Structural and Solution Chemistry, Antiproliferative Effects, and DNA and Protein Binding Properties of a Series of Dinuclear Gold(III) Compounds with Bipyridyl Ligands

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Received April 12, 2006

A series of six dinuclear gold(III) oxo complexes with bipyridyl ligands, of general formula $[Au_2(N,N)_2(\mu\text{-}O)_2][PF_6]_2$ (Auoxo1−Auoxo6) [where N,N = 2,2′-bipyridine (**1**), 4,4′-di-*tert*-butyl- (**2**), 6-methyl- (**3**), 6-neopentyl- (**4**), 6-(2,6-dimethylphenyl)- (**5**), 6,6′-dimethyl-2,2′-bipyridine (**6**)], were investigated as potential cytotoxic and anticancer agents, and their antiproliferative properties were evaluated in vitro toward the reference A2780 human ovarian carcinoma cell line. While five compounds manifested moderate cytotoxic properties (with $IC_{50} \approx 10-30$ μM), the sixth one (Auoxo6), turned out to be ∼5−15 times more active against both cell lines and will merit further pharmacological studies. The interactions of Auoxo1 and Auoxo6 with a few model proteins (serum albumin, cytochrome *c*, ubiquitin) and with calf thymus DNA were analyzed in detail by various spectroscopic methods. Both tested compounds manifested a high and peculiar reactivity toward the mentioned model proteins; specific differences were detected in their reactivity with DNA. The mechanistic implications of these results are discussed.

Introduction

During the past decade, gold(III) compounds have attracted the interest of various research groups for their encouraging cytotoxic and antitumor properties.[1−3] Through implementation of appropriate ligand selection strategies, a number of gold(III) compounds have been obtained, exhibiting sufficient stability under physiological-like conditions and manifesting, in some cases, relevant cytotoxic properties in vitro.

The following classes of mononuclear gold(III) complexes were characterized in detail: gold(III) polyamines,[4] gold(III) polypyridines,[5,6] gold(III) porphyrins,[7] various organogold(III) compounds, and gold(III) dithiocarbamate complexes.[8,10] Notably, most of the above-mentioned compounds turned out to be highly cytotoxic when tested in vitro on selected human tumor cell lines, with IC_{50} values generally falling in the low micromolar range.[11,12]

The initial efforts toward the synthesis and the biological evaluation of anticancer gold(III) compounds were mainly driven by the analogy with platinum(II)-based drugs. However, in contrast to early expectations, a large body of experimental evidence now suggests that gold(III) compounds produce their biological and antiproliferative actions through mechanisms that are profoundly distinct from those of platinum(II) compounds.[1,13] Most likely, DNA does not represent the primary target for many gold(III) compounds; a rather poor affinity toward calf thymus DNA was indeed measured for a number of gold(III) compounds.[14] Accordingly, various cytotoxic gold(III) compounds were indeed reported to affect, only scarcely, the cell cycle of treated cells.[15] On the basis of some new experimental evidence, the large proapoptotic effects that gold(III) compounds cause in vitro were proposed to be the consequence of a direct interference with mitochondrial functions.[16]

A common strategy in the field of anticancer metallodrugs consists of the design and preparation of dimetallic or polymetallic compounds derived from the "fusion" of two or more monometallic molecular fragments in which the specific reactivity of each metal center is further controlled by its interactions with the nearby metal center(s) and by the overall molecular framework. This strategy has been successfully pursued by Farrell and his group in the case of anticancer platinum(II) compounds.[17] Notably, incorporation of two (or more) metal centers within an extended molecular framework may greatly affect the overall charge of the resulting polynuclear compound, its redox properties, the kinetics of hydrolysis, and its specific reactivity toward biomolecules in comparison to mononuclear analogues.

We report here on a series of six, structurally related, dinuclear gold(III) compounds derived from the condensation of two square-planar gold(III) moieties whose chemical characterization was achieved both in the solid state and in solution.[18−23] In all cases, each gold(III) center is linked to two nitrogen atoms of a bidentate 2,2′-bipyridine ligand and to two adjacent "bridging" oxygen atoms.[18]

The antiproliferative properties of these compounds have been measured on the reference human ovarian carcinoma cell line A2780. Moreover, for two representative compounds of this series, the reactivity with calf thymus DNA and with a few model proteins has been analyzed in detail according to specific protocols developed in our laboratory. These latter studies are specifically directed to elucidate the molecular mechanisms of interaction of dinuclear gold(III) compounds with probable biomolecular targets.

