



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi
di Firenze

MECHANISMS OF CYTOTOXICITY OF SELECTED ORGANOGOLD(III) COMPOUNDS

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

MECHANISMS OF CYTOTOXICITY OF SELECTED ORGANOGOLD(III) COMPOUNDS / CORONNELLO M.; MINI E.; CACIAGLI B.; CINELLU; M.A.; BINDOLI A.; GABBIANI C.; L. MESSORI. - In: JOURNAL OF MEDICINAL CHEMISTRY. - ISSN 0022-2623. - STAMPA. - 48:(2005), pp. 6761-6765. [10.1021/jm050493o]

Availability:

This version is available at: 2158/216974 since: 2019-07-22T10:08:38Z

Publisher:

American Chemical Society:1155 Sixteenth Street Northwest:Washington, DC 20036:(800)227-5558,

Published version:

DOI: 10.1021/jm050493o

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

(Article begins on next page)

Mechanisms of Cytotoxicity of Selected Organogold(III) Compounds

Marcella Coronello,^{*,†} Enrico Mini,[†] Barbara Caciagli,[†] Maria Agostina Cinellu,[‡] Alberto Bindoli,[§] Chiara Gabbiani,^{||} and Luigi Messori^{*,||}

Departments of Pharmacology and Chemistry, University of Florence, Florence, Italy, Department of Chemistry, University of Sassari, Sassari, Italy, and Institute of Neuroscience, Section of Biomembranes (CNR) c/o Department of Biological Chemistry, University of Padova, Padova, Italy

Received May 25, 2005

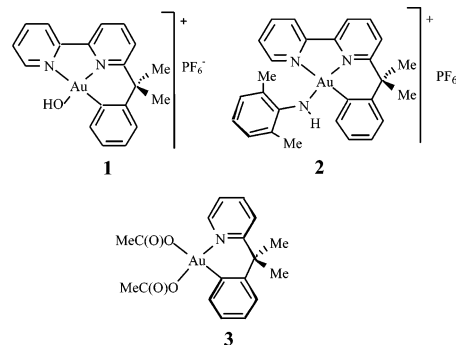
The effects of a few cytotoxic organogold(III) compounds on ovarian A2780 human cancer cells were investigated in comparison to cisplatin and oxaliplatin. The tested compounds produced significant antiproliferative effects and promoted apoptosis to a greater extent than platinum drugs while causing only modest cell cycle modifications. The mechanistic implications of these findings are discussed: mitochondrial pathways are proposed to be directly involved in the apoptotic process in relation to selective inhibition of thioredoxin reductase.

Introduction

In the past few years, second-generation gold(III) compounds have greatly attracted the interest of researchers for their encouraging antitumor properties.^{1–3} Indeed, a variety of structurally different gold(III) compounds such as gold(III) porphyrins,⁴ gold(III) dithiocarbamates,⁵ and gold(III) polyamines⁶ were shown to be chemically suitable for pharmacological testing and to produce important cytotoxic effects when assayed in vitro on various human tumor cell lines; for some of them promising in vivo results have been obtained as well.^{7–9} Also, a number of organogold(III) compounds were recently reported to exhibit favorable chemical and biological profiles; in particular, we found that a few organogold(III) compounds, bearing the bipyridyl motif, are endowed with outstanding anticancer properties against selected human tumor cell lines and thus deserve further pharmacological investigations.^{10,11}

Yet, very little is known concerning the molecular mechanisms underlying the pharmacological effects of gold(III)-based antitumor metallodrugs.¹ The initial efforts toward the synthesis and the biological evaluation of anticancer gold(III) compounds were mainly driven by their chemical analogy with platinum(II)-based drugs. It was postulated that the biological actions of gold(III) compounds might be a consequence of direct DNA damage as it is the case for cisplatin and its analogues. However, in contrast to general expectations, a number of subsequent experimental results suggested that gold(III) compounds exert their biological and antiproliferative effects through mechanisms that are substantially distinct from those of platinum(II) compounds.¹ Various gold(III) and organogold(III) compounds were shown to interact very weakly with calf thymus DNA and to produce only modest modifications of the double helix.¹²

