collected on a Finnigan LCQ ion trap instrument operating in positive ion mode (sheath gas flow N_2, 50 au; source voltage, 4 kV; capillary voltage, 3 V; capillary temperature, 200 °C); sample solutions (10^{-5} M in CH_3CN and $CHCl_3$) were directly injected by a syringe pump with a flow rate of 8 $\mu L \cdot min^{-1}$.

Thermogravimetric (TG) and thermodifferential (DT) curves were obtained using a Netzsh STA429 thermoanalyzer. The measurements were carried out in the range 35−1200 °C in alumina crucibles under air (flux rate, 30 $cm^3 \cdot min^{-1}$), using alumina as reference.

Density-functional calculations have been run by using the ADF 2002.2 code. Calculations were done within the generalized gradient approximation (GGA) included through Becke−Perdew formulas.[41,42] A triple-ξ Slater-type basis set was used for all the atoms in the molecule, and also the relativistic effect has been taken into account by using the ZORA Hamiltonian, which included the scalar relativistic coupling. The inner cores of gold (1s−4d), sulfur (1s−2p), oxygen (1s), carbon (1s), and bromine (1s−3d) were treated by the frozen-core approximation.

Synthesis of MSDTM Ligand. The MSDM ligand was prepared according to a modified literature method[43] by dropwise addition of CS_2 (7.2×10^{-3} mol) to an ethanol solution (4 mL) of methylsarcosine hydrochloride (1.4×10^{-2}) under continuous stirring. Once CS_2 was dissolved, an aqueous solution (0.5 mL) of NaOH (1.4×10^{-2} mol) was added, the solution turning from clear yellow into dark brown with the precipitation of a white solid (NaCl) that was filtered off. After 1 h of stirring, the addition of CH_3I (1.4×10^{-2} mol) yielded to a red oil that was resolubilized in ethanol and still left stirring for another hour; the solution thus obtained was joined with water to give incipient precipitation and, after one night at 4 °C, a white solid was obtained. A further fraction of MSDTM was obtained by treating the mother solution with CS_2 (3.6×10^{-3} mol), NaOH (7.0×10^{-3} mol), and CH_3I (7.0×10^{-3} mol) following the above exposed procedure. The obtained white solid was then recrystallized from ethanol/water, the final yield being 84%.

Scheme 5. Reaction Leading to the Synthesis of Methylsarcosinedithiocarbamic Acid.



N-Methyl-*N*-[(methylthio)thioxomethyl]glycine methyl ester: Anal. ($C_6H_{11}NO_2S_2$) C, H, N; S: calcd 33.18; found, 33.34.

Synthesis of [M(MSDTM)Br$_2$] (M = Pt(II), Pd(II)). In a typical preparation, a chloroform solution (2 mL) of MSDTM (7.7×10^{-4} mol) was dropwise added to a suspension of MBr_2 (M = Pt(II), Pd(II); 7.76×10^{-4} mol) in chloroform (2 mL) under continuous stirring. The solution was then cooled to 0 °C and kept in the dark for 24 h, leading to the precipitation of a yellow/orange solid (for platinum(II) and palladium(II) derivatives, respectively) which was filtered off, washed with *n*-pentane, and dried under reduced pressure (final yield, 72−75%).

Dibromo[methyl *N*-methyl-*N*-[(methylthio-k*S*)(thioxo-k*S*)-methyl]glycinate platinum(II): Anal. ($C_6H_{11}Br_2NO_2PtS_2$) C, H, N; S: calcd 11.68; found, 11.60.

Dibromo[methyl *N*-methyl-*N*-[(methylthio-k*S*)(thioxo-k*S*)-methyl]glycinate palladium(II): Anal. ($C_6H_{11}Br_2NO_2PdS_2$) C, H, N, S.

Synthesis of [M(MSDT)Br]$_n$ (M = Pt(II), Pd(II)). The species [Pt(MSDT)Br]$_n$ and [Pd(MSDT)Br]$_n$ were obtained by a quantitative evolution of CH_3Br due to successive heating of the solid [Pt(MSDTM)Br_2] in oil bath at 120 °C for 2 h and the solid [Pd(MSDT)Br_2] in an oil bath at 75 °C for 1 h, respectively yielding, in both cases, a dark orange powder.

Pure [M(MSDT)Br]$_n$ species were also obtained from the mother solution of the corresponding [M(MSDTM)Br_2] reaction on standing in time.

Bromo[methyl *N*-(dithiocarboxy-k*S*,k*S*′)-*N*-methylglycinato]-platinum(II): Anal. ($C_5H_8BrNO_2PtS_2$) C, H, N; S: calcd 14.15; found, 13.95.

Bromo[methyl *N*-(dithiocarboxy-k*S*,k*S*′)-*N*-methylglycinato]-palladium(II): Anal. ($C_5H_8BrNO_2PdS_2$) C, H, N, S.

Synthesis of [M(MSDT)Cl]$_n$ (M = Pt(II), Pd(II)). A benzene (or chloroform) solution (4 mL) of MSDTM) (6.06×10^{-4} mol) was dropwise added to a suspension of MCl_2 (M = Pt(II), Pd(II); 6.06×10^{-4} mol) in benzene (or chloroform) (2 mL) under continuous stirring. The solution was then cooled at 0 °C and kept in the dark for 24 h, leading to the precipitation of a yellow/light orange solid (for platinum(II) and palladium(II) derivatives, respectively) which was filtered off, washed with *n*-pentane, and dried under reduced pressure. The obtained crude solid was a mixture of [M(MSDTM)Cl_2] and the corresponding demethylated species [M(MSDT)Cl]$_n$. The species [Pt(MSDT)Cl]$_n$ and [Pd(MSDT)Cl]$_n$ were obtained by quantitative evolution of CH_3Cl due to successive heating of the crude solid mixture in an oil bath at 90 °C for 1 h and at 110 °C for 50 min, respectively yielding, in both cases, a dark orange powder (final yield, 82−84%).