Results

1. Chemistry.

1.1. Structural Chemistry. The six dinuclear gold(III) compounds of this study are shown in Scheme 1. Their synthesis and characterization were carried out as reported in the Experimental Section. Remarkably, all compounds of this series are characterized by the presence of a common structural motif, i.e., the $[Au_2O_2]$ diamond core, supported by two bidentate bipyridyl ligands.

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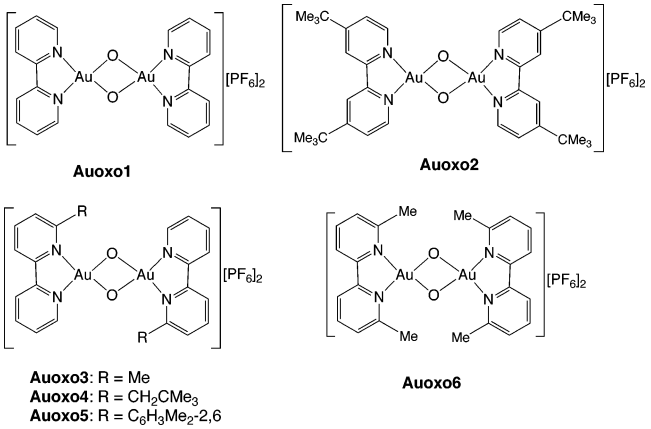
10.1021/jm060436a CCC: $33.50
Published on Web 08/05/2006

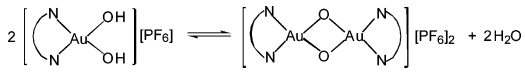
Scheme 1. Schematic Drawings of the Dinuclear Gold(III) Complexes Auoxo[a]



Auoxo3: R = Me
Auoxo4: R = CH_2CMe_3
Auoxo5: R = $C_6H_3Me_2$-2,6

[a] Auoxo3 is a ~1:1 mixture of the cis and trans isomer, while Auoxo4 and Auoxo5 are, as depicted, only trans isomers.

Scheme 2. Proposed Reaction Scheme for the Formation of the Dinuclear Gold(III) Compounds



These dinuclear compounds may be considered as the result of the condensation of two mononuclear square-planar gold(III) units, each bearing an *endo*-bidentate bipyridyl ligand, through formal loss of two water molecules according to the reaction represented in Scheme 2.

The two gold(III) centers are connected through two bridging oxygen atoms; this results in an extended planar system that comprises the two gold atoms and the two bipyridyl moieties. The crystal structure of the trans isomer of Auoxo4 was previously solved by X-ray diffraction.[18] The structure of the other complexes is proposed to be essentially the same on the grounds of a number of analytical and spectroscopic data, the exception being Auoxo3 actually consisting of a ~1:1 mixture of the cis and trans isomers. Thus, the various dinuclear gold(III) complexes only differ for the nature and the position of the substituent on the bipyridyl ligand that confers them, notwithstanding, a significant variability in terms of hydrophobicity/hydrophilicity balance and of overall chemical behavior. For instance, the mononuclear dihydroxo species, $[Au(N,N)(OH)_2][PF_6]$, may be obtained if Auoxo1 and Auoxo2 are refluxed in water over several hours (the reverse of the reaction in Scheme 2).[19] In contrast, the presence of at least one substituent in position 6, i.e., in the proximity of the bridging oxo ligand, prevents the isolation of mononuclear precursors as found for Auoxo3−Auoxo6.[18]

1.2. Solution Chemistry. The solution chemistry of the above compounds was analyzed by absorption UV−visible spectroscopy. The various compounds were first dissolved in DMSO. Notably, all six compounds are highly soluble and stable within this medium, with full retention of their dinuclear structure. Afterward, concentrated DMSO solutions of each dinuclear gold(III) compound (1×10^{-2} M) were diluted in the reference phosphate buffer, at pH 7.4, to final concentrations of 5×10^{-5} to 1×10^{-4} M, and the samples were analyzed spectrophotometrically over 24 h at 37 °C. The resulting spectral profiles

