Gold(III) Complexes as Potential Antitumor Agents: Solution Chemistry and Cytotoxic Properties of Some Selected Gold(III) Compounds

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Gold(III) complexes generally exhibit interesting cytotoxic and antitumor properties, but until now, their development has been heavily hampered by their poor stability under physiological conditions. To enhance the stability of the gold(III) center, we prepared a number of gold(III) complexes with multidentate ligands − namely $[Au(en)_2]Cl_3$, $[Au(dien)Cl]Cl_2$, $[Au(cyclam)](ClO_4)_2Cl$, $[Au(terpy)Cl]Cl_2$, and $[Au(phen)Cl_2]Cl$ − and analyzed their behavior in solution. The solution properties of these complexes were monitored by visible absorption spectroscopy, mass spectrometry, and chloride-selective potentiometric measurements; the electrochemical properties were also studied by cyclic voltammetry and coulometry. Since all the investigated compounds exhibited sufficient stability under physiological conditions, their cytotoxic properties were tested in vitro, via the sulforhodamine B assay, on the representative human ovarian tumor cell line A2780, either sensitive or resistant to cisplatin. In most cases the investigated compounds showed relevant cell-killing properties with IC_{50} values falling in the 0.2−10 μM range; noticeably most investigated gold(III) complexes were able to overcome, to a large extent, resistance to cisplatin when tested on the corresponding cisplatin-resistant cell line. The cytotoxic properties of the free ligands were also determined under the same solution conditions. Ethylenediamine, diethylenetriamine, and cyclam were virtually nontoxic (IC_{50} values > 100 μM) so that the relevant cytotoxic effects observed for $[Au(en)_2]Cl_3$ and $[Au(dien)Cl]Cl_2$ could be quite unambiguously ascribed to the presence of the gold(III) center. In contrast the phenanthroline and terpyridine ligands turned out to be even more cytotoxic than the corresponding gold(III) complexes rendering the interpretation of the cytotoxicity profiles of the latter complexes less straightforward. The implications of the present findings for the development of novel gold(III) complexes as possible cytotoxic and antitumor drugs are discussed.

Introduction

Gold(III) complexes, isoelectronic and isostructural to platinum(II) complexes, hold promise as possible antitumor agents.[1−3] Surprisingly, only a few reports exist in the literature describing the cytotoxic properties and the in vivo antitumor effects of gold(III) complexes;[4−6] some preliminary data suggesting a direct interaction of gold(III) complexes with DNA as the basis for their cytotoxic effects were previously reported.[7,8] However, until now, any extensive pharmacological investigation of these compounds as possible antitumor agents was severely hindered by their poor kinetic and redox stabilities under physiological conditions making them of difficult use for pharmaceutical purposes.[9,10]

Recently, we have shown that the gold(III) center may be significantly stabilized, even at neutral pH, through an appropriate choice of the ligands[11] while preserving its peculiar biological properties. We report here on the solution chemistry and the cytotoxic properties of five representative gold(III) complexes − $[Au(en)_2]Cl_3$ (Auen), $[Au(dien)Cl]Cl_2$ (Audien), $[Au(cyclam)](ClO_4)_2Cl$ (Aucyclam), $[Au(terpy)Cl]Cl_2$ (Auterpy), and $[Au(phen)Cl_2]Cl$ (Auphen)[12−15] − in which the stability of the gold(III) center is enhanced by coordination to one (or more) multidentate ligand(s). In all cases at least two nitrogen atoms are directly coordinated to the gold(III) center leading to a significant decrease of the reduction potentials.[16]

Interesting cytotoxic properties in vitro have been detected for most investigated complexes. Since our complexes entail a variety of different structural arrangements of the gold(III) ion, attempts are made to define preliminary structure−function relationships within this new class of cytotoxic and potentially antitumor compounds.

Experimental Section

Preparation of Auen, Audien, Aucyclam, Auphen, and Auterpy. $[Au(en)_2]Cl_3$ was prepared according to ref 12. A gummy yellow precipitate was formed by addition of a solution of 1,2-ethylenediamine monohydrate in ether to a solution of $HAuCl_4$ in ether; the yellow precipitate was dissolved in water giving an orange solution. A white precipitate of $[Au(en)_2]Cl_3$ formed upon adding ethyl alcohol to the latter solution.

$[AuCl(dien)]Cl_2$ was prepared according to ref 13. A solution of diethylenetriamine·3HCl in water was added slowly and

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with stirring to a solution of $HAuCl_4$ (20% w/v); a yellow precipitate immediately formed. Then a solution of NaOH was added to the mixture and left stirring at 0 °C for 2 h. The yellow precipitate was then filtered off and washed with ethanol.

$[Au(cyclam)](ClO_4)_2Cl$ was prepared following the procedure reported by Kimura et al.:[14] treatment of $NaAuCl_4 \cdot 2H_2O$ with equimolar amounts of cyclam (1,4,8,11-tetraazacyclotetradecane) in CH_3CN for 1 h yielded the "Au(III)-in" complex $(ClO_4)_2Cl$.

Auterpy was prepared by addition of terpyridine to a $HAuCl_4$ solution under a 1:1 stoichiometry according to ref 15.

Auphen was prepared by addition of phenanthroline·HCl to a $HAuCl_4$ solution at a 1:1 stoichiometry following the procedure reported in ref 12.

All the obtained products were checked by elemental analysis; in all cases the purity of the compounds was higher than 90%. Further evidence for the correct identification of the obtained compounds is provided by electronic spectra and mass spectra (vide infra).

Electronic Spectra and Potentiometric Studies of Chloride Release. The absorption spectra in the UV−visible region were recorded on a Perkin-Elmer Bio 20 spectrophotometer operating at room temperature. All the investigated gold(III) complexes are soluble in water (10^{-2} M solutions of all complexes are easily obtained); electronic spectra were recorded on freshly prepared buffered solutions of each complex at room temperature. The hydrolysis experiments were carried out by adding small amounts of freshly prepared, concentrated water solutions of the various gold(III) complexes to the reference buffer and monitoring the electronic spectra of the resulting mixtures over 72 h. Potentiometric measurements of chloride release were performed by using a chloride-selective electrode interfaced to a Hanna potentiometer. The apparatus was calibrated with sodium chloride solutions of known concentration.