Chart 1. Chemical Structures of the Tested Organogold(III) Compounds



To further elucidate these issues and describe the main cellular effects induced by novel organogold(III) compounds, new experiments were carried out in our laboratory. Specifically, the following organogold(III) compounds were selected for the present investigation: [Au(bipy^{dmb}-H)(OH)](PF₆)⁻ (**1**), Au(bipy^{dmb}-H)(2,6-xylylidene-H)](PF₆)⁻ (**2**) (in which bipy^{dmb} = 6-(1,1-dimethylbenzyl)-2,2'-bipyridine), and [Au(py^{dmb}-H)(AcO)₂] (**3**) (in which py^{dmb} = 2-(1,1-dimethylbenzyl)-pyridine) (see Chart 1). These compounds were previously characterized and found to cause significant antiproliferative effects on the human tumor cell lines A2780, MCF7, HT29, and A549.^{10,11} We analyze here the effects produced by these organogold(III) compounds on ovarian A2780 carcinoma cells, either sensitive (A2780/S) or resistant (A2780/R) to cisplatin, in terms of cytotoxicity, cell cycle modifications, and induction of apoptosis. For comparison purposes, parallel experiments were carried out with cisplatin (CDDP) and oxaliplatin (OHP), under identical solution conditions. Our investigations are principally aimed at describing the cellular responses to organogold(III) compounds in order to gain specific mechanistic information on these novel anticancer agents.

Results

Cytotoxicity of Organogold(III) Compounds. The antiproliferative effects, in vitro, of the mentioned

* Authors to whom correspondence should be addressed. M.C.: phone, 39-55-4271307; fax, 39-55-4271280; e-mail, marcella.coronnello@unifi.it. L.M.: phone, 39-55-4573284; fax, 39-55-4573385; e-mail, luigi.messori@unifi.it.

[†] Department of Pharmacology, University of Florence.

[‡] University of Sassari.

[§] University of Padova.

^{||} Department of Chemistry, University of Florence.

Table 1. IC₅₀ Values of Organogold(III) Compounds Compared to Platinum Complexes

| compound | IC ₅₀ (μM) ^a | |
|----------|------------------------------------|-----------|
| | A2780/S | A2780/R |
| CDDP | 1.0 | 7.0 (7.0) |
| OHP | 0.1 | 0.3 (3.0) |
| 1 | 1.0 | 7.0 (7.0) |
| 2 | 3.0 | 7.0 (2.3) |
| 3 | 3.5 | 9.0 (2.6) |

^a Amount of drug necessary to inhibit the growth of parental (A2780/S) and cisplatin-resistant (A2780/R) cells by 50% in 48 h. Data are the means of two determinations; values in parentheses indicate the ratio of IC₅₀ of cisplatin-resistant cell line and IC₅₀ of parental sensitive cell line.

organogold(III) compounds were measured as described in the Experimental Section. The concentrations required to inhibit growth of A2780/S and A2780/R cells by 50% (IC₅₀), following 48 h incubation, were determined (Table 1). Notably, all three organogold(III) compounds cause relevant antiproliferative effects with IC₅₀ values falling in the 1–10 μM range, thus being comparable with cisplatin. Significant cross-resistance effects were detected for these organogold(III) compounds: the resistance factors measured in the A2780/R line were 7.0 for **1**, 2.3 for **2**, and 2.6 for **3**.

Cell Cycle and Apoptosis Analysis. After determination of their IC₅₀ values, the three organogold(III) compounds were presented to cell cultures at equitoxic concentrations (drug concentrations were equal to the respective IC₅₀ values) and their effects analyzed by flow cytometry, after 48 h incubation. Remarkably, no significant modifications of the cell cycle phases were observed under the present experimental conditions, differently from the cases of cisplatin and oxaliplatin (Figure 1A,B). However, a relatively intense sub-G₁ peak appeared in organogold(III)-treated A2780/S cells, that represents cells undergoing apoptosis. This latter feature was far more evident for **2** and **3** than for **1**. In contrast, treatment of A2780 cells with larger amounts of organogold(III) compounds (drug concentrations equal to IC₇₀ values were employed in this new series of experiments, roughly corresponding to 5 × IC₅₀), for 48 h, resulted in deeply perturbed flow cytometry profiles, most likely in relation to extensive cell death (data not shown).