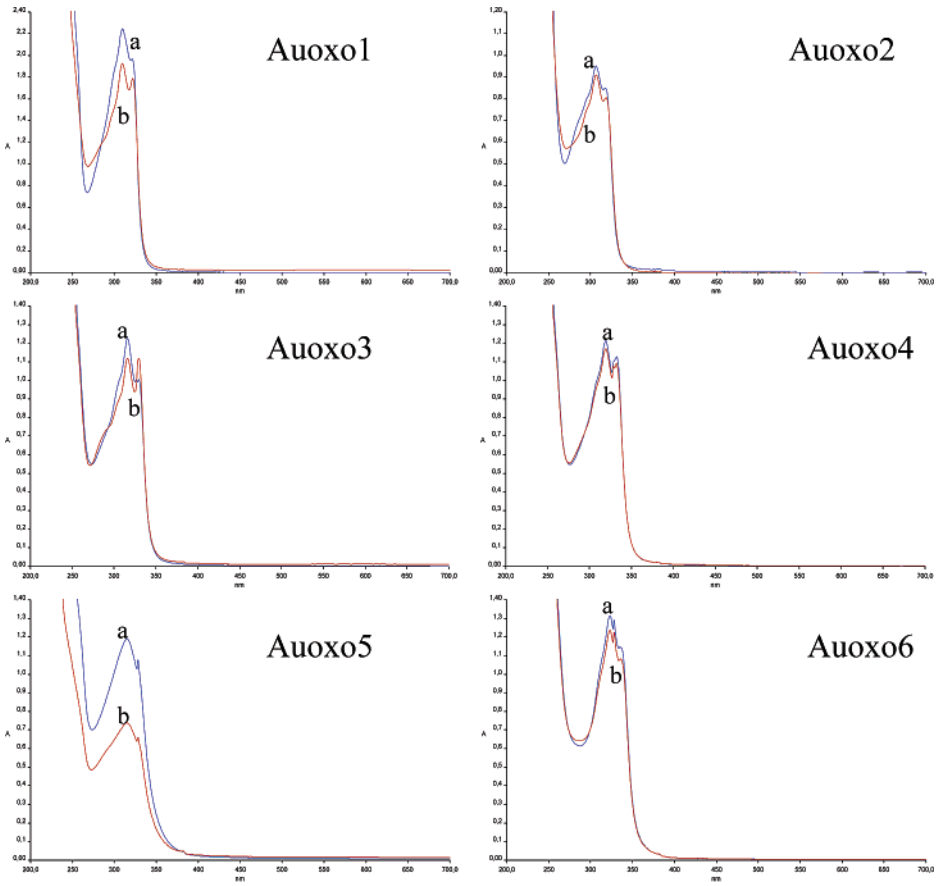


Figure 1. Hydrolysis profiles of the dinuclear Auoxo complexes dissolved in 10 mM phosphate buffer, pH 7.4, at 37 °C at $t = 0$ (a) and after 24 h (b). Concentration of the complex is 10^{-4} M for Auoxo1, Auoxo2, and Auoxo5, while it is 5×10^{-5} M for Auoxo3 and Auoxo4 and is 10^{-5} M for Auoxo6.

Table 1. IC_{50} Values of Dinuclear Organogold(III) Compounds, Auoxo, Compared to the Mononuclear $[Au(bipy)(OH)_2]^+$ (AuOH1) and Cisplatin (CDDP)

	IC_{50} (μM)[a]							
cell lines	CDDP	AuOH1	Auoxo1	Auoxo2	Auoxo3	Auoxo4	Auoxo5	Auoxo6
A2780/S	2.1 ± 0.2	8.4 ± 0.1	22.8 ± 1.53	12.1 ± 1.5	25.4 ± 2.47	12.7 ±1.06	11 ± 1.5	1.79 ± 0.17
A2780/R	24.4 ± 0.1	14.9 ± 0.3	23.3 ± 0.35	13.5 ± 1.8	29.8 ± 3.1	19.8 ± 1.8	13.2 ± 1.2	4.81 ± 0.5