Mass Spectra. Electrospray mass spectra were taken on a Perkin-Elmer Sciex API 365 triple quadrupole mass spectrometer (Sciex, Thornhill, Canada) equipped with a Turbo ion spray interface. The operative parameters were set as follows: ionspray voltage 5.8 kV, orifice voltage 40 V, scan range 150−1000 amu, scan speed 4.26 s, resolution > 1 amu. Solutions of the analyte in water were introduced into the interface by a syringe pump at a flow of 5 μL/min.

Electrochemical Measurements. The apparatus for electrochemical measurements has been described elsewhere.[17] All the measurements were performed on solutions deaerated by bubbling ultrapure nitrogen for 15 min. The potential values here reported were measured using a saturated calomel electrode (SCE). The oxidation of ferrocene is assumed to occur at $E^{\circ\prime} = +0.17$ V (vs SCE) in aqueous solution,[18] whereas it occurs at $E^{\circ\prime} = +0.40$ V in DMSO solution containing $[NEt_4][ClO_4]$ (0.1 mol dm^{-3}). Conversion to values vs ENH was obtained upon adding +0.24 V to the relevant SCE values.

Cytotoxicity Studies. For cytotoxicity studies the representative cisplatin-sensitive ovarian carcinoma A2780/S human cell line was used; the corresponding cisplatin-resistant A2780/R cell line was produced by repeated 1-h weekly exposure to 50 μM cisplatin of the sensitive parental cell line.[19] Cell lines were maintained in RPMI 1640 medium supplemented with fetal bovine serum (FBS) and antibiotics at 37 °C in a 5% CO_2 atmosphere and subcultured twice weekly. Experiments were conducted on exponentially growing cells. Inhibition of cell growth by the various complexes was monitored through the SRB assay. The SRB assay was performed in 96-well plates using RPMI 1640 medium + 5% FBS, according to the procedure described by Skehan et al.[20]

Results

Structural Features of the Investigated Gold(III) Complexes. Five gold(III) complexes, of different chemical structure, were considered in the present study: Auen, Audien, Aucyclam, Auphen, and Auterpy

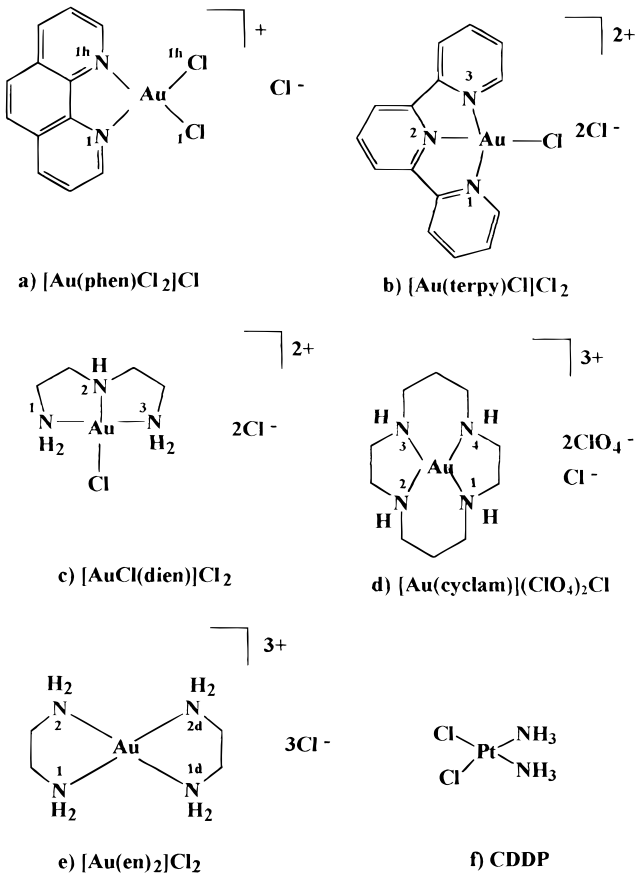

Figure 1. Schematic drawing of the gold(III) complexes used in the present study: (a) $[Au(en)_2]Cl_3$, (b) [AudienCl]Cl_2, (c) $[Aucyclam](ClO_4)_2Cl$, (d) [AuphenCl_2]Cl, and (e) [AuterpyCl]-Cl_2.

(Figure 1). The X-ray crystal structures are available for all compounds − Auen,[21] Audien,[22] Aucyclam,[14] Auterpy[15] − but Auphen; the structure of the latter compound is assumed to match the structure of the cation dicyanogold(III) phenanthroline.[23] Notably, all complexes are characterized by the classical square-planar arrangement of the gold(III) chromophore.[16] Deviations from square-planar geometry are quite large for Auterpy, intermediate for Audien, and generally small for Auen and Aucyclam as shown in Table 1. In all cases the gold(III) center is coordinated to a polydentate ligand with nitrogen donors; in three cases (Audien, Auterpy, and Auphen) there is at least one chloride ion directly bound to the gold(III) center that may act as a good leaving group; in the remaining cases (Auen and Aucyclam) all donors are nitrogen atoms. Au−N distances vary from 1.91 and 2.14 Å; Au−Cl distances fall in the range 2.23−2.28 Å.

