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Clinical report

Synthesis and biological activity of gold and tin compounds in ovarian cancer cells

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We have investigated the patterns of *in vitro* cytotoxicity, induced by six newly synthesized gold and tin compounds, in three human ovarian cancer cell lines (SW 626, IGROV 1 and OVCAR-3). Four gold compounds, i.e. gold(I)lupinylsulfide hydrochloride [1] (containing a naked gold atom), triethylphosphinogold(I)lupinylsulfide hydrochloride [2], triphenyl-phosphinogold(I)lupinylsulfide hydrochloride [3] and 1,2-bis(diphenylphosphino)ethane bis[gold(I)lupinylsulfide] dihydrochloride [4] (all containing a gold atom coordinated with different phosphines), were prepared. Moreover, the triethylphosphinogold(I)(2-diethylamino)ethylsulfide hydrochloride [5] in which the simple diethylaminoethylthiol replaced the bulky lupinylthiol was synthesized. The tin compound, triethyltin(IV)lupinylsulfide hydrochloride [6], was also studied. Comparative tests with cisplatin, the most widely used antitumor agent in ovarian cancer, were carried out in biological investigations. *In vitro* cytotoxicity, by MTT assay, showed that compound [4] and compound [6] exhibited interesting antiproliferative activity in all the three cell lines (mean IC_{50}=1.3 and 0.7 μM, respectively) compared to cisplatin (mean IC_{50}=4.8 μM). In addition, the PA-1 cell line, more sensitive to cisplatin (IC_{50}=0.6 μM), was included as a comparison in the study. Cell count assays confirmed the cytotoxic properties of compounds [4] and [6] against the four cell lines, reporting higher growth inhibition potency than cisplatin, with IC_{50} values in the sub-micromolar range.

Key words: Antitumor activity, gold, ovarian cancer, tin.

Introduction

Despite extensive surgical and chemotherapeutic efforts, the long-term survival rate of ovarian cancer patients remains poor.[1-3] The most widely used chemotherapeutic regimen includes platinum compounds;[4] however, drug resistance of tumor cells,[5] toxicity (nephrotoxicity, ototoxicity, neurotoxicity) and side effects (emesis) frequently limit the clinical usefullness of cisplatin. Chemical modifications of cisplatin have been carried out to reduce toxic effects, and improve their antitumor efficacy and pharmacokinetic properties.[6] Many cisplatin analogs have been investigated in recent years but, at present, carboplatin is the only derivative that has entered into clinical trials.[7,8] Moreover, several non-platinum metal complexes have been extensively investigated in the last decades:[9] antitumor activity has been found in derivatives of rhodium, iridium and palladium, and other compounds containing copper, gallium, titanium, tin and germanium were tested for their biological activity in human cancer cells.[10-12] Among them, spirogermanium[13] and budotitane[14] have shown some clinical interest.

In the group of gold derivatives only auranofin, a compound originally used as an oral antirheumatic agent, demonstrated high cytotoxic activity against a variety of tumor cell types (*in vitro*) and in a limited number of *in vivo* mouse tumor models.[15,16]

More interesting antitumor properties exhibited in several cancer cell lines have been demonstrated by the gold complex containing 1,2-bis(diphenylphosphino)ethane (ddpe).[17] As far as compounds active on ovarian carcinoma are concerned, the tin(IV) dialkyldithiocarbammates were considered interesting for their innovative mechanism of action not related to DNA alkylation.[18] On this basis, we thought it worthy to undertake a study on new metal complexes as potential therapeutic agents in ovarian cancer, displaying chemical and physico-chemical characteristics suitable for their interaction with different cellular targets and for increasing their intracellular distribu-

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tion. This aim was pursued through the binding of phosphine-gold(I) complexes, or alkyltin(IV) derivatives, to *tert*-aminoalkylthiols. Such compounds are endowed with an increased degree of lipophilicity as the number of carbon atoms in the aminothiol mojety is increased. Moreover, due to their basicity, water soluble salts could be obtained.

The *tert*-aminothiol selected for the present study was quinolizidinyl-methylthiol (lupinylthiol or thiolupinine) previously described by Novelli and Sparatore.[19] From this bulky and highly lipophilic aminothiol four compounds, i.e. gold(I)lupinylsulfide hydrochloride [1], triethylphosphinogold(I)lupinylsulfide hydrochloride [2], triphenylphosphinogold(I)lupinylsulfide hydrochloride [3] and 1,2-bis (diphenylphosphino) ethane bis [gold (I) lupinylsulfide] dihydrochloride [4] containing a gold atom naked or coordinated with different phosphines, were prepared.