[a] Mean ± SE of at least three determinations or mean of three independent experiments performed with quadruplicate cultures at each concentration tested.

for all six compounds are shown in Figure 1. All six compounds exhibit intense transitions in the 300−400 nm range, characteristic of the gold(III) chromophore, that may be straightforwardly assigned as LMCT bands. In most cases, these transitions remain substantially stable over 24 h (when gold(III) compounds are diluted within the phosphate buffer), implying a substantial stability of the dinuclear gold(III) chromophore under the present solution conditions. In most cases, some very small spectral changes are slowly observed with time that might be related to the occurrence of partial hydrolysis processes and/or to formation of oligomeric species. In any case, the dinuclear species are the dominant ones in buffered aqueous solutions. Extensive cleavage of the oxo bridges could only be achieved by applying far more drastic solution conditions. For instance, nearly complete conversion of Auoxo1 into its monomeric $[Au(bipy)(OH)_2]^+$ species (AuOH1) [24] was obtained at 70 °C after ~2 h of incubation.

At variance with the other complexes, Auoxo5 at 37 °C was found to manifest a large, progressive decrease in intensity of its major LMCT band, at 310 nm. After 24 h, this band drops to 60% of its initial intensity, without significant shape modifications. However, these effects could be later ascribed to the occurrence of precipitation phenomena and could be largely reversed by changing the composition of the medium (i.e., by increasing the amount of DMSO).

Finally, the stability of the various dimetallic compounds toward biologically occurring reductants was evaluated. Ascorbic acid (AsA) was selected as the reference reducing agent. We found that AsA, presented at a 2:1 molar ratio, is able to reduce quickly all six dinuclear gold(III) compounds. Occurrence of gold(III) reduction is clearly witnessed by complete disappearance of the LMCT bands characteristic of the gold(III) bipyridyl chromophore. Concomitantly, a broad absorption band appears around 550 nm that may be ascribed to formation of colloidal gold, the final product of gold(III) reduction. For comparison purposes, the effects of glutathione (GSH) were also analyzed. Similar to ascorbic acid, GSH causes the rapid disappearance of the LMCT bands typical of the gold(III) centers, in agreement with the idea of gold(III) reduction. However, in contrast to the case of ascorbic acid, no formation of colloidal gold is observed. Probably the presence of excess glutathione favors the formation of soluble gold(I) thiolate species as a major product of gold(III) reduction.

2. Antiproliferative Properties. The cytotoxic properties of the above dinuclear gold(III) compounds were analyzed in vitro according to the standard procedure described by Skehan et al.[25] working on the A2780 ovarian carcinoma human cell line either sensitive (A2780/S) or resistant (A2780/R) to cisplatin. Results are shown in Figure 2 and Table 1. For comparison purposes the antiproliferative effects of AuOH1 (the mononuclear precursor of Auoxo1) and of CDDP on the same cell lines are also reported. Notably, compounds Auoxo1−Auoxo5 manifested a roughly similar biological behavior and appreciable antiproliferative properties, with IC_{50} values falling in the 10−30 μM range; no relevant cross resistance effects with cisplatin were

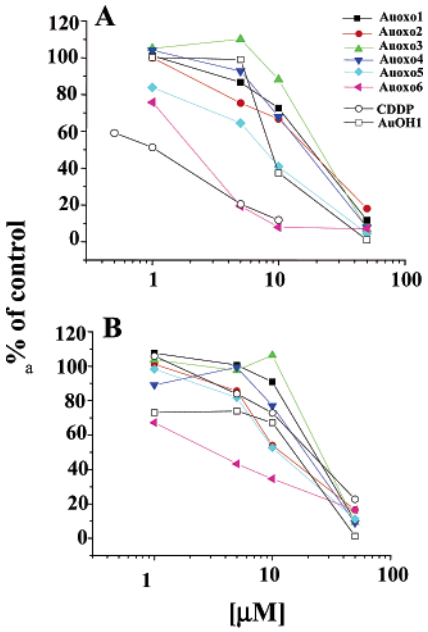


Figure 2. Effects of Auoxo compounds on cell growth of A2780/S (A) and A2780/R (B) cell lines. Cisplatin (CDDP) and AuOH1 sensitivity profiles are also shown. Reported data are from a single representative experiment.

observed. They also turned out to be slightly less effective than AuOH1 on the cisplatin sensitive line. In contrast, Auoxo6 was far more active on both cell lines with an IC_{50} value of ~2 μM for the sensitive line and ~5 μM for the resistant one. Notably, Auoxo6 turns to be about 5 times more active than cisplatin on the CDDP resistant line.