Behavior of the Complexes in Solution: Mass Spectra. The behavior in solution of the investigated gold(III) complexes was first studied through electrospray mass spectrometry (ESMS), a very powerful and appropriate technique for the analysis and the identification of inorganic species existing in solution.[24] ES mass spectra were carried out on freshly prepared aqueous solutions of the various gold(III) complexes. In the case of Auen the ES mass spectrum clearly reveals intense peaks corresponding to the $[Au(en)_2]^+$ species (m/z 315) and to the species deriving from the addition of one or two HCl moieties to $[Au(en)_2]^+$ (m/z 351 and

Table 1. Crystallographic Data: Bond Lengths and Bond Angles for the Investigated Gold(III) Complexes

compd	angles (deg)				bond lengths (Å)			ref
Auterpy	N1	Au	N2	81.36	N1	Au	2.029	15
	N2	Au	N3	81.37	N2	Au	1.932	
	N3	Au	Cl	98.49	N3	Au	2.018	
	Cl	Au	N1	98.75	Cl	Au	2.268	
	N2	Au	Cl	176.95				
	N1	Au	N3	162.73				
Audien	N1	Au	N2	84.52	N1	Au	2.048	22
	N2	Au	N3	84.55	N2	Au	2.010	
	N3	Au	Cl	95.83	N3	Au	2.052	
	Cl	Au	N1	95.39	Cl	Au	2.279	
	N2	Au	Cl	177.74				
	N1	Au	N3	166.72				
Aucyclam	N1	Au	N2	86.89−85.43	N1	Au	2.023−2.050	14
	N2	Au	N3	94.00−96.10	N2	Au	1.959−1.928	
	N3	Au	N4	84.14−83.26	N3	Au	2.108−2.075	
	N4	Au	N1	94.98−94.93	N4	Au	2.103−2.090	
	N1	Au	N3	179.11−174.27				
	N2	Au	N4	177.89−177.04				
Auen	N1	Au	N2	84.58	N1	Au	2.042	21
	N2	Au	N2d	95.48	N2	Au	2.035	
	N2d	Au	N1d	84.52	N1d	Au	2.035	
	N1d	Au	N1	95.48	N2d	Au	2.042	
	N1	Au	N2d	180.00				
	N2	Au	N1d	179.97				

387). The ES mass spectrum of Audien is dominated by the peak corresponding to the $[Au(dien)Cl]^+$ species (*m*/*z* 334). In the case of Auterpy intense peaks are detected corresponding either to $[Au(terpy)]^+$ (*m*/*z* 430) or $[Au(terpy)Cl]^+$ (*m*/*z* 465). In the case of Auphen a rather weak peak is observed corresponding to $[Au(phen)Cl_2]^+$ (*m*/*z* 447) as well as a more intense peak corresponding to the $[Au(phen)ClOH]^+$ species (*m*/*z* 429).

Behavior of Gold(III) Complexes in the Buffer: Electronic Spectra. When dissolved in the reference physiological buffer (50 mM sodium phosphate, 4 mM NaCl, pH 7.4) all complexes are characterized by rather intense bands in the visible, which are assigned as ligand-to-metal charge-transfer transitions characteristically associated to the gold(III) center.[14] In the cases of Auen, Audien, and Aucyclam the main band in the visible was previously assigned as NH^- to a gold(III) charge-transfer band.[14] Notably these spectral features appear only at relatively high pH values (pH > 6−7) at which ligand deprotonation has fully occurred. The electronic spectra of the various complexes in the buffer were monitored over 72 h after mixing; electronic spectra for all compounds at mixing and after 72 h are shown in Figure 2. It is apparent that the electronic spectra of these compounds are essentially stable with time pointing out that, in all cases, the gold center remains in the 3+ oxidation state. The minor spectral changes that are generally observed within the first hours may be ascribed either to progressive hydrolysis of gold(III)-bound chloride ions or to partial reduction of gold(III) to metallic gold. In all cases, however, loss of spectral intensity is lower than 10% of the original intensity within the observation period of 72 h.

Potentiometric Studies. The hydrolysis of the three complexes bearing gold-coordinated chloride groups (Audien, Auterpy, and Auphen) was followed potentiometrically. We found that, upon dissolution of the three complexes within a chloride-free medium (50 mM phosphate buffer, pH 7.4), at millimolar concentration, chloride hydrolysis may be directly monitored by use of a chloride-selective electrode. At room temperature chloride hydrolysis reaches completion within about 40 min for Auterpy and 60 min for Auphen. In the latter case the process is clearly biphasic with a fast phase of about 10 min and a slow phase of 60 min. Notably, no change in chloride concentration is observed in the case of Audien suggesting that the dominant species in solution is $[AudienCl]^{2+}$; this finding is in nice agreement with the above mass spectrometry results and with previous literature results.[13]

Table 2. Peak Potential Values (vs ENH) for the Reduction of the Present Gold(III) Complexes at a Platinum Electrode

complex	E_p[a] (V)	solvent
$[Au(dien)Cl]^{2+}$	+0.19	aqueous solution[b]
$[Au(en)Cl]^{2+}$	+0.15	aqueous solution[b]
$[Au(terpy)Cl]^{2+}$	+0.62	aqueous solution[b]
	+0.31	DMSO
$[Au(phen)Cl_2]^+$	+0.80	aqueous solution[b]
	+0.66	DMSO
$[Au(cyclam)]^{3+}$	−0.20	aqueous solution[b]
$[Au(Cl)_4]^-$	+0.55	aqueous solution[b]
	+0.30	DMSO
	+0.15	CH_2Cl_2[c]

[a] Measured at 0.2 V s^{-1}. [b] Buffered at pH 7.4 (see Experimental Section). [c] From ref 24.

Electrochemistry. The inherent electrochemical properties of the investigated gold(III) complexes within a physiological environment were studied through cyclic voltammetry soon after dissolution. As exemplified in Figure 3, which shows the cyclic voltammetric behavior of $[Au(dien)Cl]^{2+}$ in aqueous solution (pH 7.4), all the Au(III) complexes here studied undergo an irreversible reduction, which in controlled potential coulometry involves three electrons per molecule. The occurrence of the Au(III)/Au(0) reduction is confirmed by the appearance of a gold metal deposit at the platinum electrode surface after exhaustive electrolysis (E_w = −0.2 V). The behavior is somewhat different from that found in nonaqueous solution, in which the Au(III) reduction proceeds through two separated Au(III)/Au(I) and Au(I)/Au(0) steps.[25] This arises from the well-known disproportionation of Au(I) in aqueous solution. Table 2 summarizes the electrode potentials of the Au(III) reduction of the present complexes. In some cases the redox pathway was also tested in nonaqueous DMSO solution. In general, the present gold(III) complexes are relatively stable under physiological conditions while retaining, in some cases, significant oxidizing properties. The most stable complexes are those with polyamine ligands: i.e., $[Au(en)_2]^{3+}$ and $[Au(dien)Cl]^{2+}$; particularly stable is $[Au(cyclam)]^{3+}$, in which the macrocyclic effect strongly stabilizes the Au(III) oxidation state.