Chloro[methyl *N*-(dithiocarboxy-k*S*,k*S*′)-*N*-methylglycinato]-platinum(II): Anal. ($C_5H_8ClNO_2PtS_2$) C, H, N, S.

Chloro[methyl *N*-(dithiocarboxy-k*S*,k*S*′)-*N*-methylglycinato]-palladium(II): Anal. ($C_5H_8ClNO_2PdS_2$) C, H, N; S: calcd 20.03; found, 20.22.

Synthesis of [Au(MSDT)X$_2$](X = Cl, Br). A water solution (3 mL) of methylsarcosine hydrochloride (1.58×10^{-3} mol) cooled at 0 °C was dropwise treated under continuous stirring with cool CS_2 (1.61×10^{-3} mol) and an aqueous solution (1 mL) of NaOH (1.59×10^{-3} mol). After 1 h, the pH value turned from 10 to 6 according to the reaction shown in Scheme 5. The solution thus obtained was slowly added under stirring to an aqueous cool (0 °C) solution (2 mL) of $KAuX_4$ (X = Cl, Br; 0.79 $\times 10^{-3}$ mol), leading to the immediate precipitation of a yellow-ochre/reddish-brown solid (for chloro and bromo derivatives respectively) that was filtered off, washed with water, and dried in a desiccator with P_4O_{10}, the final yield being 82−85%.

Dichloro[methyl *N*-(dithiocarboxy -k*S*,k*S*′)-*N*-methylglycinato]gold(III): Anal. ($C_5H_8AuCl_2NO_2S_2$) C, H, N; S: calcd 14.38; found, 14.25.

Dibromo[methyl *N*-(dithiocarboxy-k*S*,k*S*′)-*N*-methylglycinato]-gold(III): Anal. ($C_5H_8AuBr_2NO_2S_2$) C, H, N, S.

Cytotoxicity Assay (Trypan Blue Dye Exclusion Test). All the tested compounds were dissolved in dimethyl sulfoxide, aliquoted, and stored at −80 °C. The synthesized complexes and cisplatin (Pharmacia & Upjohn), previously dissolved in DMSO, were subsequently dissolved in IMDM and filter sterilized (0.2 μM) immediately before use. The final DMSO concentration (0.1%) had no effect on cell killing.

Cells (5.0×10^4 HeLa cells·mL^{-1} or 2.0×10^5 HL60 cells·mL^{-1}) were incubated in IMD growth medium in the presence of different concentrations (2.5−15 μM) of the compounds to be tested. After 72 h, cells were then incubated for 2 min with 0.25% trypan blue and 5% fetal calf serum. Viable cells were identified by their ability to exclude dye: dead cells take up the trypan blue, whereas live cells have yellow nuclei. Cell counting was performed with a phase contrast microscope.

Measurement of in Vitro Apoptosis. Cells (5.0×10^4 HeLa cells·mL^{-1} or 2.0×10^5 HL60 cells·mL^{-1}) were incubated in IMD growth medium in the presence of 10 μM of the compounds to be tested, previously dissolved in DMSO. After 72 h, apoptosis was measured by staining cells with PE-conjugated APO2.7 mAb[44] (Coulter-Immunotech), according to the manufacturer's instructions. Briefly, cells were fixed with 1.0% *p*-formaldehyde and permeabilized for 20 min on ice with digitonin (100 μg·ml^{-1}; Sigma-Aldrich), washed once in cold phosphate-buffered saline (PBS) containing 2.5% FCS and 0.01% NaN_3, and incubated for 15 min at room temperature in the dark with 10 μL of PE-conjugated APO2.7 mAb. Cells were then washed twice in PBS and analyzed by flow cytometry. Nonspecific isotype-matched immunoglobulins (Becton-Dickinson) were used as control. Viable antibody-labeled cells were identified according to their forward and right-angle scattering, electronically gated, and analyzed on a FACScan flow cytometer (Becton-Dickinson), by means of the CellQuest software (Becton-Dickinson).

Abbreviations: MSHCl = methylsarcosine hydrochloride/*N*-methylglycine methyl ester hydrochloride ($CH_3O(O)CCH_2N(CH_3)H{\cdot}HCl$); MSDT$^-$ = methylsarcosinedithiocarbamate anion/methyl *N*-(dithiocarboxy)-*N*-methylglycinato anion ($CH_3O(O)CCH_2N(CH_3)CSS^-$); MSDTM = methylsarcosinedithiocarbamate *S*-methyl ester/*N*-methyl-*N*-[(methylthio)thioxomethyl]glycine methyl ester ($CH_3O(O)CCH_2N(CH_3)C(S)SCH_3$); DMSO = dimethyl sulfoxide ($(CH_3)_2SO$); cisplatin = *cis*-diamminedichloroplatinum(II) (*cis*-[$(NH_3)_2PtCl_2$])

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Supporting Information Available: Elemental analyses of the ligand and the synthesized complexes and ESI mass spectrum of [Pt(MSDT)Br]$_n$ before complete demethylation. This material is available free of charge via the Internet at http://pubs.acs.org.

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