3. Mechanistic Studies. To gain further insight into the specific reactivity of these dinuclear gold(III) compounds with potential biomolecular targets, the reaction patterns of two representative members of the series (i.e., Auoxo1 and Auoxo6) with calf thymus DNA and with a few model proteins were analyzed in detail by various spectroscopic methods.

3.1. Reactions with Calf Thymus DNA. The interactions of Auoxo1 and Auoxo6 with calf thymus DNA were investigated according to established procedures of our laboratory that couple spectroscopic and analytical determinations. Auoxo1 and Auoxo6 were dissolved in the reference phosphate buffer at 37 °C. Afterward, an appropriate amount of calf thymus DNA was added in order to achieve an $r = 0.1$ final stoichiometry (where r is the gold compound/base pair ratio) and the samples were analyzed continuously over 24 h. Remarkably, substantially different spectroscopic patterns were found in the two cases as detailed below.

Indeed, very modest perturbations of the characteristic spectral features of Auoxo1 were observed soon after addition of calf thymus DNA (Figure 3A). The absorption bands characteristic of this gold(III) chromophore turned out to be substantially stable over 24 h of observation. After this period, extensive ultrafiltration of the sample resulted in nearly complete separa-

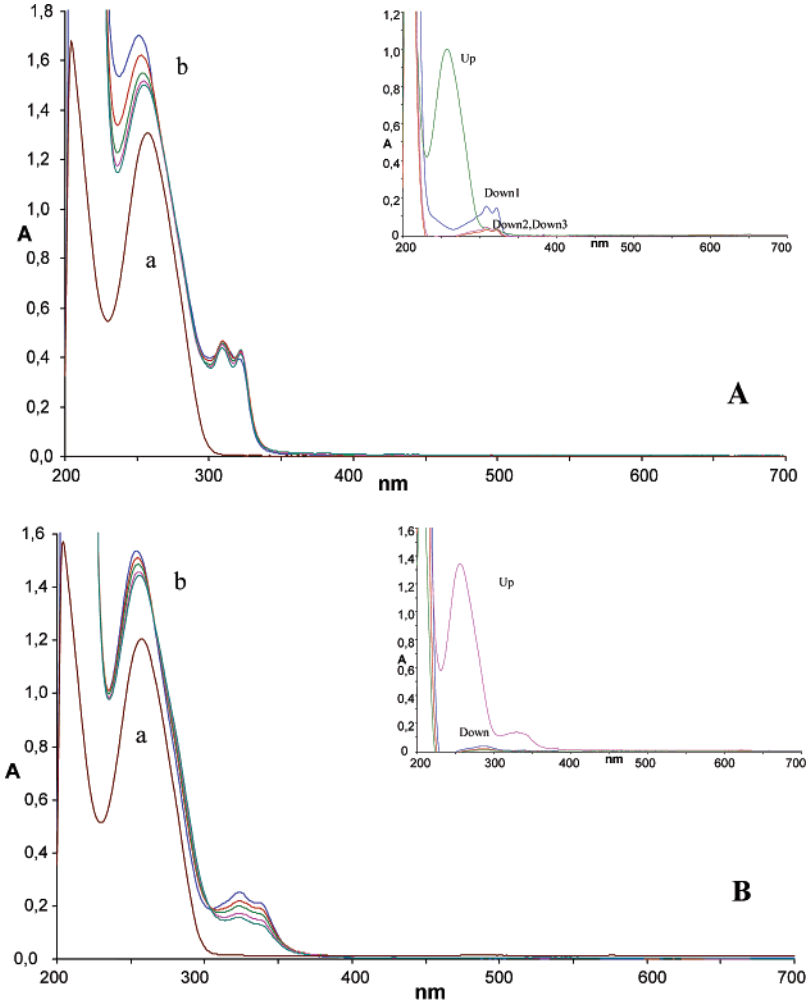


Figure 3. Time-dependent spectral profiles of dinuclear gold(III) compounds/DNA calf thymus adducts at the $r = 0.1$ stoichiometric ratio. The electronic spectra of 10^{-5} M calf thymus DNA were recorded before (a) and after (b) the addition of Auoxo1 (A) and Auoxo6 (B) complexes and were followed for 24 h at 37 °C. The buffer was 10 mM phosphate, pH 7.4, 20 mM NaCl. The concentration of DNA is expressed in base pairs (bp). Insets show the results of ultrafiltration experiments reporting the spectra of the fraction containing DNA (top) and those of the separated fractions (bottom).

tion of the gold(III) species (which crosses the membrane and passes into the lower fraction) from calf thymus DNA (which remains in the upper fraction) (Figure 3A, inset). Notably, the UV−visible spectrum of the gold(III) species that is recovered in the lower fraction matches quite closely the spectrum of Auoxo1 in the phosphate buffer.