We also checked the stability of the five gold(III) compounds in the reference buffer following addition of stoichiometric amounts of the biologically important reducing agent sodium ascorbate. It is observed that sodium ascorbate causes rapid reduction of both Auphen and Auterpy (accompanied by disappearance of the respective LMCT bands) but does not affect the electronic spectra of Auen, Audien, and Aucyclam, in nice agreement with the electrochemical results (data not shown). A more careful analysis of the spectral profiles obtained by treatment of Auphen and Auterpy with ascorbate and comparison with the spectral profiles of the same complexes upon reaction with other reductants

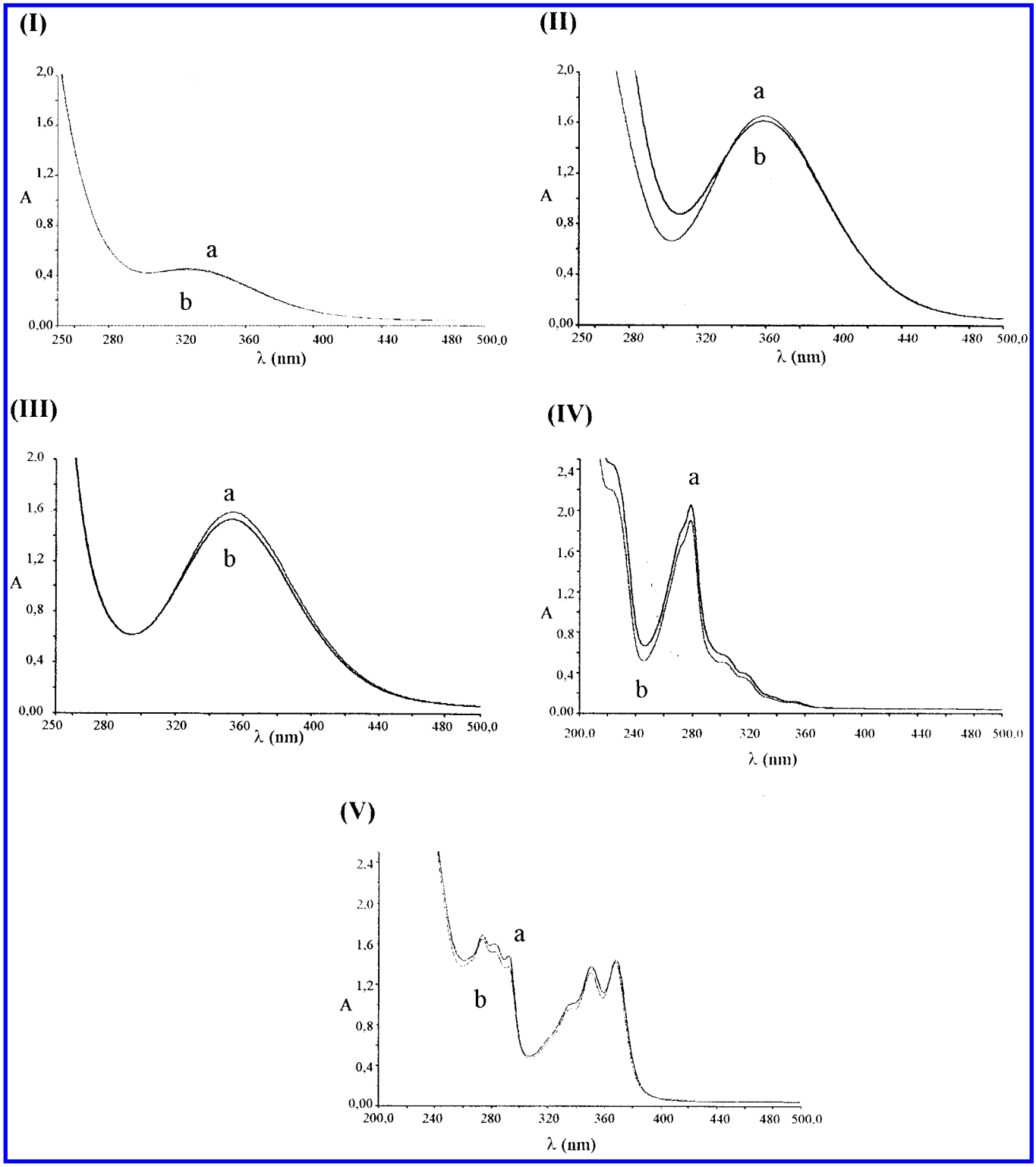

Figure 2. Electronic spectra with time of the five gold(III) complexes following dissolution in the buffer: (I) $[Au(en)_2]Cl_3$, 1×10^{-3} M at mixing (a) and after 72 h (b); (II) $[AudienCl]Cl_2$, 1×10^{-3} M at mixing (a) and after 1 h (b); (III) $[Aucyclam](ClO_4)_2Cl$, 1×10^{-3} M at mixing (a) and after 72 h (b); (IV) $[AuphenCl_2]Cl$, 6×10^{-5} M at mixing (a) and after 72 h (b); (V) $[AuterpyCl]Cl_2$, 1.2×10^{-4} M at mixing (a) and after 72 h (b).

(such as sodium thiosulfate) permit to establish that, upon reduction, the free terpyridine and phenanthroline ligands are released, while gold(III) ions are reduced to colloidal gold (data not shown).

