Antiproliferative and apoptotic effects of two new gold(III) methylsarcosinedithiocarbamate derivatives on human acute myeloid leukemia cells *in vitro*

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[Au(MSDT)Cl_2] (dichloro[methyl *N*-(dithiocarboxy-kS,kS′)-*N*-methylglicinato]gold(III)) and [Au(MSDT)Br_2] (dibromo[methyl *N*-(dithiocarboxy-kS,kS′)-*N*-methylglicinato]gold(III)) gold(III) dithiocarbamate derivatives are two newly synthesized gold(III) derivatives of methylsarcosinedithiocarbamate, containing a sulfur chelating ligand that is able to bind the metal center strongly, so preventing interactions with sulfur-containing enzymes; in fact these reactions are believed to be responsible for the nephrotoxicity induced by the platinum(II)-based drugs. Their activity has been compared with the well-known platinum-based anticancer agent cisplatin on a panel of acute myelogenous leukemia cell lines representing different French–American–British subtypes and in the Philadelphia-positive cell line K562. Both compounds suppressed, in a dose-dependent manner, colony formation in methylcellulose with ID_{50} values of about 10-fold lower than that of the reference drug. After a short exposure (18 h), our compounds, but not cisplatin, were able to: downregulate the antiapoptotic molecule Bcl-2, upregulate the proapoptotic molecule Bax and induce apoptosis, as determined by a strong induction of APO2.7 and phosphatidylserine exposure. Finally, after a 72-h exposure, both gold(III) dithiocarbamate derivatives determined modest cell cycle modifications, but induced DNA fragmentation in all myeloid cell lines tested. Altogether, our results indicate that these new gold(III) dithiocarbamate derivatives might represent novel potentially active drugs for the management of myeloid leukemia, able to combine cytostatic and apoptotic activity with reduced nephrotoxicity. *Anti-Cancer Drugs* 18:323–332 © 2007 Lippincott Williams & Wilkins.

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Introduction

Acute myelogenous leukemia (AML) is usually treated with chemotherapeutic regimens that may include cytosine–arabinoside and anthracycline analogs. Although these standard treatments induce remissions in most patients, there is still the likelihood of relapse and the development of resistant disease [1]. Therefore, the discovery of more effective treatments for AML, particularly the ones that exploit apoptosis pathways, is essential, because apoptosis resistance is a mechanism that can contribute to leukemogenesis and drug resistance [1,2].

Recent advances in medicinal inorganic chemistry demonstrate significant prospects for the utilization of metal complexes as drugs, presenting a flourishing arena for bioinorganic chemistry [3]. Significant progress in platinum-based anticancer agents has been achieved, dependent in part on the understanding of the DNA-binding mechanisms and pharmacological effects of cisplatin. Therefore, much attention has been focused on designing new platinum compounds with improved pharmacological properties, a broad range of antitumor activities and lower toxic side effects (nephrotoxicity, myelosuppression and neurotoxicity) [4]. In addition to cisplatin, three other compounds have been approved for clinical use (carboplatin, nedaplatin and oxaliplatin), and a number of interesting platinum compounds including JM216, ZD0473, BBR3464 and lipoplatin are under clinical evaluation [4,5]. Ruthenium complexes with antitumor activity are also emerging rapidly [6,7] and, besides their established use to treat arthritis, gold complexes [8–13].

Recently, with the aim of modulating activity and toxicity of potential drugs, some platinum(II) [Pt(II)] and

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palladium(II) dithiocarbamate derivatives have been synthesized [14,15], in which both sulfur and nitrogen donor atoms are present inside the coordination sphere. In-vitro studies and in-vivo nephrotoxicity tests have confirmed that these compounds are highly cytotoxic towards the HL-60 and HeLa cell lines and show very low renal toxicity as compared with cisplatin itself [16]. The choice of dithiocarbamate ligands is not accidental; in fact, dithiocarbamates were shown to protect against cisplatin-induced nephrotoxicity in several animal models [15,17]. Afterwards, in order to obtain compounds with superior chemotherapeutic index in terms of increased bioavailability, higher cytotoxicity and lower nephrotoxicity than cisplatin, we have also synthesized new Pt(II), palladium(II) and gold(III) derivatives. These new compounds are sufficiently stable under physiological conditions and represent promising candidates for pharmacological testing as antitumor agents as they are highly cytotoxic towards cultured human tumor cell lines [10–11].

We analyzed the in-vitro cytotoxicity of two new gold(III) methylsarcosinedithiocarbamate derivatives on a panel of AML cell lines and we compared these drugs with cisplatin. Moreover, as one of the main purposes of cancer chemotherapy is to commit tumor cells to apoptosis [18], we have examined the apoptotic activity of these new compounds.

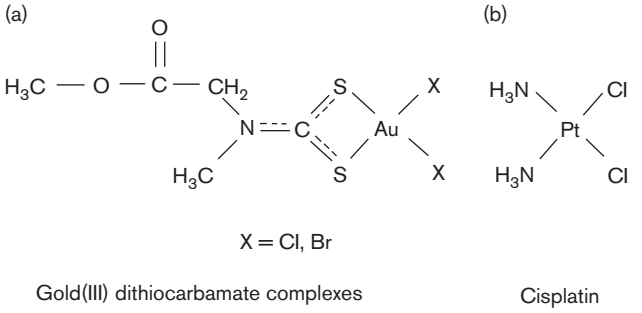
In the present study, we show that the new gold(III) dithiocarbamate derivatives exert a potent antiproliferative and apoptotic effect on a panel of AML cell lines representing different French–American–British (FAB) subtypes and in the Philadelphia positive (Ph+) cell line (K562) carrying the BCR–ABL fusion gene product. Our results suggest that these new compounds may represent potentially active new agents for AML treatment.