This led us to establish a further administration scheme that might be a reasonable compromise between the two above extremes. In the new scheme, A2780 cells were treated for 4 h with drug concentrations matching the IC₇₀ values (~5 × IC₅₀), followed by 20 h postincubation in drug-free medium. Cells exhibiting apoptotic fragmentation were detected by TUNEL assay. Percentages of S, G₁, G₂ cell phases, and of apoptotic cells, determined either by TUNEL assay or by sub-G₁ peak quantitation, in organogold(III) treated A2780/S and A2780/R cells, are reported in Figure 2A and Figure 2B, respectively.

In the new series of experiments, the cell cycle effects of **1** turned out to be similar to those of OHP, with a significant decrease of S phase; also, a concomitant slight increase in G₂ phase was noted. Pairwise, the intensity of the sub-G₁ peak was similar to OHP whereas results of TUNEL were lower. The behavior of compounds **2** and **3** was more similar to that of cisplatin; increases of G₂ and S/G₂M phases were respectively

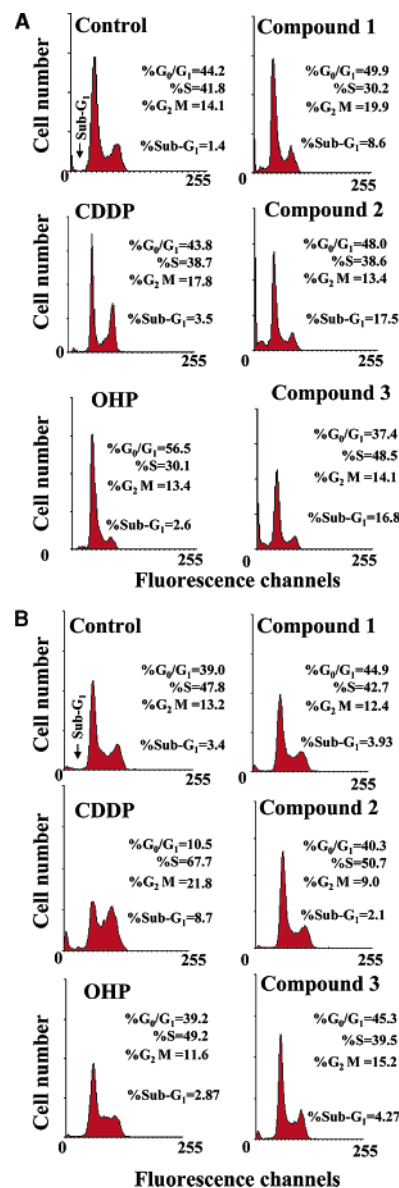


Figure 1. Cell cycle analysis. A2780/S (panel A) and A2780/R (panel B) cells were treated with IC₅₀ concentrations of the various cytotoxic agents and processed at 48 h for flow cytometric analysis as described in the Experimental Section. Percentages of G₀/G₁ phase, S phase, and G₂ phase cells are shown; percentages of sub-G₁ cells are also reported. Data refer to a representative experiment, repeated twice.

found for these compounds. However, **2** and **3** differed markedly from cisplatin for the large increase of the sub-G₁ peak and for the high scores of the TUNEL test.

These results suggest that cells in the G₂M phase are the most susceptible to apoptosis induction. Significant apoptotic breaks could be seen with compound **1** at IC₇₀ values, after 48 h incubation but not after a 4 h treatment, showing a clear dependence of the apoptotic events on the exposure time. Treatment of A2780/R cells, in accordance with the 4/20 h scheme, resulted in accumulation of cells in the S-phase, that is particularly evident for **3**; in addition, we noticed, for compounds **1** and **3**, the appearance of cells with significant DNA fragmentation that are better evidenced by TUNEL assay than by sub-G₁ peak quantitation.

Overall, these experimental results reveal a great apoptotic potential for all the investigated organogold-