The triethylphosphinogold-lupinylsulfide was formerly designed as a potential auranofin-like antiarthritic drug, being active after oral administration in adjuvant-induced arthritis in rats.[20]

In order to assess the possible contribution of the quinolizidinylmethylthiol mojety to the antitumor activity, triethylphosphinogold(I)(2-diethylamino)ethyl sulfide hydrochloride [5], in which the simple diethylaminoethylthiol replaced the bulky lupinylthiol, was prepared. In addition, the tin compound, triethyltin-(IV)lupinylsulfide hydrochloride [6], was also studied. Although the triethyltin derivatives are generally considered too toxic for therapeutic use, the presence of a sulfide bond to saturate the fourth valence of tin should strongly reduce the toxicity.

In the current paper the patterns of *in vitro* cytotoxicity, induced by the above-mentioned compounds against human ovarian cancer cell lines, were investigated. The potential implications as novel therapeutic drugs are discussed.

Materials and methods

Physical methods

Melting points were determined by the capillary method on a Büchi apparatus and are uncorrected. Elemental analyses were performed on a CE Instrument, model EA1110, CHNS-0 and the analytical results, for the indicated elements, were within $\pm 0.3\%$ of the calculated values.

Synthetic routes

The structures of the compounds studied are shown in Figure 1.

Gold(I)lupinylsulfide hydrochloride [1] and triethylphosphinogold (I) lupinylsulfide hydrochloride [2]. These compounds were prepared as already described by Novelli and Sparatore.[19] The gold (I)lupinylsulfide was dissolved in the stoichiometric volume of 1 N ethanolic hydrogen chloride; the solution was evaporated to dryness under reduced pressure and the residue was washed several times

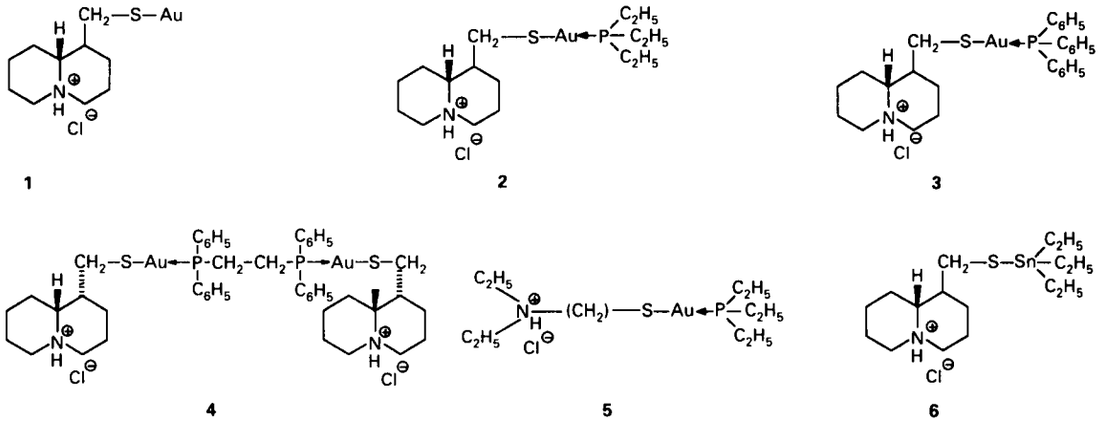

Figure 1. Structural formulae of the complexes studied.

with dry ether to give the corresponding hydrochloride.

Triphenylphosphinogold (I) lupinylsulfide hydrochloride [3]. Commercially available triphenylphosphinogold(I)chloride (500 mg=1 mM) was dissolved, with mild heating, in 30 ml of dry benzene; 187 mg (1 mM) of freshly distilled thiolupinine[19] was added to this solution and the mixture was stirred in a closed tube, protected from light, at room temperature for 3 days. Solvent was removed under reduced pressure and the residue was washed several times with dry ether leaving 550 mg (80% yield) of a white solid, melting at 114–127°C with decomposition (vacuum sealed capillary). Analysis (C, H, N) for $C_{28}H_{33}AuNPS{\cdot}HCl{\cdot}0.5H_2O$.