Materials and methods

Drugs

[Au(MSDT)Cl_2] (dichloro[methyl *N*-(dithiocarboxy-kS, kS′)-*N*-methylglicinato]gold(III)) and [Au(MSDT)Br_2] (dibromo[methyl *N*-(dithiocarboxy-kS,kS′)-*N*-methylglicinato]gold(III)) (Fig. 1a) were prepared as previously reported [11]. Compounds were dissolved in dimethyl sulfoxide (Sigma Aldrich, Milan, Italy), aliquoted and stored at −80°C. Compounds, cisplatin (Pharmacia & Upjohn, Milano, Italy), citarabine (Ara-C) (Mayne Pharma, Napoli, Italy) and Doxorubicin (Pharmacia Italia, Milano, Italy) were dissolved in Iscove's-modified Dulbecco's medium (IMDM; Biochrome, Berlin, Germany) and filter sterilized (0.2 µmol/l) immediately before use. The final dimethyl sulfoxide concentration had no effect on cell killing or apoptosis.

Fig. 1



Chemical structures of gold(III) dithiocarbamate derivatives (a) and cisplatin (b).

Cell lines and culture conditions

K562 [human chronic myeloid leukemia (CML) in blast crisis, Ph+, carrying the BCR–ABL fusion gene product], HEL (myeloblastic-erythroblastic), KG-1a (early myeloblasts, $CD34^+$), KG-1 (early myeloblasts, CD34), HL-60 (intermediate myeloid-promyelocytes), U-937 (originally classified as histiocytic lymphoma cells, and described to express markers and properties of monocytes), ML-3 (myelo-monoblasts), THP-1 (monoblasts) and NB-4 (leukemia promyelocytes), harboring the t(15,17) translocation, were maintained in IMDM (Biochrome) supplemented with 10% heat-inactivated fetal calf serum (FCS; Biochrome), 0.2 mg/ml penicillin/streptomycin and 0.1% (w/v) L-glutamine (Biochrome) at 37°C in a 5% CO_2 fully humidified atmosphere. Sources and phenotypic characterization of all the above cell lines have been reported in detail elsewhere [19].

Colony assay

Clonogenic growth of AML cell lines was assayed as previously described [20]. Briefly, 5.0×10^3/ml cells, were suspended in 1 ml of IMDM medium containing 0.8% methylcellulose and 15% FCS, in the presence of increasing drug concentrations in 96-well flat-bottomed micro-plates. After 14 days of incubation, plates were observed under phase-contrast microscopy and aggregates with ≥ 40 cells were scored as colonies. ID_{50} represent the concentration of the drug required to cause 50% clonogenic growth inhibition of treated cells compared with control cells.

Measurement of cell cycle and apoptosis

AML cell lines in the exponential growth phase were incubated at 2×10^5 cells/ml on six-well flat-bottomed micro-plates in IMDM supplemented with 10% FCS in the presence of the drugs (5 µmol/l). After 72 h, cells were harvested, and DNA fragmentation and cell cycle were determined by propidium iodide (PI) staining. Briefly, cells were washed twice with phosphate-buffered saline (PBS), resuspended in a solution containing

50 µg/ml PI, 0.1% sodium citrate, 0.1% Nonidet P-40 and 6.2 µg/ml RNase for 30 min at room temperature. Samples were then analyzed by flow cytometery by gating out cell debris and fixation artifacts, and scoring the number of apoptotic cells as the percentage of events falling in an area immediately preceding the G_0–G_1 peak of DNA content histograms. The percentage of cells in each phase of the cell cycle was obtained using Mod-FIT LT 3.0 program (Becton-Dickinson, Immunocytometry System, San Jose, California, USA). APO2.7 expression and Annexin-V binding were detected by flow cytometry, as described previously [21]. Briefly, cells were fixed with 1% paraformaldehyde at 4°C, permeabilized for 20 min on ice with digitonin (100 µg/ml; Sigma), washed once in cold PBS containing 2.5% FCS and 0.01% NaN_3 (PBSA), and incubated with 10 µl of phycoerythrin (PE)-conjugated antiAPO2.7 monoclonal antibodies (Coulter-Immunotech, Fullerton, California, USA) for 15 min at room temperature in the dark. Cells were then washed twice in PBSA and analyzed. For Annexin-V binding, cells were resuspended in 100 µl of binding buffer (10 mmol/l *N-2*-hydroxyl piperazine-*N′-2*-ethane sulfonic acid/NaOH pH 7.4, 140 mmol/l NaCl, 2.5 mmol/l $CaCl_2$), incubated with 10 µl of Annexin-V–fluorescein isothiocyanate (Pharmingen, Immunocytometry System, San Jose, California, USA) and 10 µl of PI (10 µg/ml in binding buffer) in the dark for 15 min, and assayed after the addition of 300 µl binding buffer to each sample. For Bcl-2 and Bax analysis, cells were fixed with 2% paraformaldehyde in PBS for 15 min at 4°C, then permeabilized with 1% Tween 20 for 30 min at 4°C and finally incubated with fluorescein isothiocyanate-conjugated mouse antihuman Bcl-2 (clone 124) (DAKO Citomation, Glostrup, Denmark) or with 1 µg/ml mouse antiBax generated from Bax-α (BD-Pharmingen), followed by PE-conjugated goat antimouse IgG (Becton-Dickinson). Irrelevant isotype-matched antibodies (Jackson's Immunoresearch Laboratories, WestGrove, Pennsylvania) were used to determine the background fluorescence. Viable, antibody-labeled cells were identified according to their forward and right-angle scattering, electronically gated and analyzed on a FACSCalibur flow cytometer (Becton Dickinson), by means of the CellQuest software (Becton Dickinson).