Cytotoxicity Results on the A2780 Cell Line. Given their reasonable stability under physiological conditions, the cytotoxic properties of the investigated gold(III) complexes were evaluated toward the established A2780 ovarian human cell line either sensitive (A2780/S) or resistant (A2780/R) to cisplatin. The experiments were carried out following the sulforhodamine B procedure[20] and data analyzed through standard methods. Cells were exposed to the cytotoxic agent for 72 h. Results are shown in Table 3 and Figure 4. It is apparent that all the investigated complexes but Aucyclam exhibit relevant cytotoxic properties with IC_{50}

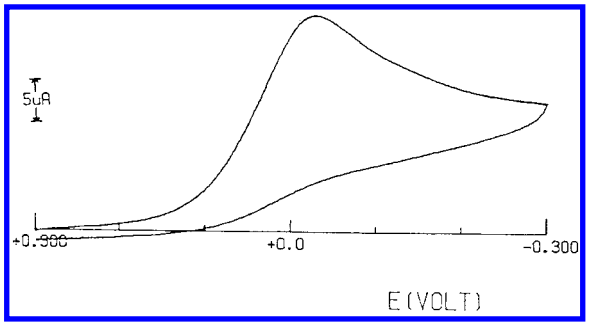

Figure 3. Cyclic voltammogram recorded at a platinum electrode on an aqueous solution (50 mM sodium phosphate, 4 mM NaCl, pH 7.4) of $[Au(dien)Cl]^{2+}$ (1.1×10^{-3} mol dm^{-3}); scan rate 0.2 V s^{-1}.

Table 3. IC_{50} Values of the Various Gold(III) Complexes against the Human Ovarian Carcinoma A2780 Cell Lines Sensitive or Resistant to Cisplatin

	IC_{50} (μM) (±SE)[a]				
complex	A2780/S	expt no.	A2780/R	expt no.	R/S
$AuCl_3(en)_2$	8.36 ± 0.77	3	17.0 ± 4.24	3	2.03
AuCl(dien)	8.2 ± 0.93	3	18.7 ± 2.16	3	2.28
Au(cyclam)	99.0	2	>120.0	3	
en, dien, cyclam	>120.0	2	>120.0	2	
Au(terpy)	0.2	1	0.37 ± 0.032	3	1.23
Au(phen)	3.8 ± 1.1	5	3.49 ± 0.91	5	0.92
terpy	0.125	1	0.36	2	2.88
phen	3.66 ± 1.52	3	2.73 ± 0.16	3	0.75
CDDP	1.22 ± 0.43	8	14.16 ± 2.72	8	11.6

[a] Expressed as mean ± SE of at least three determinations or mean of two determinations.

values falling between 9 and 0.2 μM for the cisplatin-sensitive line. Notably, Auterpy showed the highest cytotoxicity; to determine with more accuracy the IC_{50} value of the latter compound, an additional experiment was carried out to cover the 1×10^{-8} to 1×10^{-6} M concentration range (Figure 5).

The order of the cytotoxic potency of the investigated gold(III) complexes coming out from our experiments is the following: Auterpy ≫ Auphen > Auen, Audien ≫ Aucyclam. Remarkably Auen, Auphen, Auterpy, and Audien retain most of their cytotoxic properties when tested on the cisplatin-resistant line, ruling out the occurrence of important cross-resistance phenomena.

The cytotoxic properties of the free ligands were also tested. We found that terpyridine and *o*-phenanthroline used as such also exhibit important cytotoxic properties, whereas the other three ligands (ethylenediamine, diethylenetriamine, and cyclam) are virtually devoid of any intrinsic cytotoxicity (see Table 3). These results strongly support the view that cytotoxicity, for Auen and Audien, can be quite safely ascribed to the presence of the gold(III) center.

Discussion

Owing to their structural and electronic relatedness to platinum(II) complexes, gold(III) complexes represent potentially attractive agents for cancer treatment.[1,2] We have investigated here the solution chemistry and the cytotoxic properties of five representative gold(III) complexes, of different chemical structure, to gain preliminary insight into structure−function relationships for this class of compounds.

Solution Chemistry of the Investigated Compounds. The chemical characterization carried out in aqueous solution allowed us to establish that all the investigated gold(III) complexes are reasonably stable within a physiological-like environment, this representing the essential prerequisite for any further pharmacological evaluation. The minor spectral changes observed with time in the visible spectra of samples dissolved in the buffer are ascribed either to chloride hydrolysis or to partial reduction to metallic gold or to possible oligomerization phenomena.

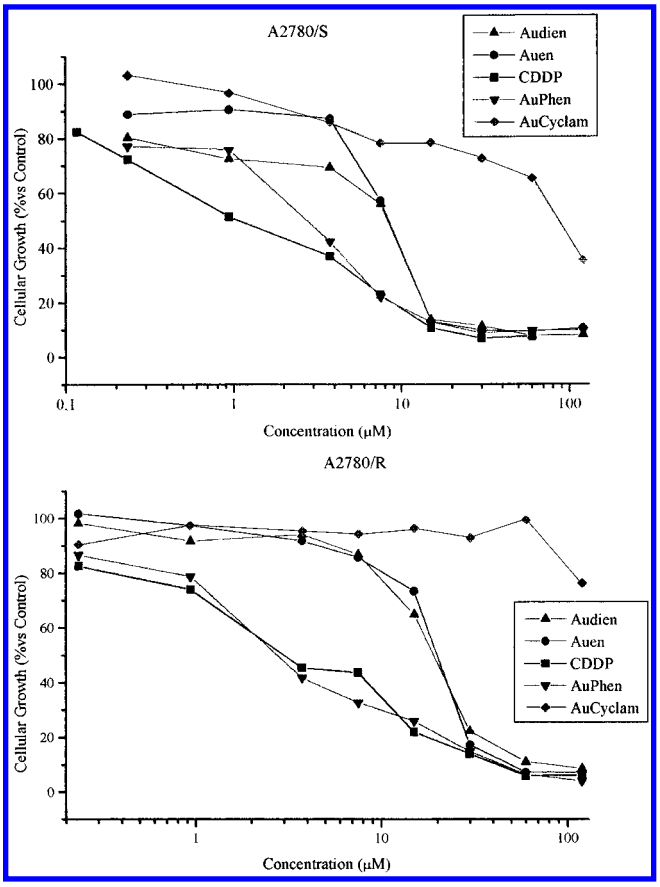

Figure 4. Drug sensitivity profiles of cisplatin-sensitive and -resistant human tumor cell lines (A2780/S and A2780/R ovarian carcinoma) toward the complexes $[Au(en)_2]Cl_3$, $[AudienCl]Cl_2$, $[Aucyclam](ClO_4)_2Cl$, and $[AuphenCl_2]Cl$. Graphs show the percentage of growth compared to control upon incubation of increasing amounts of the gold(III) complexes. For comparison purposes the CDDP curves are reported.