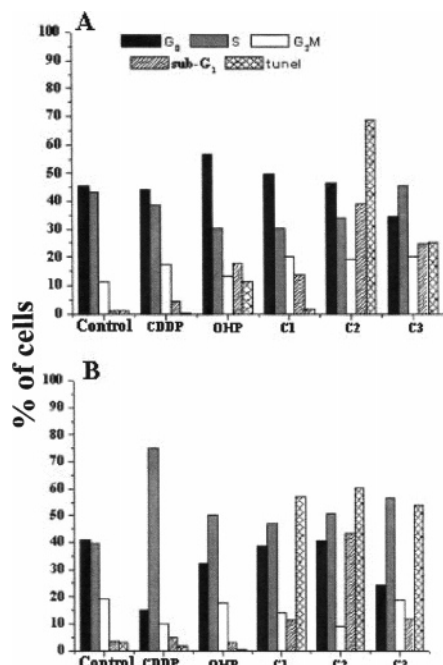


Figure 2. Cell cycle and apoptosis analysis. Percentages of S, G₁, G₂ cell phases, and of apoptotic cells, determined either by TUNEL assay or by sub-G₁ peak quantitation, in organogold(III) treated A2780/S and A2780/R cells, are reported in panel A and panel B, respectively. In this experiment, A2780 cells were treated for 4 h with drug concentrations matching the IC₇₀ values ($\sim 5 \times IC_{50}$), followed by 20 h postincubation in drug-free medium. Cells exhibiting apoptotic fragmentation were detected by TUNEL assay.

(III) compounds in the presence of only modest cell cycle alterations.

A further insight into the apoptotic potential of organogold(III) compounds is provided by multiparametric flow cytometry determinations. Specifically, an experiment was carried out in which cells or cell fragments positive for apoptotic strand breaks (determined by TUNEL) were measured against the forward scatter. Data are presented as bivariate histograms (Figure 3) according to previous literature.¹³ In the case of cells treated with the organogold(III) compounds, profound increases in cells with a high signal for apoptotic breaks were generally observed. Notably, forward scatter data suggest dramatic morphological changes in compound 2 treated A2780/S cells. Occurrence of extensive apoptosis is confirmed by direct observation of cells at the microscope (Figure 4) where bright fluorescence upon HOECHST 33342 staining is indicative of nuclear fragmentation.

Discussion

The present study was mainly directed to gain further mechanistic information on novel gold(III) metallodrugs; specifically, we focused our attention on a few organogold(III) compounds that were recently reported to display encouraging chemical and biological profiles as possible cytotoxic and antitumor agents.

The cellular effects of these compounds on the reference ovarian A2780 human cell line were analyzed in detail, by using different concentrations and different administration schedules. These organogold(III) compounds manifest relevant cytotoxic effects with IC₅₀ values falling in the low micromolar range; nonetheless,

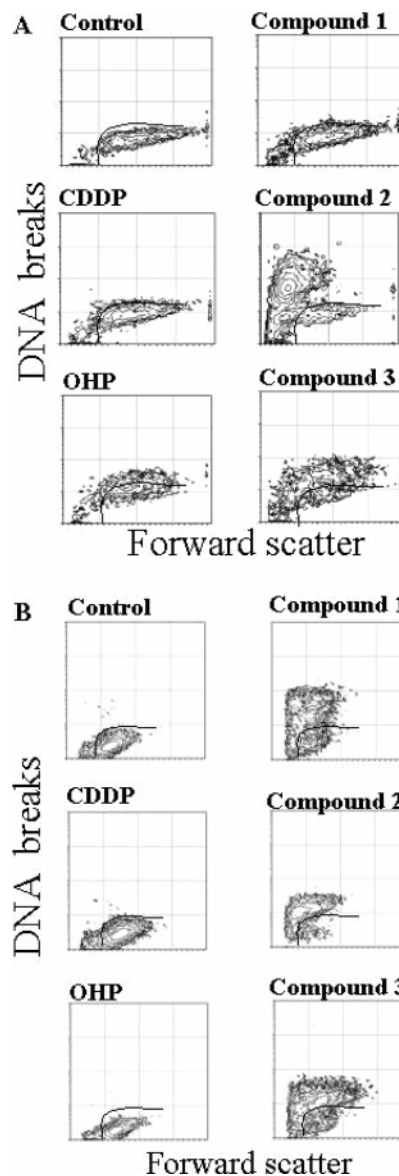


Figure 3. Apoptosis analysis. Bivariate histograms, relative to A2780/S (panel A) and A2780/R (panel B) cells, are shown for control cells and cells treated with IC₇₀ drug concentrations for 4 h followed by a 20 h postincubation in drug-free medium. Apoptotic breakage data (by TUNEL assay) are plotted against forward scatter.

significant cross-resistance phenomena were detected implying that the resistance mechanisms that are effective toward platinum(II) compounds are still largely effective toward gold(III) compounds. All the investigated organogold(III) compounds, when presented at concentrations matching their respective IC₅₀ values, did not produce significant alterations of the cell cycle distribution. This behavior largely differs from that of classical platinum(II) complexes that are known to induce characteristic cell cycle alterations.¹⁴ It was also evident that these organogold(III) compounds are able to promote apoptosis to a greater extent than cisplatin and oxaliplatin. These findings probably imply that apoptosis is not a consequence of direct damage produced on nuclear DNA, as it is the case for platinum compounds.