Statistical analysis

Mean data values are presented with their standard deviation (mean ± SD). Statistical comparisons were performed using the two-sided Student's *t*-test. Differences were considered significant at $P < 0.05$.

Results

Effects of gold(III) dithiocarbamate derivatives on clonogenic growth of acute myelogenous leukemia cell lines

Traditional antineoplastic therapy is based on the use of chemotherapeutic compounds, which exert a cytotoxic effect on proliferating cells and promote the destruction of sensitive tumors. As a preliminary screening of their antiproliferative activity, the in-vitro cytotoxic effect of gold(III) dithiocarbamate derivatives has been evaluated on a panel of AML cell lines representing different FAB subtypes. For comparison, the cytotoxic activity of cisplatin has been evaluated under the same experimental conditions.

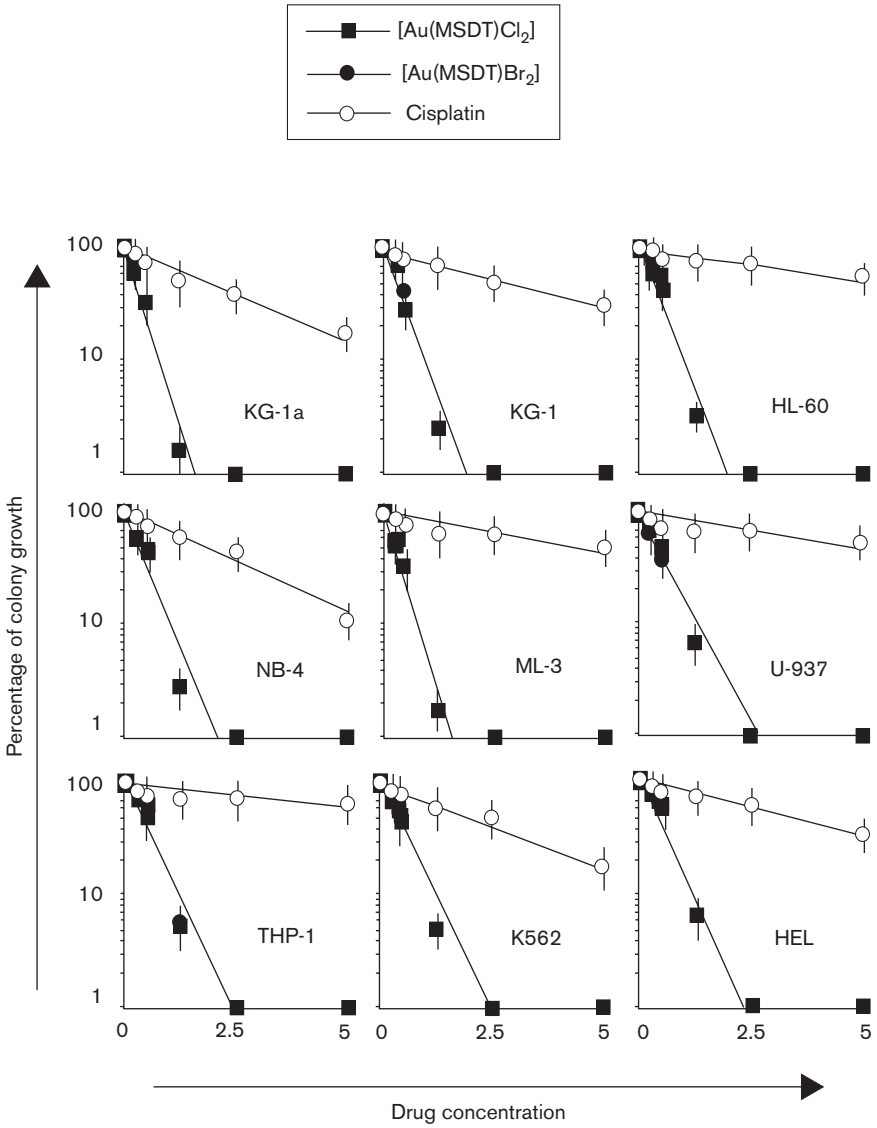
Exposure of AML cells to increasing concentrations of both [Au(MSDT)Cl_2] and [Au(MSDT)Br_2] resulted in a strong dose-dependent clonogenic growth inhibition (Fig. 2). ID_{50} values, obtained from dose–response curves, showed that both gold(III) complexes were significantly more active than cisplatin in inhibiting the clonogenic growth of all AML cell lines tested and always resulted in statistically different ID_{50} ($P < 0.05$). As shown in Table 1, to obtain a 50% inhibition of clonogenic growth inhibition of AML cells, drug concentrations ranging from 0.25 to 0.45 µmol/l (mean 0.33) for both gold(III) dithiocarbamate derivatives and from 1.7 to 7.8 µmol/l (mean 3.7) for cisplatin were used. In addition, we examined the activity of two drugs (Ara-C and doxorubicin) that are currently used to treat AML, but characterized by a different mechanism of action compared with metal complexes. As shown in Table 1, ID_{50} values ranged from 2 to 30 nmol/l (mean 14) for Ara-C and from 3 to 37 nmol/l (mean 18) for doxorubicin.

Taken together, our results demonstrate that gold(III) dithiocarbamate derivatives determine a strong dose-dependent clonogenic growth inhibition, with ID_{50} values much lower than those of the reference antitumor agent cisplatin in all the AML cell lines tested.