More detailed information on the solution chemistry of the various gold(III) complexes has been gained by selective potentiometric measurements monitoring the release of gold-coordinated chlorides. Notably hydrolysis studies point out that complete chloride hydrolysis takes place within 40 min for Auphen and within 60 min for Auterpy; at variance no significant hydrolysis is detected in the case of Audien. These results suggest that the dominant species in physiological solution are $[AudienCl]^{2+}$, $[Auphen(OH)_2]^{+}$, and $[AuterpyOH]^{2+}$. In all cases, gold remains in the 3+ oxidation state due to the stabilization effects played by the polydentate ligands. These expectations have been largely confirmed by the ESMS studies.

The acceptable stability of the present gold(III) complexes in solution is further confirmed by electrochemical measurements showing that the reduction potentials of these compounds lie in the 600−200 mV range, well

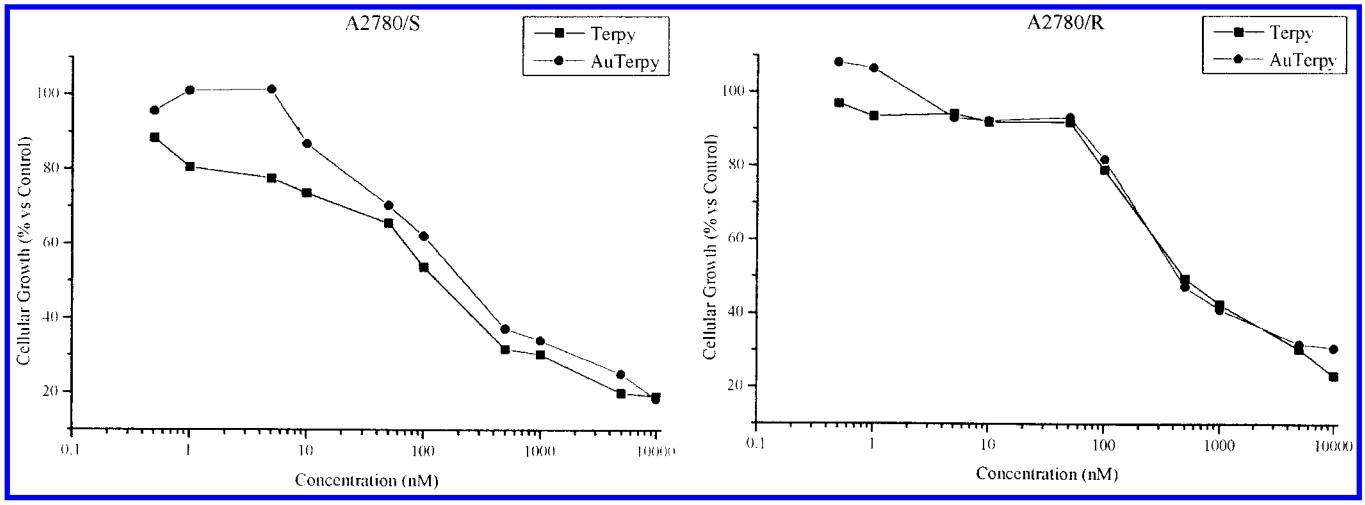

Figure 5. Cytotoxicity profiles of cisplatin-sensitive and -resistant human tumor cell lines (A2780/S and A2780/R) toward the complex [AuterpyCl]Cl_2 are shown apart, since the range of concentration used is larger than the previous one.

below the typical value of the Au(III)/Au(0) couple ($E°$ = 1.43 V). Indeed, coordination by polyamines induces a large stabilization of the 3+ oxidation state of the gold center owing to the electron-donating ability of the amine ligand;[26] as expected, cyclam induces an even larger stabilization as a consequence of the "chelate effect".[27] At variance the less basic terpy and phen ligands induce a significantly lower stabilization of gold(III): Auterpy and Auphen with reduction potentials of 0.62 and 0.80 V still exhibit discrete oxidizing properties and are quickly reduced by sodium ascorbate.

In Vitro Cytotoxic Properties. Cytotoxicity studies, carried out according to the protocol of Skehan, point out that all the investigated complexes but Aucyclam exhibit important cell-killing properties toward the reference A2780 line, with IC_{50} values falling in the micromolar range (from 0.3 to 10 μM). Notably the IC_{50} value of Auterpy even exceeds that of cisplatin; instead the IC_{50} values measured for Auen, Audien, and Auphen are slightly higher than those of cisplatin. When tested on the corresponding cisplatin-resistant line A2780/R, the latter three complexes showed only minor cross-resistance effects and turned out to be significantly more toxic than cisplatin itself.

The cytotoxicity of the free ligands was measured on the same cell line as well; whereas en, dien, and cyclam are virtually nontoxic, terpy and phen were found to be highly cytotoxic with IC_{50} values matching or even exceeding those of the corresponding gold(III) complexes. In light of these findings the analysis of the cytotoxic properties of Auen, Audien, and Aucyclam is quite straightforward; much more caution must be used in interpreting the results obtained with Auterpy and Auphen.

Cytotoxic Properties of Auen, Audien, and Aucyclam. The results obtained for this group of polyamine complexes suggest that the presence of hydrolyzable chloride groups or, more in general, of good leaving groups on the gold(III) center does not represent *an essential requirement* for biological activity. In fact Auen, with no gold-bound chlorides, is as cytotoxic as Audien, bearing a gold-coordinated chloride. Also, it is worth observing that Auen and Audien are significantly more cytotoxic than the isostructural $[Pt(en)_2]^{2+}$ and [Pt(dien)Cl]$^+$ compounds; indeed, IC_{50} values of 1684 and 271 μM were previously determined respectively for $[Pt(en)_2]Cl_2$ and [Pt(dien)Cl]Cl on the cell line L1210 (cisplatin IC_{50} value against the same line is 2.3 μM);[28] this observation suggests that the gold(III) center plays a specific role in producing the cytotoxic effects. Conversely, Aucyclam, with the gold(III) center tightly bound to the macrocycle cage, is poorly cytotoxic probably as a consequence of its low reactivity.