In contrast, in the case of Auoxo6, a progressive decrease in intensity and some significant modifications of the bands assigned to the gold(III) chromophore were observed over the first 24 h (Figure 3B). At the end of this period, extensive ultrafiltration failed to separate the gold-containing species from DNA. Substantial gold binding to DNA was instead observed and then independently confirmed by inductively coupled plasma optical emission spectroscopy (ICP-OES) measurements (more than 80% of total gold was found associated with DNA).

These differences were further confirmed by circular dichroism measurements in the UV (Figure 4). Circular dichroism is indeed a very powerful technique to monitor the conformational state of the DNA double helix in solution. Calf thymus DNA, in its native B-type conformation, is characterized by a positive band at 275 nm and a negative one at ~242 nm. Remarkably, addition of Auoxo1 to a solution of calf thymus DNA at $r = 0.1$ causes only some modest alterations of the CD spectrum. We observe some decrease in intensity of the negative band at 242 nm, which we attribute to a partial loss of DNA helicity (possibly due to occurrence of electrostatic interactions between the exposed phosphate groups at the positively charged metal complex). These CD spectral changes may be reversed by ultrafiltration against the buffer, implying that the interaction is weak and reversible.

At variance, Auoxo6 is more effective than Auoxo1 in altering the CD spectra of B-type DNA. For example, at $r = 0.1$, a more pronounced decrease of the negative CD band is observed coupled with a significant change of the positive band at 275 nm. This latter modification is ascribed to an alteration in the overall base stacking of the double helix. Most remarkably, in the case of Auoxo6, a weak "induced" CD band appears at 345 nm. All these spectral modifications are diagnostic of the formation of a relatively tight Auoxo6/DNA adduct. Accordingly, no significant changes of the CD spectrum were observed following extensive ultrafiltration against the buffer.

3.2. Reactions with Representative Proteins. Subsequently, the reactions of either Auoxo1 or Auoxo6 with a few model proteins were investigated according to established protocols.[26,27] In particular, these two compounds were challenged against human serum albumin (hSA), horse heart cytochrome *c* (cyt c), and bovine ubiquitin (Ubq, from red blood cells), and the respective reaction batches were monitored spectrophotometrically over 24 h.