Effects of gold(III) dithiocarbamate derivatives on apoptosis of acute myelogenous leukemia cells

In view of the above-mentioned growth inhibition effects, we were interested in determining whether these compounds also induced apoptosis, that represents the predominant mechanism by which cancer cells die in response to immune attack or to cytotoxic drugs [18]. First, we analyzed the early events of the apoptotic process by means of both Annexin-V and APO2.7 staining, comparing the activity of gold(III) compounds with cisplatin. A short exposure (18 h) of AML cells to a cytoxic dose (5 µmol/l) of cisplatin determined only a slight increase in Annexin staining (Fig. 3a). Conversely, a short exposure to [Au(MSDT)Br_2] induced a consistent Annexin staining together with PI staining, suggesting that the membrane damage occurred early (Fig. 3a and b). Similar results were obtained in the presence of [Au(MSDT)Cl_2] complex (data not shown). To further demonstrate the efficacy of gold(III) complexes to induce apoptosis, we also evaluated the expression of APO2.7, a protein confined to the mitochondrial membrane that can be detected during the early stages of apoptosis. As shown in Fig. 4a, in the presence of

Fig. 2



Representative concentration survival curves of acute myelogenous leukemia cells exposed to increasing concentrations of gold(III) dithiocarbamate derivatives. Acute myelogenous leukemia cells (5×10^3) were cultured in semisolid medium in the presence of increasing concentrations of $[Au(MSDT)Cl_2]$, $[Au(MSDT)Br_2]$ and cisplatin. After 14 days of incubation, plates were observed under phase contrast microscopy and aggregates with $\geq$ 40 cells were scored as colonies. Results represent the mean ± SD of eight replicate wells. The negative exponential dose–response survival curves are drawn by linear regression and may be described as a single parameter (the ID_{50} value). Comparison between linear regression curves for cisplatin and gold(III) derivatives reached statistical significance ($P < 0.05$).

$[Au(MSDT)Br_2]$, APO2.7 expression was strongly upregulated in all myeloid cell lines tested, thus confirming the apoptotic potential of gold(III) dithiocarbamate derivatives. Similar results were obtained in the presence of $[Au(MSDT)Cl_2]$ complex (data not shown). Cisplatin did not significantly change the expression of APO2.7. As shown in Fig. 4b, cisplatin induced APO2.7 expression only in the K-G1a cell line, even if in a minor extent in comparison with $[Au(MSDT)Br_2]$.

The ability of gold(III) dithiocarbamate derivatives to inhibit the cell cycle progression and to promote DNA fragmentation was determined using PI staining and FACS analysis. As shown in Fig. 5, after 72 h of treatment, gold(III) complexes determined an increase of apoptotic nuclei in all the myeloid cell lines tested, identified in flow cytometry as a sub-diploid peak in DNA content histograms. Moreover, gold(III) complexes determined a block in the S phase of THP-1 cells, and a slight increase of G_1 phase of ML-3, K562 and HEL cells, while cisplatin strongly affected the cell cycle: determined a significant ($P < 0.05$) increase of G_1 phase of NB4 and HEL cells, of S phase of HL-60 cells, and of G_2/M phase in KG-1, U-937, THP-1 and K562 cells (Fig. 5).

Table 1 Growth inhibition of AML cells by gold(III) dithiocarbamate derivatives

Cell line	ID_{50} (μmol/l)			ID_{50} (nmol/l)	
	[Au(MSDT)Cl_2]	[Au(MSDT)Br_2]	Cisplatin	Ara-C	Doxorubicin
KG-1a	0.25 ± 0.02	0.25 ± 0.02	1.9 ± 0.2	2 ± 0.2	25 ± 2.4
KG-1	0.25 ± 0.02	0.25 ± 0.02	2.9 ± 0.3	19 ± 1.7	10 ± 0.9
HL-60	0.30 ± 0.03	0.30 ± 0.03	5.2 ± 0.4	20 ± 2.2	25 ± 1.9
NB-4	0.30 ± 0.02	0.30 ± 0.03	1.7 ± 0.1	11 ± 1.0	20 ± 1.7
ML-3	0.25 ± 0.02	0.25 ± 0.02	4.2 ± 0.3	23 ± 1.5	10 ± 1.1
U-937	0.40 ± 0.03	0.40 ± 0.04	4.7 ± 0.5	30 ± 2.7	37 ± 3.2
K562	0.40 ± 0.03	0.40 ± 0.03	2.1 ± 0.3	11 ± 0.9	10 ± 0.8
THP-1	0.40 ± 0.04	0.40 ± 0.03	7.8 ± 0.6	4 ± 0.3	3 ± 0.3
HEL	0.45 ± 0.04	0.45 ± 0.04	3.1 ± 0.3	11 ± 0.9	25 ± 2.0
Mean	0.33 ± 0.07	0.33 ± 0.07	3.7 ± 1.9	14 ± 9.1	18 ± 10.7

AML cells were cultured in semisolid medium in the presence of increasing concentrations of gold(III) complexes and cisplatin (0.10–15 μmol/l), Ara-C and doxorubicin (0.5–100 nmol/l). After 14 days of incubation, aggregates with $\geq$ 40 cells were scored as colonies. Results represent the mean ± SD of eight replicate wells from three different experiments. The negative exponential dose–response survival curves are drawn by linear regression and may be described by a single slope parameter (ID_{50} value).
AML, acute myeloid leukemia.

Effects of gold(III) dithiocarbamate derivatives on Bcl-2 and Bax levels

Bcl-*2* family proteins are evolutionarily well-conserved apoptosis-regulating proteins [*22*]. The balance between proapoptotic and antiapoptotic Bcl-*2* proteins is crucial for determining the fate of a cell. To test the possibility that changes in the levels of Bcl-*2* family proteins were involved in the gold(III) derivative-induced apoptosis of AML cells, both Bcl-*2* and the integral membrane form of Bax (Bax-α) were analyzed by flow cytometry.