Given the generally high reduction potentials of gold(III) complexes, it might be hypothesized that the observed cell-killing effects are somehow correlated to oxidative cell damage; however, a direct comparison of the IC_{50} values with the measured reduction potentials shows that there is not always a positive correlation between these two series of parameters: for instance, Auen and Audien with low reduction potentials are equally (or even more) cytotoxic as $[AuCl_4]^-$ or Auhpm[5] despite the significant differences in the redox potentials. IC_{50} values of 11.0 ± 2.0 and 10.1 ± 1.0 μM were previously measured for $[AuCl_4]^-$ and Auhpm on the A2780/S cell line ($E°$ of Auhpm in aqueous solution is ≈0.57 V, unpublished result of this laboratory). Moreover one must consider that exceedingly high redox potentials will lead most likely to rapid in vivo reduction of the compound by physiologically occurring reducing agents and/or to direct membrane oxidative damage.

Overall the results reported here point out that: (i) the cytotoxicity of these gold(III) complexes is associated with the presence of the gold(III) center (indeed Auen and Audien are significantly more cytotoxic than the corresponding platinum compounds); (ii) the presence of good leaving groups such as metal-bonded chlorides is not essential for activity; (iii) there is no direct correlation between cytotoxicity and the reduction potentials; (iv) excessive stabilization of the gold(III) center results into loss of biological activity (see the case of Aucyclam).

Auphen and Auterpy. The cases of Auphen and Auterpy deserve more extensive analysis. Given the high toxicity of the free ligands, it is possible that the biological effects of these gold(III) complexes are mediated by the ligand itself following complex disruption.

The cytotoxic properties of the ligands terpyridine and *o*-phenanthroline, previously documented on similar in

vitro models,[29,30] have been confirmed by our study. The mechanisms mediating the cytotoxic effects of terpyridine and *o*-phenanthroline yet are not fully understood. For phenanthroline it is hypothesized that the biological effects should be ascribed to formation of the cuprous complex characterized by relevant DNA-damaging properties.[31] The biological effects of terpyridine may be due to its ability to induce metal-deficiency states or to the formation of metal complexes that themselves are growth inhibitory.[29]

Also, we have shown that the 3+ oxidation state of the gold center in both Auphen and Auterpy is pretty stable under physiological conditions (in the absence of reducing agents) but undergoes facile reduction when exposed to ascorbate or thiosulfate. Thus, the important cytotoxic effects observed for Auphen and Auterpy might well arise from the free ligand following in vivo reduction of the complex and release of the ligand itself. A similar situation was previously described by McFayden et al. when comparing the cytotoxic properties of a series of bipyridine, *o*-phenanthroline, and terpyridine platinum complexes to those of the free ligands.[29] In any case an intrinsic and specific cytotoxic effect of the gold(III) complex, or of some related species, cannot be ruled out at the present state of the knowledge. More extensive experimental work is required to shed light on the molecular mechanisms of the cytotoxic action of these compounds and to separate the specific biological effects of the free ligands and of the corresponding gold(III) complexes.

Concluding Remarks

In conclusion this work demonstrates that some selected gold(III) complexes are reasonably stable under physiological conditions and exhibit relevant cytotoxic properties when tested on the reference human tumor cell line A2780. Notably gold(III) complexes retain most of their cytotoxic properties when tested on the corresponding cisplatin-resistant line (A2780/R) suggesting that they are able to overcome platinum-resistance mechanisms. Some preliminary correlations between cytotoxicity and the chemical structure have been proposed. It is worth noting that preliminary COMET results obtained in our laboratory on Audien and Auphen suggest that both complexes are capable of producing direct DNA damage. Occurrence of a direct interaction of the present gold(III) complexes with DNA is also supported by CD and DNA melting experiments on calf thymus DNA.

In light of the obtained results Auen and Audien seem to be good candidates for further pharmacological evaluation and for in vivo testing, whereas more extensive investigations are needed, in our opinion, for Auterpy and Auphen to better understand the basis of the cytotoxic effects.