1,2-Bis (diphenylphosphino) ethane bis [(gold (I)-lupinylsulfide] dihydrochloride [4]. To a solution of 1,2-bis(diphenylphosphino)ethane (370 mg=1.8 mM) in peroxide-free tetrahydrofuran (7 ml) was added 370 mg (1.8 mM) of gold(I)lupinylsulfide[19] and the mixture was stirred in a closed tube, protected from light, at room temperature for 22 h. A small amount of insoluble material was filtered and the solution was evaporated to dryness under reduced pressure. The residue was washed several times with dry ether leaving a white, impalpable powder (about 700 mg=65.7% yield). Analysis (C, H, N) for $C_{46}H_{60}Au_2N_2P_2S_2$. Part of this compound was converted to the hydrochloride: analysis (C, H, N) for $C_{46}H_{60}Au_2N_2P_2S_2{\cdot}2HCl{\cdot}0.5H_2O$.

Triethylphosphinogold(I)(2-diethylamino)ethylsulfide hydrochloride [5]. To a solution of freshly distilled 2-diethylaminoethanthiol (190 mg=1.4 mM) in 3 ml of methanol was added a solution of 500 mg (1.4 mM) of triethylphosphinogold chloride in 7 ml of methanol and the mixture was stirred in a closed tube, at room temperature, for 30 min. The reaction mixture was filtered and the solution was evaporated to dryness under reduced pressure; the residue was washed several times with dry ether giving 230 mg (34% yield) of **[5]**. Analysis (C, H, N) for $C_{12}H_{29}$ AuNPS·HCl.

Triethyltin (IV) lupinylsulfide hydrochloride [6]. Triethyltin(IV) bromide (1 g=3.5 mM) and freshly distilled thiolupinine (650 mg=3.5 mM) were dissolved in peroxide-free tetrahydrofuran (6 ml); the mixture was stirred in a closed tube, protected from light, at room temperature for 24 h. Solvent was removed under reduced pressure and the oily residue was dissolved in water, treated with 6 N sodium hydroxide solution and extracted with ether. After evaporation of the ether extract 1.27 g of free base as yellowish oil was obtained, chromatographed on neutral alumina (1:15) eluting with dry ether. The colorless oily free base (940 mg=68.7% yield) was converted into the hydrochloride. Analysis (C, H, N) for $C_{16}H_{33}NSSn{\cdot}HCl$.

Cell culture and treatment

The human ovarian cancer cell lines used in the present investigation were: SW 626 (primary poorly differentiated cystoadenocarcinoma), OVCAR-3 (progressive papillary adenocarcinoma, resistant to clinical relevant concentrations of doxorubicin, melphalan and cisplatin), PA-1 (teratocarcinoma of the ovary) from ATCC (Rockville, MD) and IGROV 1, primary moderately differentiated polymorphous adenocarcinoma,[21] kindly provided by Dr Ferrini from our Institute.

Cultures were maintained in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin-streptomycin and 0.25 mg/ml amphotericin-B (all obtained from ICN Biochemicals, Costa Mesa, CA), at 37°C in a 5% CO_2 and 95% air atmosphere.

For growth inhibition studies, the compounds were freshly dissolved in distilled water [**1**, **2** and **5**], in absolute ethanol [**3** and **4**] or in 30% ethanol/water (v/v) [**6**] at 10 mM, and then diluted with distilled water to reach the planned concentrations. The final percentage of ethanol did not exceed 1% and preliminary experiments demonstrated that this concentration did not affect cell growth. Cisplatin (Sigma, St Louis, MO) was freshly dissolved in saline solution at the stock concentration of 100 μM.