These initial results prompted us to implement a new experimental protocol; the 4/20 h scheme was, in fact, chosen to highlight early drug effects while minimizing

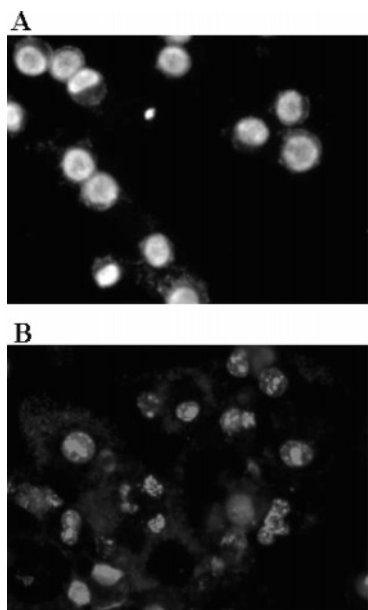


Figure 4. Apoptotic cell morphology evaluation by Hoechst 33342 staining. A2780/S and A2780/R cells were treated according to the 4/20 h scheme and stained with Hoechst 33342 dye as described in the Experimental Section. The morphology of untreated (A) and compound **2** treated (B) A2780/S cells is shown.

late effects such as secondary necrosis and/or apoptosis, resulting from lethal mitosis. DNA cleavage into high molecular weight fragments (HMW) and the appearance of oligonucleosomes (LMW) characterize the apoptotic process. The rate of generation and the abundance of HMW fragments can vary significantly according to the apoptotic agent used, the duration of the stimulus, and the cell type considered.¹⁵ High values of the TUNEL test were obtained with compounds **2** and **3**, in sensitive cells, and with all organogold(III) compounds, in resistant cells. In all cases, the increases of the sub-G₁ peak are less pronounced. These observations are suggestive of extensive DNA cleavage into high molecular weight fragments associated with weak chromatin condensation (see Figure 4). The lower values of hypodiploidy might be indicative of the lack of low molecular weight fragments responsible for the emission of apoptotic bodies.¹⁵ Thus, these results highlight a great proapoptotic potential for these novel organogold(III) compounds; this interpretation was further supported by multiparametric flow cytometry determinations and by direct observation at the microscope.

The above observation prompted us to consider alternative pathways for induction of apoptosis by organogold(III) drugs. An attractive hypothesis is that apoptosis is induced by direct activation of a mitochondrial pathway. Indeed, over the past few years, mitochondria have become known for promoting apoptosis through release of cytochrome *c* and other proapoptotic factors during mitochondria permeability transition or via other release mechanisms.¹⁶ Rigobello et al. have recently provided further experimental support for the linkages existing among the thioredoxin reductase/thioredoxin system, the mitochondrial membrane permeability transition, and selective metal toxicity.¹⁷ In collaboration with the group of Alberto Bindoli we have recently shown that various gold(I) and gold(III) compounds as well as other toxic agents behave as specific

Table 2. Inhibitory Effects of Organogold(III) Compounds on Mitochondrial Thioredoxin Reductase

| | CDDP | Auranofin | Aubipy | AuXil | Aupy |
|-----------------------|------|-----------|--------|-------|------|
| IC ₅₀ (μM) | 36.0 | 0.020 | 0.28 | 0.21 | 1.42 |

inhibitors of mitochondrial thioredoxin reductase; in particular, the effects of **1**, **2**, and **3** as inhibitors of thioredoxin reductase were evaluated.¹⁸ The measured IC₅₀ values obtained for thioredoxin reductase inhibition, reported in Table 2, suggest that these compounds are indeed highly selective inhibitors of this crucial selenoenzyme although less potent than auranofin. For comparison purposes, inhibition produced by cisplatin was also measured and found to be orders of magnitude lower.