As shown in Fig. 6 (left panels), a short treatment (18 h) with [Au(MSDT)Br_2] determined a strong decrease of the antiapoptotic molecule Bcl-*2* in all cell lines examined. Conversely, the proapoptotic molecule Bax always resulted in upregulated or induced (Fig. 6, right panels). Similar results were also obtained in the presence of the [Au(MSDT)Cl_2] complex (data not shown).

Taken together, our results clearly demonstrate that apoptosis is a mechanism involved in the growth inhibition observed in the presence of gold(III) dithiocarbamate derivatives.

Discussion

AML is a malignant disease characterized by an aberrant accumulation of immature myeloid haematopoietic cells. Although the remission can be achieved in most patients, the relapse is common and the long-term survival is poor for most cases [1]. For this reason, the discovery of better antileukemic drugs is essential.

In fact, despite their high effectiveness, there are some clinical problems related to the use of platinum compounds in the curative therapy, such as severe normal tissue toxicity, and the frequent occurrence of initial and acquired resistance to the treatment. The most important adverse side effect of cisplatin is nephrotoxicity, which is correlated to platinum binding and inactivation of thiol-containing enzymes [5,17].

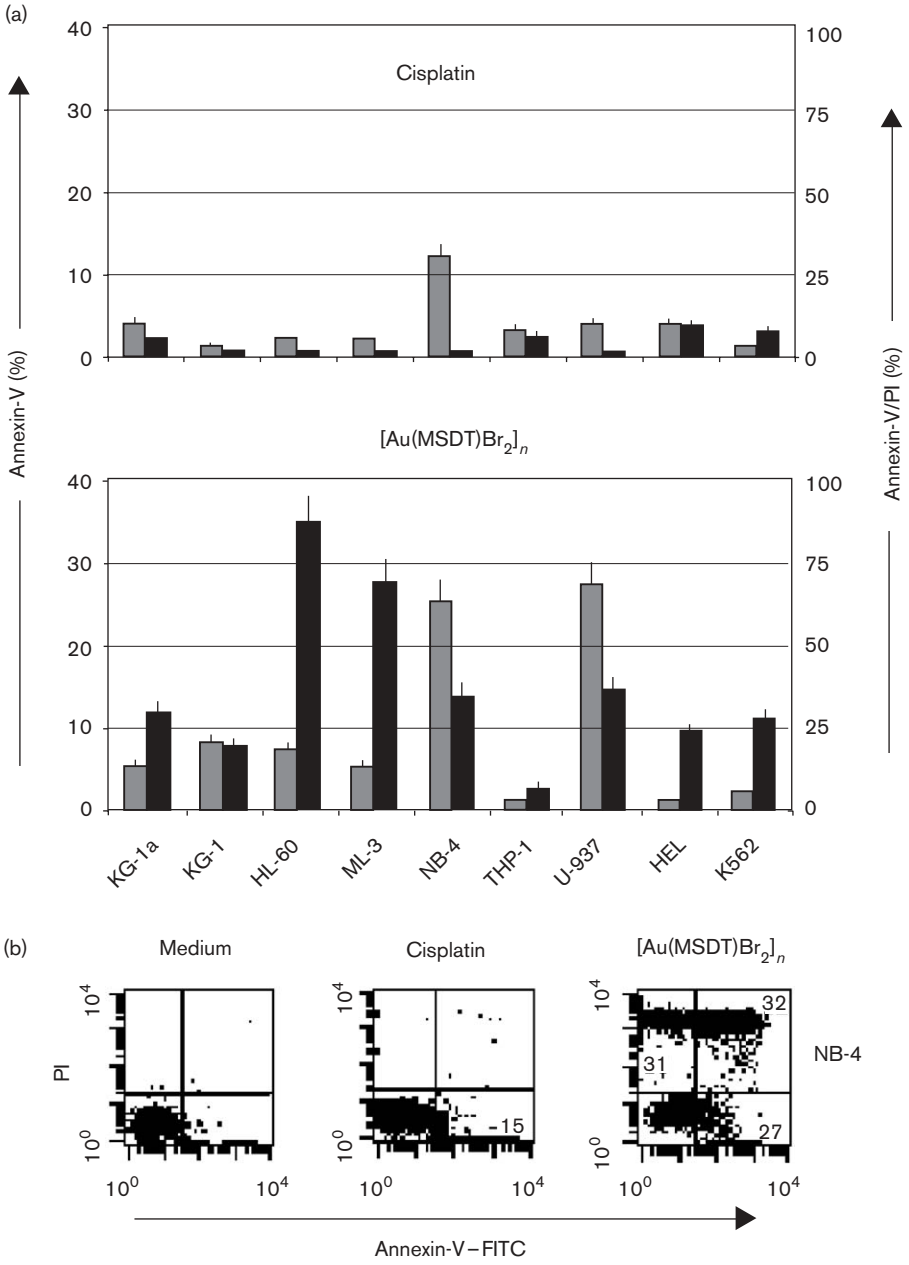
Several new compounds with reduced toxicity and high specificity have been developed, such as ruthenium complexes [6,7] or the second-generation platinum drug carboplatin that is active in treating AML [23,24] or blast crisis of CML [25]. Finally, the potential application of gold compounds as a new class of anticancer drugs has been explored recently [8–13].

In order to obtain compounds with superior chemotherapeutic index in terms of increased bioavailability, but minor nephrotoxicity, new gold(III) dithiocarbamate derivatives have been synthesized, purified and fully characterized [10,11].