Cytotoxicity

MTT assay. Cytoxicity of tested compounds against tumor cells was measured by the MTT colorimetric test[22,23] according to the *in vitro* antitumor screen protocol from the NCI described by Boyd.[24,25] The tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma. Exponentially growing cell lines were seeded in quadruplicate into 96-well flat-bottomed plates in 200 μl of complete medium at the concentration of 5×10^3 cells/well and allowed to attach overnight. After 24 h, 20 μl of increasing concentrations of compounds (final concentration in the medium: range 0.01–100 μM) was added to wells. After 48 h, 50 μl of MTT solution (2 mg/ml in phosphate-buffered solution) were added to the culture medium and incubated at

37°C for a further 4 h. The plates were then centrifuged for 5 min at 200 *g* and reinverted to remove unconverted MTT. Dimethylsulfoxide (150 μl) was added to each well and the plates were shaken to dissolve the reduced MTT crystals (formazan); the optical density (OD) was measured on a microtiter reader (Medgenix-Technogenetics, Milan, Italy) at a wavelength of 540 nm. Control wells (100% viability), in which the tested compounds were absent, were included in all the experiments. All data points represent an average of triplicate assays. The mean, the standard deviation and the percentage of error from quadruplicate samples were determined for each drug concentration. The average 50% inhibitory concentration (IC_{50}) was determined graphically from the dose-response curves.

Cell count. Sensitivity to drugs was further determined by cell count. The cells (1×10^5) were seeded into 35 mm tissue culture dishes in 2 ml of complete medium and treated 24 h later with compounds at the final concentration ranging from 0.01 to 10 μM. The cultures were set in duplicate and exposed to the new complexes for 48 h. After trypsinization, viable cells were counted by a Thoma hemocytometer using the Trypan blue dye exclusion method. The percentage of growth inhibition was the ratio between the number of treated cells and that of the untreated control.

Results

Six newly synthesized compounds (five gold complexes and one tin derivative) were prepared (see Figure 1), and their potential cytotoxic effects against two primary (SW 626 and IGROV 1) and one recurrent (OVCAR-3) ovarian cancer cell lines were studied. The choice of these complexes was motivated by the known antitumor activity of some metal-derived drugs[12] and by the search for new more active compounds in ovarian cancer.

Table 1. IC_{50} values determined by MTT assay in ovarian cancer cell lines

Compound	IC_{50} (μM)		
	SW 626	IGROV 1	OVCAR-3
1	40	20	100
2	5.0	4.5	8.0
3	100	50	30
4	1.8	1.9	0.3
5	3.0	2.0	4.0
6	0.9	0.7	0.5

Dose-response curves, evaluated by the MTT assay, were drawn to assess the drug concentration reducing survival by 50% of control values (IC_{50}). The current results demonstrated that the IC_{50} values differed for each compound as shown in Table 1.

Among the gold complexes derived from lupinylthiol, compounds [**1**] and [**3**] were poorly active, displaying significant activity at IC_{50} values ranging from 20 to 100 μM, while compound [**2**] was just modestly active with IC_{50} values between 4.5 and 8.0 μM. Moreover, although the complex derived from diethylaminoethylthiol [**5**] exhibited some interesting cytotoxic activity with IC_{50} values in the range of 2.0 and 4.0 μM; among the gold-derived compounds, the most interesting cytotoxicity seemed to be exerted by the 1,2-bis(diphenylphosphino)ethane bis[gold(I)lupinylsulfide]dihydrochloride [**4**]. Indeed, while a greater sensitivity was observed in OVCAR-3 cells with an IC_{50} of 0.3 μM, the IC_{50} values reported in SW 626 and IGROV 1 (1.8 and 1.9 μM, respectively) were still the lowest among all the gold-derivatives.

An outstanding activity inhibiting survival by 50% was shown after testing the tin derivative, triethyltin-(IV)lupinylsulfide hydrochloride [**6**]. As in Table 1, the *in vitro* cytotoxicity was in the sub-micromolar region with IC_{50} values of 0.9, 0.7 and 0.5 μM in the SW 626, IGROV 1 and OVCAR-3 cell lines, respectively.

In order to further assess and confirm the inhibitory potential of the two selected compounds, [**4**] and [**6**], comparative *in vitro* assays, including cisplatin, were carried out. In addition PA-1, the most cisplatin-sensitive ovarian cell line studied, was introduced in the above-mentioned assays together with the relevant cell lines.

As reported in Figure 2, PA-1 cells did display more sensitivity to cisplatin (IC_{50}=0.6 μM) as compared to SW 626 (IC_{50}=4.0), IGROV 1 (IC_{50}=4.5 μM) and OVCAR-3 (IC_{50}=6.0 μM). Nevertheless, while the cytotoxic activity (in terms of IC_{50}) induced by compound [**4**] in PA-1 was equivalent to cisplatin (0.6 μM), the activity of compound [**6**] was significantly higher, having an IC_{50} of 0.04 μM.