These results strongly qualify thioredoxin reductase as a probable target for novel organogold(III) compounds. Although the effects of **1**, **2**, and **3** on the mitochondrial functions and, specifically, on the induction of mitochondrial swelling are less pronounced than those of other toxic metal ions, e.g., cadmium, it is very likely that the observed mitochondrial alterations may eventually lead to release of cytochrome *c*, thus initiating the apoptotic process. However, gold compounds are scarcely effective on mitochondrial respiration, at variance with metal ions and metal complexes that exhibit a marked inhibition. This suggests that, while the various metal ions act on a number of different targets, gold compounds are highly specific and their action seems to be restricted to a single target identified as the mitochondrial isoform of thioredoxin reductase¹⁸

In conclusion, we have shown that a few novel organogold(III) compounds produce relevant cytotoxic and apoptotic effects on the reference A2780 cell line, either sensitive or resistant to cisplatin, without affecting importantly the cell cycle distribution. The scarce effects produced on the cell cycle and the reported poor binding toward purified DNA *in vitro* led us to consider nonclassical pathways for induction of apoptosis. Since these compounds behave as strong and selective inhibitors of mitochondrial thioredoxin reductase, we propose that a direct interference with mitochondrial functions might explain the observed large proapoptotic effects and the relevant antiproliferative effects.

Experimental Section

Materials. Sterile tissue-culture plates and other tissue-culture plastic wares were purchased from Corning (U.S.A.). Dimethyl sulfoxide (DMSO) and propidium iodide (PI) were bought from Sigma, and Hoechst 33342 was bought from Calbiochem-Behring Corp. (U.S.A.). The *in situ* cell death detection kit and fluorescein were purchased from Roche Diagnostics Corp. (U.S.A.). Human ovarian carcinoma sensitive (A2780/S) and its cisplatin-resistant clone (A2780/R) were cultured in RPMI 1640 (Euroclone Ltd, U.K.) supplemented with 10% fetal calf serum (FCS, Euroclone Ltd, U.K.). Cell lines were grown in humidified 5% CO₂ at 37 °C and were screened for mycoplasma; cells were split twice a week by trypsinization (trypsin 0.05%/EDTA 0.02% in PBS, from Euroclone Ltd, U.K.). [Au(bipy^{dm}-H)(OH)](PF₆), [Au(py^{dm}-H)(AcO)₂], and [Au(bipy^{dm}-H)(2,6-xylylidine-H)](PF₆) were synthesized according to reported procedures in refs 19, 20, and 21; cisplatin (CDDP) and oxaliplatin (OHP) were obtained from Teva Pharma Italia (Milano, Italy) and Sanofi-Synthelabo (Milano, Italy), respectively. The stock solutions (10⁻² M) of compounds were prepared by dissolving the compounds in 1 mL of DMSO; CDDP and OHP stock solutions (10⁻² M) were prepared in sterile bidistilled water.

Cytotoxicity Assay. The cytotoxic effects were checked by count of the cells grown in 25 cm² plates. Cells were seeded in tissue-culture plates at 5 × 10⁴ cells/mL (total volume 10 mL) and incubated overnight. Standard drugs and compounds were diluted to desired concentrations in RPMI 1640 medium, added to plates, and incubated for 48 h. At the end of the incubation, the medium was decanted and the IC₅₀ values (drug concentration inhibiting cell growth by 50%) were determined by cell counting (model D Coulter counter, Coulter electronics, Ltd, Luton, Bedfordshire, England).

Cell Cycle Analysis. A2780/S and A2780/R cells were exposed to concentrations of the test compounds equal to IC₅₀. At 24 h and 48 h, cells were fixed in 70% ice-cold ethanol and stored at 4 °C. Cells were then rehydrated in PBS (phosphate buffered saline) and stained in propidium iodide (PI, 50 µg/mL) solution containing RNase A (5 units/mL) for 30 min.²² PI-stained cells were analyzed for DNA content with a FAC-Star cell sorter (Becton-Dickinson, Mountain View, CA) equipped with an argon ion laser (model Innova 90, Coherent, Palo Alto, CA) operating at 500 mW output at 488 nm. The red fluorescence emitted by PI was collected by a 620 nm long-pass filter, as a measure of the amount of DNA-bound PI, and displayed on a linear scale. Data from 20 000 cells were analyzed with forward and scattered light, and red fluorescence was recorded with Consort 30 software. Cell cycle distribution was determined on a linear scale. The percentage of cells in cycle phases was determined using WinMDI 2.8 Windows Multiple Document Interface Flow Cytometry Application (Cylchred Windows 95, version 1.02).