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References

(1) Sadler, P. J. The biological chemistry of gold: a metallo-drug and heavy-atom label with variable valency. *Struct. Bond.* **1976**, *29*, 171−210.
(2) Ni Dhubhghaill, O. M.; Sadler, P. J. Gold complexes in cancer chemoterapy. In *Metal Complexes in Cancer Chemoterapy*; Keppler, B. K., Ed.; VCH: Weinheim, 1993; pp 221−248.
(3) Sadler, P. J.; Sue, R. E. The Chemistry of gold drugs. *Metal Based Drugs* **1994**, *1*, 107−144.
(4) Buckley, R. G.; Elsome, A. M.; Fricker, S. P.; Henderson, G. R.; Theobald, B. R. C.; 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.
(5) Calamai, P.; Carotti, S.; Guerri, A.; Mazzei, T.; Messori, L.; Mini, E.; Orioli, P.; Speroni, G. P. Cytotoxic effects of gold(III) complexes on established human tumor cell lines sensitive and resistant to cisplatin. *Anticancer Drug Des.* **1998**, *13*, 67−80.
(6) Cossu, F.; Matovic, Z.; Radanovic, D.; Ponticelli, G. Cytotoxic activity of some gold(III) complexes. *Farmaco* **1994**, *49*, 301−302.
(7) Mirabelli, C. K.; Johnson, R. K.; Sung, C. M.; Faucette, L. F.; Muirhead, K.; Crooke, S. T. Evaluation of the in vivo antitumor activity and in vitro cytotoxic properties of auranofin, a coordinated gold compound, in murine tumor models. *Cancer Res.* **1985**, *45*, 32−39.
(8) Crooke, S. T.; Mirabelli, C. K. Molecular mechanisms of action of auranofin and other gold complexes as related to their biological activities. *Am. J. Med.* **1983**, *75*, 109−113.
(9) Parish, R. V.; Howe, B. P.; Wright, J. P.; Mack, J.; Pritchard, R. G.; Buckley, R. G.; Elsome, A. M.; Fricker, S. P. Chemical and biological studies of dichloro(2-((dimethylamino)methyl)phenyl)-gold(III). *Inorg. Chem.* **1996**, *35*, 1659−1666.
(10) Calamai, P.; Guerri, A.; Messori, L.; Orioli, P.; Speroni, G. P. Structure and DNA binding properties of the gold(III) complex $[AuCl_2(esal)]$. *Inorg. Chim. Acta* **1999**, *285*, 309−312.
(11) Carotti, S.; Guerri, A.; Mazzei, T.; Messori, L.; Mini, E.; Orioli, P. Gold(III) compounds as potential antitumor agents: cytotoxicity and DNA binding properties of some selected polyamine-gold(III) complexes. *Inorg. Chim. Acta* **1998**, *281*, 90−94.
(12) Block, B. P.; Bailar, J. C. The reaction of gold(III) with some bidentate coordinating groups. *J. Am. Chem. Soc.* **1951**, *73*, 4722−4725.
(13) Nardin, G.; Randaccio, L.; Annibale, G.; Natile, G.; Pitteri, B. Comparison of structure and reactivity of bis(2-aminoethyl)-amine- and bis(2-aminoethyl)amido-chlorogold(III) complexes. *J. Chem. Soc., Dalton Trans.* **1979**, 220−223.
(14) Kimura, E.; Kurogi, Y.; Takahashi, T. The first gold(III) macrocyclic polyamine complexes and application to selective gold-(III) uptake. *Inorg. Chem.* **1991**, *30*, 4117−4121. Kimura, E.; Kurogi, Y.; Koike, T.; Shionoya, M.; Iitaka, Y. X-ray structure of $[Au(cyclam)](ClO_4)_2Cl$. *J. Coord. Chem.* **1993**, *28*, 33−36.
(15) Hollis, L. S.; Lippard, S. J. Aqueous chemistry of (2,2′,2"-Terpyridine)gold(III). Preparation and structures of $[Au(terpy)Cl]Cl_2 \cdot 3H_2O$ and the mixed valence Au(I)-Au(III) salt $[Au(terpy)Cl][AuCl_2]_3[AuCl_4]$. *J. Am. Chem. Soc.* **1983**, *105*, 4293−4299.
(16) Puddephatt, R. J. *The Chemistry of Gold*; Elsevier: Amsterdam, 1978.
(17) Zanello, P.; Laschi, F.; Fontani, M.; Mealli, C.; Ienco, A.; Tang, K.; Jin, X.; Li, L. Redox behavior of the molybdenum and tungsten metallafullerenes $M(\eta^2\text{-}C_{60})(CO)_2(phen)(dbm)$ (phen = 1,10-phenanthroline; dbm = dibutyl maleate): (spectro)electrochemistry and theoretical consideration. *J. Chem. Soc., Dalton Trans.* **1999**, 965−970.
(18) Diggle, J. W.; Parker, A. J. Ferricinium couple and its role in the estimation of free energies of transfer of single ions. *Electrochim. Acta* **1973**, *18*, 975−979.
(19) Lu, Y.; Han, J.; Scanlon, K. J. Biochemical and molecular properties of cisplatin-resistant A2780 cells grown in folinic acid. *J. Biol. Chem.* **1988**, *263*, 4891−4894.
(20) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; Mcmahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.
(21) Minacheva, L. Kh.; Gladkaya, A. Sh.; Sakharova, V. G.; Don, G. M.; Shchelkov, R. N.; Porai-Koshits, M. A. Structure of bis-(ethylendiamine)gold(III) determined by single-crystal X-ray Diffraction. *Zh. Neorg. Khim.* **1988**, *233*, 683−687.

(22) Elder, R. C.; Watkins, J. W., II. Structure of trichloro(diethylenetriamine)gold(III), [Au(dien)Cl]Cl_2, determined by single-crystal X-ray Diffraction, Raman, and EXAFS spectroscopies: an EXAFS caveat. *Inorg. Chem.* **1986**, *25*, 223.
(23) Marangoni, G.; Pittieri, B.; Bertolasi, V.; Ferretti, V.; Gilli, G. Crystal structures and properties of [Au(phen)(CN){$(CN)_{0.82}$-$Br_{0.18}$}]·0.5trans-[Au$(CN)_2Br_2$]·0.5Br·phen (phen = 1,10-phenanthroline) obtained by disproportionation of five-coordinate bromodicyano(1,10-phenanthroline)gold(III). Two examples of secondary coordination and CN/Br disorder in square-planar gold(III) complexes. *J. Chem. Soc., Dalton Trans.* **1987**, 2235−2240.
(24) Hop, C. E. C. A.; Bakhtiar, R. Electrospray Ionization Mass Spectrometry. *J. Chem. Educ.* **1996**, *73*, A162−A169.
(25) Koelle, U.; Laguna, A. Electrochemistry of Au-complexes. *Inorg. Chim. Acta* **1999**, *290*, 44−50.
(26) Solomons, T. W. G. *Fundam. Org. Chem.* **1994**.
(27) Kettle, S. F. A. *Phys. Inorg. Chem.* **1998**.
(28) Macquet, J. P.; Butour J. L. Platinum-amine compounds: importance of the labile and inert ligands for their pharmacological activities toward L1210 leukemia cells. *J. Natl. Cancer Inst.* **1983**, *70*, 899−905.
(29) McFayden, W. D.; Wakelin, L. P.; Roos, I. A.; Leopold, V. A. Activity of platinum(II) intercalating agents against murine leukemia L1210. *J. Med. Chem.* **1985**, *28*, 1113−1116.
(30) Veal, J. M.; Rill, R. L. Noncovalent DNA binding of bis(1,10-phenanthroline)copper(I) and related compounds. *Biochemistry* **1991**, *30*, 1132−1140.
(31) Sigman, D. S.; Landgraf, R.; Perrin, D. M.; Pearson, L. Nucleic acid chemistry of the cuprous complexes of 1,10-Phenanthroline and derivatives. *Metal Ions Biol. Syst.* **1996**, *33*, 485−510.

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