Collectively, results from plotted MTT dose-response curves indicated that the order of cytotoxicity induced by compounds [**4**], [**6**] and cisplatin in the four cell lines was as follows: in SW 626, [**6**] > [**4**]=cisplatin; in IGROV 1, [**6**] > [**4**] > cisplatin; in OVCAR-3, [**6**] = [**4**] > cisplatin; in PA-1, [**6**] > [**4**] = cisplatin. Further studies were conducted to determine the inhibitory effects exerted by the relevant complexes on cell growth. Cultures were exposed to compound [**4**] or [**6**] or cisplatin 24 h after plating and then incubated for further 48 h before cell count.

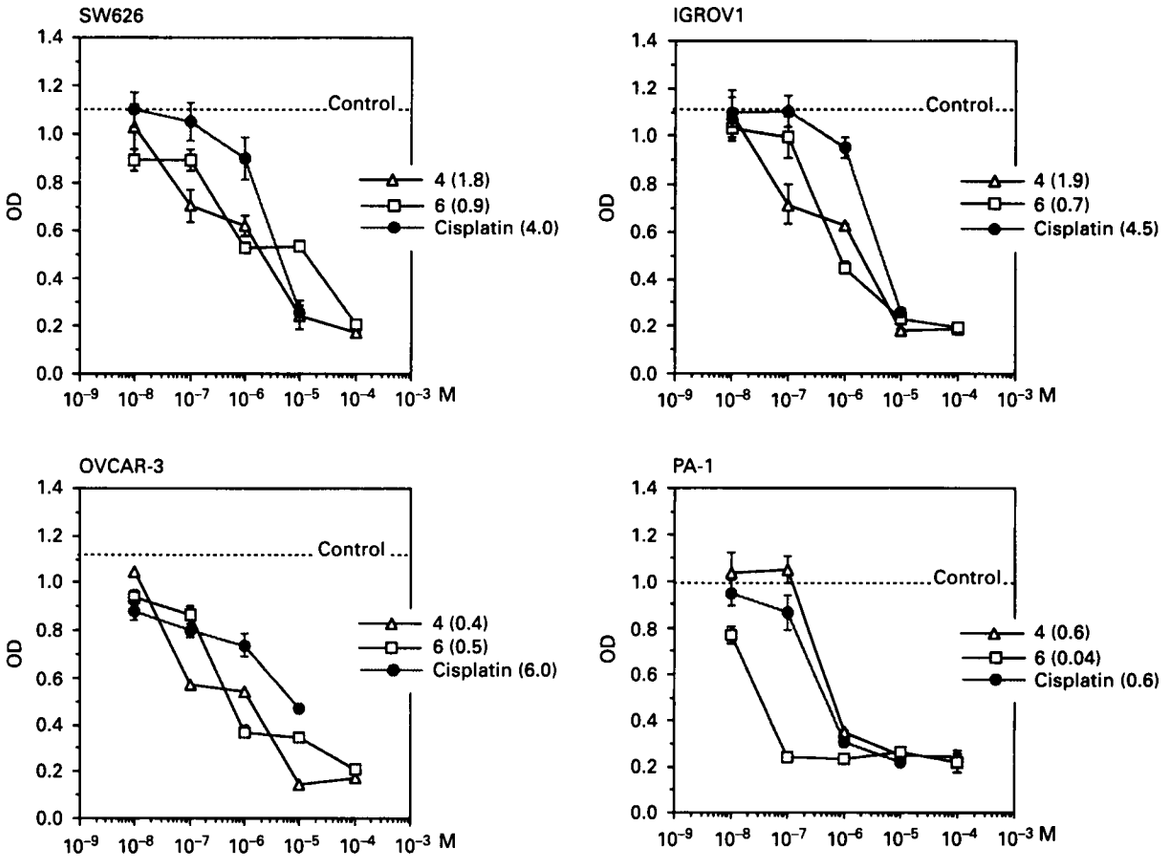

Figure 2. Dose–response curves from MTT assay after 48 h of treatment with [4], [6] and cisplatin. Points and bars represent the mean and the standard deviation of at least three replicate determinations. Parenthesis: IC_{50} in μM.