TUNEL Assay for Identification of Apoptotic Cells. Apoptotic DNA fragmentation was monitored based on fluorescent nucleotide-tagging of 3'-OH ends with terminal deoxynucleotidyl transferase (TdT) followed by cytofluorimetric detection. Briefly, A2780/S and A2780/R cells were exposed to concentrations of the test compounds equal to IC₇₀ (at 48 h) for 4 h and postincubated in drug-free medium for an additional 20 h. Aliquots of 2 × 10⁷ drug-treated and control cells were fixed with 1% paraformaldehyde (Sigma) in PBS at room temperature for 15 min'. Fixed cells were washed with PBS, resuspended in cold PBS, mixed with cold (-20 °C) absolute ethanol (70% final concentration), and stored at 4 °C in the dark. Cells were washed twice with cold PBS, and pellets were resuspended in 50 µL of TUNEL reaction mixture (in situ cell death detection kit, fluorescein, from Roche Kit No. 1-684-795) and incubated for 60 min at 37 °C in a humidified atmosphere in the dark. For each cell line, a parallel reaction without the enzyme was run to provide background staining. Finally, cells were washed with PBS and counterstained in 1.0 mL of 5.0 µg/mL PI in PBS. Fluorescein isothiocyanate (FITC) and PI fluorescence emissions were collected through a 525–530 filter and 620 nm long-pass filter, respectively. Data from 20 000 cells were analyzed with forward and scattered light, and green and red fluorescence were recorded with Consort 30 software. The percentage of apoptotic cells was determined using WinMDI 2.8.

Apoptotic Cell Morphology Evaluation by Hoechst 33342 Staining. A2780/S and A2780/R cells were harvested at 20 h following 4 h exposure to 48 h IC₇₀ values of the test compounds; control cells and treated cells were collected, centrifuged, and stained with Hoechst 33342 (2 µg mL⁻¹). After staining, morphology was determined by examining the cytospin slides using fluorescence microscopy interfaced with a computerized image analysis system.

Acknowledgment. MIUR and Ente Cassa di Risparmio di Firenze are gratefully acknowledged for financial support.