In this study, we evaluated the antitumor activity of these two new drugs on a panel of eight AML cell lines representing different FAB subtypes and in the Ph + cell line (K562) (human CML in blast crisis). Cisplatin, one of the most widely used anticancer drugs, with well understood mechanisms of action, was chosen as a control drug [26,27]. These new gold(III) compounds determined a potent dose-dependent clonogenic growth inhibition on all myeloid leukemic cell lines tested, including a Ph + cell line, carrying the BCR–ABL fusion gene product, known to confer resistance to cytotoxic drugs [28]. More importantly, both gold(III) complexes resulted around 10 times more toxic towards leukemia cells than the control molecule cisplatin, although, compared with drugs used for standard treatment of AML, such as Ara-C and doxorubicin, gold complexes inhibited clonogenic growth at a higher micro molar concentration.

Apoptosis, or programmed cell death, is a genetic program that allows the control of cellular homeostasis. Disruption of apoptosis can contribute to a number of diseases, including cancer [29,30]. It is now well established that anticancer agents induce apoptosis and that disruption of apoptotic programs can reduce treatment sensitivity [18]. Our results indicate that gold(III) dithiocarbamate derivatives induce apoptotic death, as documented by Annexin-V and APO2.7 staining. After an overnight incubation, we found Annexin-V protein associated with cytoplasmic membrane damage as evaluated by PI staining. Apoptosis was also confirmed by the strong induction of APO2.7, a mitochondrial membrane protein that appears to be exposed on cells undergoing apoptosis [31]. Conversely, after an overnight incubation in the presence of cisplatin, both APO2.7 and Annexin-V were always absent, confirming that the gold(III) dithiocarbamate derivatives activity was stronger and faster when

Fig. 3

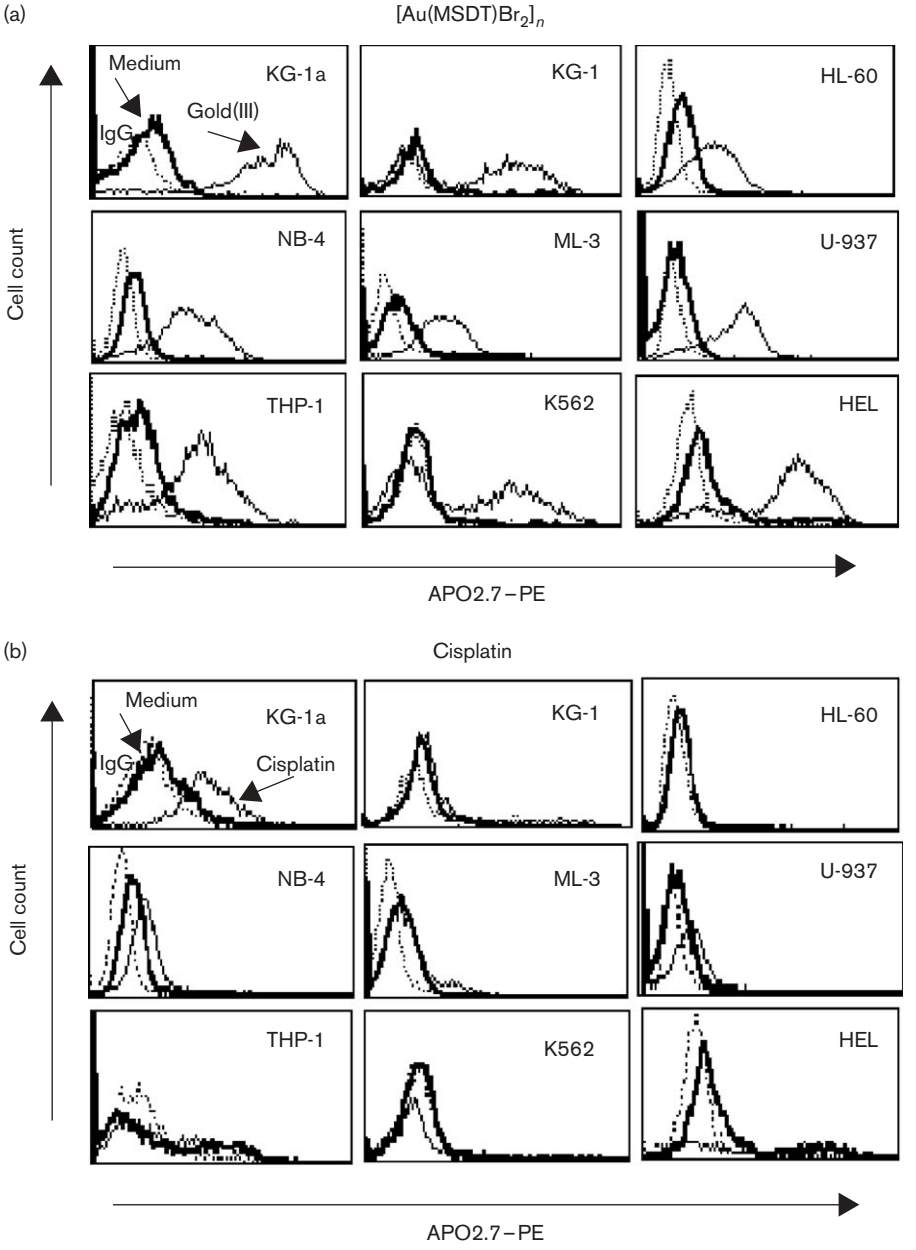


Induction of apoptosis of acute myelogenous leukemia (AML) cell lines by [Au(MSDT)Br_2]. (a) Exponentially growing AML cells were cultured in the absence (medium) and in the presence of [Au(MSDT)Br_2] (5 μmol/l) and cisplatin for 18 h. AML cells were double stained with Annexin-V–fluorescein isothiocyanate (FITC) and propidium iodide (PI). Results expressed the percentage of Annexin-V–FITC (dark bars) and Annexin-V–FITC/PI (white bars) stained cells ± SD, and are representative of three independent experiments. (b) Representative dot plots of NB-4 cells cultured in the presence of medium alone, cisplatin and [Au(MSDT)Br_2]. The percentages of stained cells are indicated in the quadrants.

compared with the reference drug. PI staining demonstrated DNA fragmentation, suggesting that the growth inhibition after treatment with gold(III) dithiocarbamate derivatives is caused by apoptosis of the leukemia cells.