As reported in Figure 3, compounds [4] and [6] mainly confirmed their interesting properties when compared to cisplatin but some differences in activity, with respect to the MTT results, were evidenced. When the concentration corresponding to 0.1 μM was taken into account, compound [4] was active in all the four cell lines, but particularly in SW 626 and PA-1. Compound [6] also showed good activity in all cell lines but was more cytotoxic in IGROV 1 and OVCAR-3, compared to cisplatin. In addition, both complexes still exerted a striking growth inhibition in IGROV 1 and PA-1 at the concentration of 0.01 μM, which was almost ineffective in the cisplatin-treated cultures.

Discussion

In the present investigation, six metal-derived compounds (five gold compounds and one tin derivative) were synthesized and screened for their antiproliferative activity in ovarian cancer cell lines. However, only one gold complex [4] and the tin derivative [6] were suitable for further investigation.

The inactivity of compounds [1] and [3] could be related, respectively, to: (i) the exposure of the gold atom that could interact with reactive groups before reaching the cellular target, and (ii) the overwhelming steric hindrance and high lipophilicity of the triphenylphosphine ligand. The latter possibility, however, is not unequivocal, since compound [4], exhibiting a still high steric hindrance around the gold atom, is actually active. Thus, some other physico-chemical characteristics concerning the phosphine might play a role in the expression of the activity. Anyhow, a direct contribution to activity from the peculiar phosphine present in compound [4], as claimed by some authors for other complexes containing dppe,[10,17] was ruled out in our experiments.

Compound [4] was more active than compound [2] on a molar basis and such a difference seemed to be