References

- Messori, L.; Marcon, G. *Met. Ions Biol. Syst.* **2004**, *42*.
- Shaw, C. F., III. Gold-based therapeutic agents. *Chem. Rev.* **1999**, *99*, 2589–2600.
- Tiekink, E. R. Gold derivatives for the treatment of cancer. *Crit. Rev. Onc. Hematol.* **2002**, *42*, 225–248.
- Che, C. M.; Sun, R. W.; Yu, W. Y.; Ko, C. B.; Zhu, N.; Sun, H. Gold(III) porphyrins as a new class of anticancer drugs: cytotoxicity, DNA binding and induction of apoptosis in human cervix epitheloid cancer cells. *Chem. Commun.* **2003**, *14*, 1718–1719.
- Ronconi, L.; Giovagnini, L.; Marzano, C.; Bettio, F.; Graziani, R.; Pilloni, G.; Fregona, D.; Gold Dithiocarbamate Derivatives as potential antineoplastic agents: design, spectroscopic properties, and in vitro antitumor activity. *Inorg. Chem.* **2005**, *44*, 1867–1881.
- Messori, L.; Abbate, F.; Marcon, G.; Orioli, P.; Fontani, M.; Mini, E.; Mazzei, T.; Carotti, S.; O'Connell, T.; Zanello, P. Gold(III) complexes as potential antitumor agents: solution chemistry and cytotoxic properties of some selected gold(III) compounds. *J. Med. Chem.* **2000**, *43*, 3541–3548.
- Giovagnini, L.; Ronconi, L.; Aldinucci, D.; Lorenzon, D.; Sitran, S.; Fregona, D. Synthesis, characterization, and comparative in vitro cytotoxicity studies of platinum(II), palladium(II), and gold(III) methylsarcosinedithiocarbamate complexes. *J. Med. Chem.* **2005**, *48*, 1588–1595.
- Buckley, R. G.; Elsome, A. M.; Fricker, S. P.; Henderson, G. R.; Theobald, B. R.; Parish, R. V.; Howe, B. P.; Kelland, L. R. Antitumor properties of some 2-[(Dimethylamino)methyl]phenylgold(III) complexes. *J. Med. Chem.* **1996**, *39*, 5208–5214.
- Fricker, S. P.; Skerj, R.; Cameron, B. R.; Mosi, R.; Zhu, Y. *GLD 2003*; AnorMED Inc.: Langley, BC, V2Y 1N5, Canada, 2003.
- Marcon, G.; Carotti, S.; Coronello, M.; Messori, L.; Mini, E.; Orioli, P.; Mazzei, T.; Cinellu, M. A.; Minghetti, G. Gold(III) complexes with bipyridyl ligands: solution chemistry, cytotoxicity, and DNA binding properties. *J. Med. Chem.* **2002**, *45*, 1672–1677.
- Messori, L.; Marcon, G.; Cinellu, M. A.; Coronello, M.; Mini, E.; Gabbiani, C.; Orioli, P. Solution chemistry and cytotoxic properties of novel organogold(III) compounds. *Bioorg. Med. Chem.* **2004**, *12*, 6039–6043.
- Messori, L.; Orioli, P.; Tempi, C.; Marcon, G. Interactions of selected gold(III) complexes with calf thymus. *Biochem. Biophys. Res. Commun.* **2001**, *281*, 352–360.
- Faivre, S.; Chan, D.; Salinas, R.; Woyannarowska, B.; and Woyannarowski, J. M. DNA strand breaks and apoptosis induced by oxaliplatin in cancer cells. *Biochem. Pharmacol.* **2003**, *66*, 225–237.
- Lippert, B. *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; John Wiley & Sons: New York, 1999.
- Lecoeur, H. Nuclear apoptosis detection by flow cytometry: influence of endogenous endonucleases. *Exp. Cell Res.* **2002**, *277*, 1–14.
- McKeage, M. J.; Maharaj, L.; Berners-Price, S. J. Mechanisms of cytotoxicity and antitumor activity of gold(I) phosphine complexes: the possible role of mitochondria. *Coord. Chem. Rev.* **2002**, *232*, 127–135.
- Rigobello, M. P.; Scutari, G.; Boscolo, R.; Bindoli, A. Induction of mitochondrial permeability transition by auranofin, a gold(I)-phosphine derivative. *Br. J. Pharmacol.* **2002**, *136*, 1162–1168.
- Rigobello, M. P.; Messori, L.; Marcon, G.; Cinellu, M. A.; Bragadin, M.; Folda, A.; Scutari, G.; Bindoli, A. Gold complexes inhibit mitochondrial thioredoxin reductase: consequences on mitochondrial functions. *J. Inorg. Biochem.* **2004**, *98*, 1634–1641.
- Cinellu, M. A.; Zucca, A.; Stoccoro, S.; Minghetti, G.; Manassero, M.; Sansoni, S. Synthesis and characterization of gold(III) adducts and cyclometallated derivatives with 6-benzyl- and 6-alkyl-2,2'-bipyridines. *J. Chem. Soc., Dalton Trans.* **1996**, *22*, 4217–4225.
- Cinellu, M. A.; Minghetti, G.; Pinna, M. V.; Stoccoro, S.; Zucca, A.; Manassero, M. Replacement of the chloride ligand in [Au(C₂N₂Cl)]PF₆ cyclometalated complexes by C, N, O and S donor ligands. *J. Chem. Soc., Dalton Trans.* **1999**, *16*, 2823–2831.
- Cinellu, M. A.; Minghetti, G.; Pinna, M. V.; Stoccoro, S.; Zucca, A.; M. Manassero Synthesis and Characterization of Mononuclear Amidogold(III) Complexes—Crystal Structure of [Au(N₂C₁₀H₇(CMe₂C₆H₄)-6)(NHC₆H₃Me₂-2,6)]PF₆—Oxidation of 4-Methylaniline to Azotoluene. *Eur. J. Inorg. Chem.* **2003**, *12*, 2304–2310.
- Coronello, M.; Marcon, G.; Carotti, S.; Caciagli, B.; Mini, E.; Mazzei, T.; et al. Cytotoxicity, DNA damage, and cell cycle perturbations induced by two representative Gold(III) complexes in human leukemic cells with different cisplatin sensitivity. *Oncol. Res.* **2001**, *12*, 361–370.