In recent years, it has become clear that one of the major events during apoptosis is the permeabilization of the mitochondrial outer membrane that is facilitated by the Bcl-2 family proteins. The primary cause of treatment failures in AML is the emergence of both resistant disease and early relapse. Among the most frequent causes of these phenomena are the defects in the mitochondrial-mediated apoptotic pathway. This pathway is regulated by the Bcl-2 family of antiapoptotic (Bcl-2, Bcl-x_L, mcl-1) and proapoptotic proteins (Bax, Bad, Bak). Bcl-2 functions as an inhibitor of mitochondrial

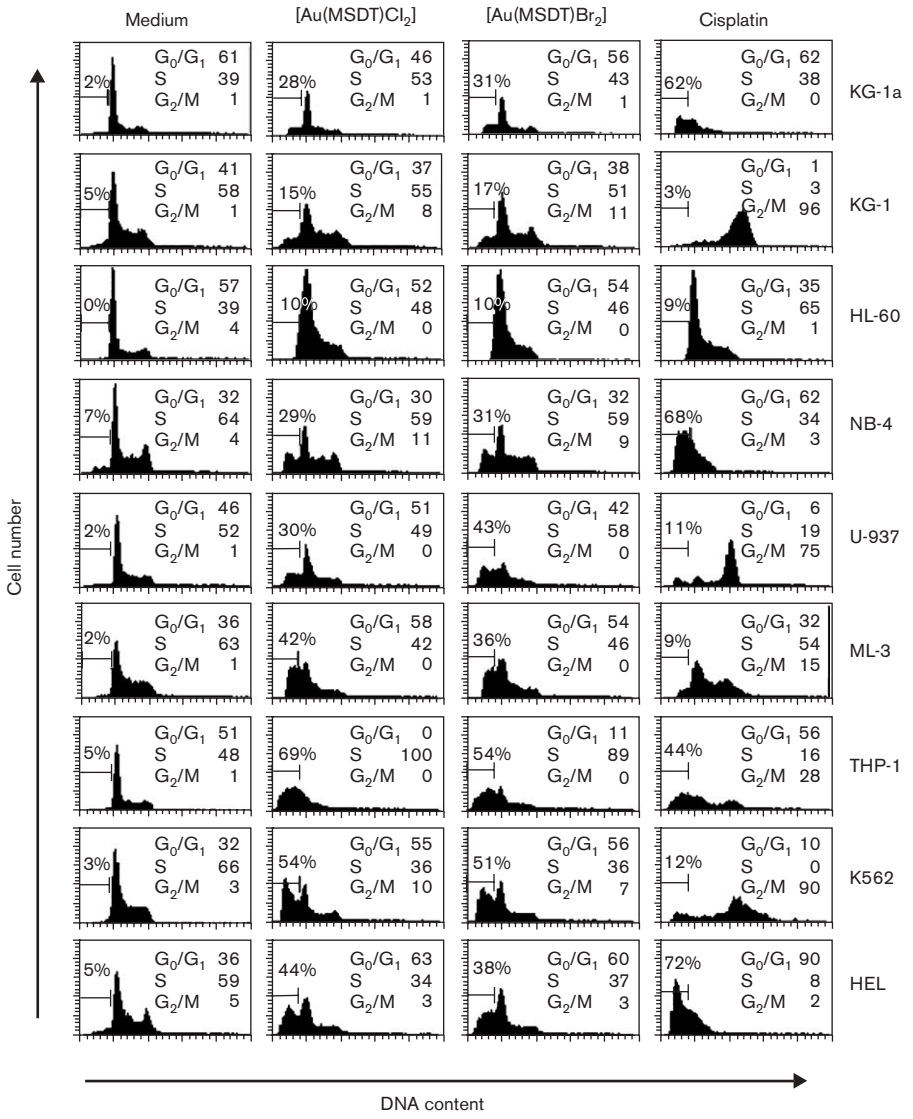
Fig. 4



Induction of apoptosis of acute myelogenous leukemia (AML) cell lines by [Au(MSDT)Br_2]. Exponentially growing AML cells were cultured in the absence (medium) and in the presence of [Au(MSDT)Br_2] (5 μmol/l) and cisplatin for 18 h. Representative fluorescence histograms showing the increase of APO2.7 expression. AML cells were stained with APO2.7–PE monoclonal antibody. Dotted lines indicate background fluorescence of cells, as determined by isotype-matched immunoglobulins. The *x*- and *y*-axis indicate the logarithm of the relative intensity of red fluorescence and relative cell number, respectively.

permeabilization, by changing its conformation on the mitochondrial membrane to bind with the membrane-inserted Bax monomers and to prevent productive oligomerization of Bax [32,33]. Likewise, reduced Bax levels have been associated with poor responses to chemotherapy and shorter overall survival in breast or colorectal carcinoma [30]. Conversely, enhanced Bax levels correlated in several cell types with response to chemotherapy *in vivo* [34]. Thus, Bax/Bcl-2 ratio, as determined by flow cytometry analysis, represents crucial clinical information in AML; in fact, a lower Bax/Bcl-2 ratio confers a very poor prognosis with decreased rates of complete remission and overall survival [27,32,35]. Therefore, Bcl-2 and Bax, represents both sensitive indicators of clinical outcome and potential targets of novel proapoptotic molecules designed to circumvent