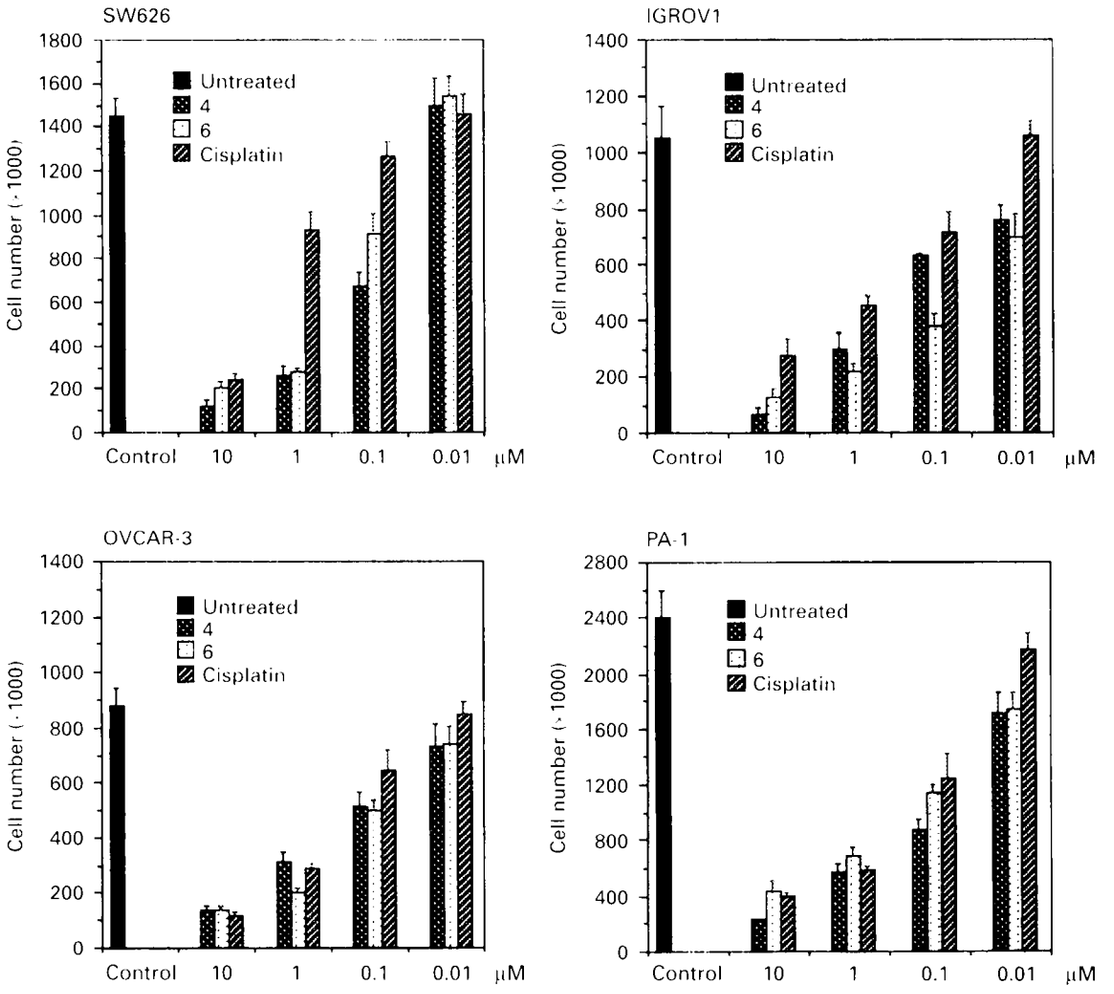

Figure 3. Growth inhibition evaluated by cell count after treatment with [**4**], [**6**] and cisplatin. Histograms and bars represent the mean and the SD of at least three replicate determinations.

related, at first, to the presence of two atoms of gold. However, the observed differences of sensitivity among the cell lines indicate a more complex role of the molecular structure. On the other hand, the possibility of some contribution to activity *in vitro*, from the nature of the aminothiol employed, is inferred from the comparison of compounds [**2**] and [**5**]. The latter, derived from diethylaminoethanethiol, was somewhat superior than the first containing the thiolupine moiety. However, it is not possible to anticipate if such a difference will be maintained in *in vitro* experiments, where a higher lipophilicity of the aminothiol might be very important.

The triethyltin(IV)lupinylsulfide [**6**], characterized by a quite novel structure, exhibited an outstanding activity inhibiting all the cell lines at sub-micromolar IC_{50} values and was, at least in these preliminary assays, more active than all other gold complexes so far studied. In addition, the different patterns of cell proliferation and doubling times as well as clinical drug resistance might contribute to the diverse chemosensitivity and IC_{50}.

Moreover, comparative tests, carried out by treating the cell cultures with cisplatin, reported little sensitivity of the relevant cell lines to this drug, having an average IC_{50} of approximately 5.0 μM. To further assess the cytotoxic relevance of the current compounds, the PA-1 cell line (sensitive to cisplatin) was also assayed in cytotoxicity experiments and preliminary data confirmed their interesting activity, particularly at lower concentrations. To summarize, the combined data from the MTT and cell count assays

showed that compounds [4] and [6], though with some differences, were more active than cisplatin, expecially at lower concentrations.

Previous studies already showed that some gold complexes and organotin compounds exhibited antitumor activity in a variety of mouse tumor models and in particular against the P388 lymphocytic leukemia.[12,16,17] Preliminary tests of the current compounds were carried out in this model as well, and a significant cytotoxic activity in the P388 cells was found (data not shown), providing IC_{50} of 1.1, 0.38 and 0.34 μM for compound [4], compound [6] and cisplatin, respectively. A recent paper by Buckley *et al.*[26] reported the antitumor activity of four gold complexes, having structural features in common with cisplatin, against a panel of human ovarian carcinoma cell lines. Although the above-mentioned complexes are structurally different from those described in the current study, the IC_{50} range of the two selected compounds (3 and 5) in the paper by Buckley[26] varied from 2.7 μM (in A2780 and CH1 cell lines for compound 5) to 107 μM (in SK-OV-3 for compound 3), whereas the IC_{50} range of the present compounds, [4] and [6], was between 0.3 μM (in OVCAR-3) and 1.9 μM (in IGROV 1).

Conclusion

The purpose of this preliminary study was to determine whether six newly synthesized gold- and tin-derived compounds could exert significant cytotoxic activity against human ovarian cancer cell lines to be exploited as potential antitumor agents. Our results indicate that one gold complex, the 1,2-bis(diphenylphosphino)ethane bis[gold(I)lupinylsulfide]dihydrochloride [4], and the tin derivative, triethyltin(IV)lupinylsulfide hydrochloride [6] in particular, were more effective than cisplatin in the four considered ovarian cancer cell lines (SW 626, IGROV 1, OVCAR-3 and PA-1) exhibiting mean IC_{50} values in the sub-micromolar range. These data were firstly obtained by the MTT colorimetric assay and then confirmed by cell growth tests. Although the current results are preliminary and need to be supported by more extensive investigations, compounds [4] and [6] might represent interesting agents as antitumor drugs in ovarian cancer.

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