Fig. 5



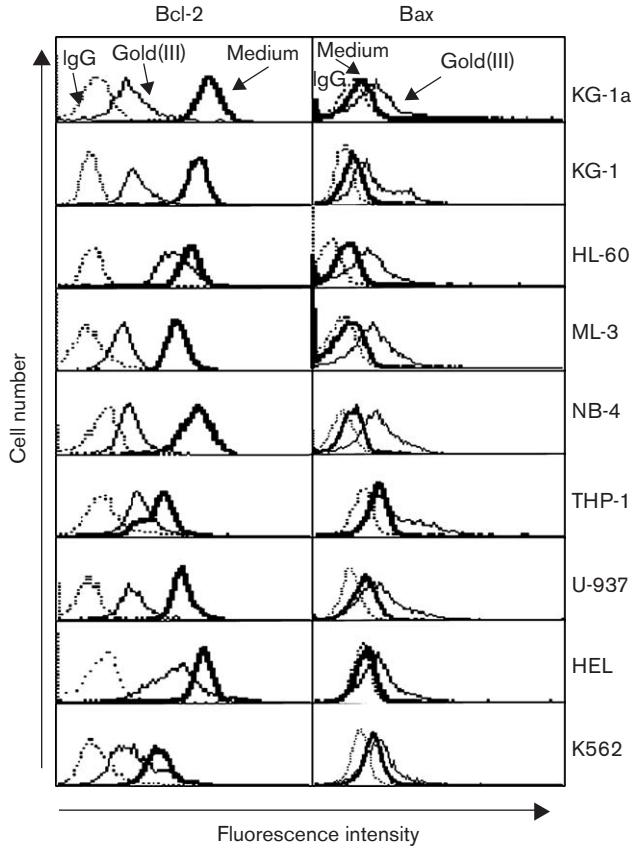
Cell cycle analysis after gold(III) complexes treatment. Acute myelogenous leukemia cells (5×10^3) were cultured in the presence of [Au(MSDT)Cl_2], [Au(MSDT)Br_2] and cisplatin (5 μmol/l). After 72 h, cell cycle and DNA fragmentation was determined by propidium iodide staining. Percentages of G_0/G_1-, S- and G_2/M-phase cells are shown; percentages of sub-G_1 cells are also reported. Data shown refer to a representative experiment, repeated twice.

chemoresistance [2,22]. The gold(III) complexes determined a strong and rapid downregulation of the antiapoptotic molecule Bcl-2 and an upregulation of Bax, decreasing the Bcl-2/Bax ratio, thus suggesting that they may represent a new therapeutic regimen, capable of inducing apoptosis at an early treatment stage, and overcame the multidrug resistance *in vivo*.

Standard treatment of leukemia has been directed towards the inhibition of proliferation through the use of antimetabolic drugs. Our compounds induce only modest cell cycle perturbations. This behavior largely differs from that of classical Pt(II) complexes that are known to induce the characteristic cell cycle alterations resulting in an increase in the G_2M cell fraction [36]. It was also evident that these gold(III) coordination compounds are able to promote early apoptosis and membrane damage to a much greater extent than by cisplatin.

Tumor cell resistance to chemotherapeutic agents is a central problem in medical oncology [1]. It is well known that an increased rate of drug inactivation in some cases is due to the high affinity of platinum and other soft heavy metals for sulfur-containing ligands such as glutathione,

Fig. 6



Modulation of Bcl-2 and Bax molecules by [Au(MSDT)Br_2] complex. Exponentially growing acute myelogenous leukemia cells were cultured in the absence (medium) and in the presence of [Au(MSDT)Br_2] (5 μmol/l) for 18 h. Dotted lines indicate background fluorescence of cells, as determined by isotype-matched immunoglobulins. The *x*- and *y*-axis indicate the logarithm of the relative intensity of red fluorescence and relative cell number, respectively. Data shown refer to a representative experiment, repeated three times.

metallothionine and other sulfur-containing biomolecules. Considering that our compounds contain a dithiocarbamate group capable to prevent the reaction with other sulfur-containing proteins [10,15], we can hypothesize a higher activity and a lower drug inactivation *in vivo* and consequently a decreased chemoresistence and nephrotoxicity (experimental in-vivo tests with similar compounds were already performed and will be published soon). In fact, as dithiocarbamates were shown to protect against cisplatin-induced nephrotoxicity in several animal models [17], recently we synthesized a new dithiocarbamate Pt(II) complex, characterized by a much lower renal toxicity than cisplatin [15,16]. Consistently, also our new gold(III) dithiocarbamate derivatives are expected to be less nephrotoxic than cisplatin, *in vivo*.

With regard to the potential clinical use of these new gold(III) dithiocarbamate derivatives as antitumor agents, it is important to note that in our study we achieved maximal growth inhibition of AML at a concentration of 2.5 μmol/l. Pharmacokinetics studies on rheumatoid arthritis patients receiving a standard regimen of gold therapy with aurothioglucose and the related compound aurothiomalate show that steady-state blood levels in the 10 μmol/l range are routinely achievable with limited adverse toxicity [13].

Further preclinical studies will be required to evaluate the efficacy of gold(III) dithiocarbamate derivatives as single agents in multiple preclinical tumor models and to assess their effectiveness in combination with existing standards therapy for AML. It will also be of interest to assess whether these compounds show efficacy against other tumor types.

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