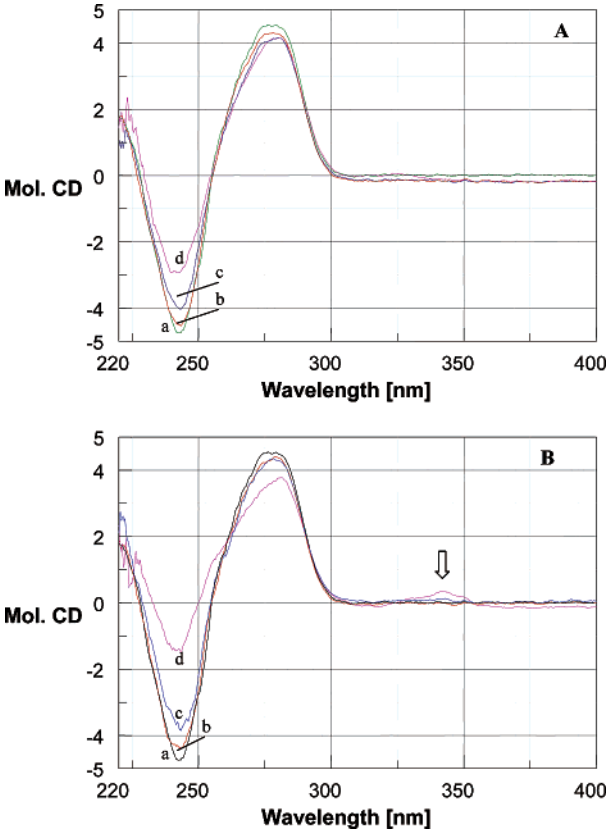


Figure 4. Circular dichroism spectral profiles of calf thymus DNA and of its adducts with Auoxo1 (A) and Auoxo6 (B) complexes at different *r* ratios (metal complex/bp): (a) 10^{-4} M calf thymus DNA alone; (b) $r = 0.01$; (c) $r = 0.05$; (d) $r = 0.1$.

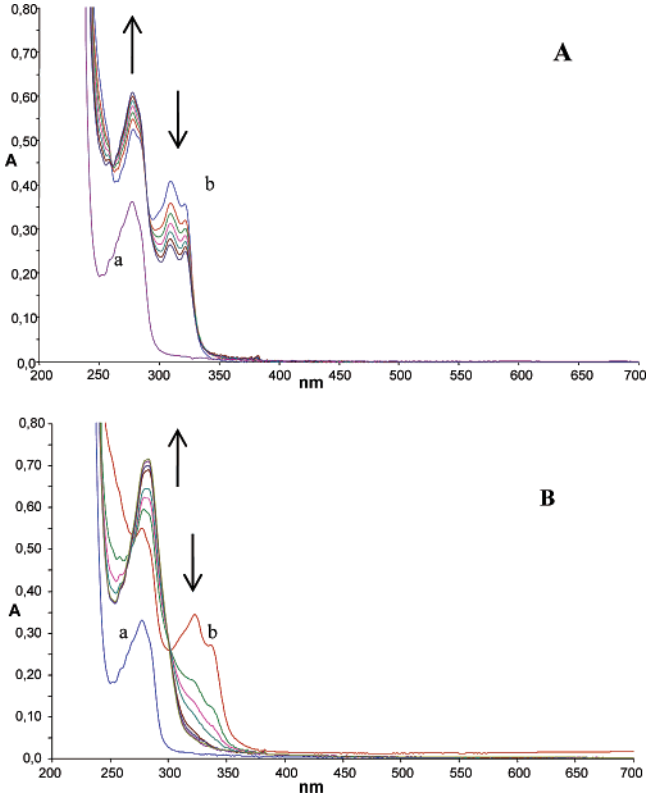


Figure 5. Time-dependent spectral profiles of dinuclear gold(III) compounds/hSA adducts. Shown are representative visible absorption spectra of buffered solutions containing gold(III) complexes and hSA in a 1:1 ratio. Spectra correspond to 10^{-5} M hSA before (a) and after (b) the addition of Auoxo1 (A) and Auoxo6 (B). The further evolution of the various systems over time is reported for up to 24 h of incubation at 37 °C. The arrows indicate the changes of the major bands during this period. The buffer was 10 mM phosphate, pH 7.4, 20 mM NaCl.

The overall spectral profiles of the reactions of Auoxo1 with hSA, cyt c, and Ubq are shown in Figures 5A, 6A, and 7A, respectively. Notably, these spectral profiles are suggestive of the occurrence of relatively slow reactions between Auoxo1 and the various model proteins. Some common trends are easily identified in the reported spectral patterns consisting of the progressive decrease of the transitions typical of Auoxo1 and of the appearance of new bands around 285 nm characteristic of the free ligand. Thus, the obtained spectral patterns may be interpreted in terms of a progressive reduction of the gold(III) centers by protein side chains. A substantial analogy with the reduction by ascorbic acid is evident although the kinetics of the reduction process with proteins is significantly slower. Notably, if the complex is presented to the protein in a large molar excess, complete oxidation of redox-active protein side chains is achieved and a certain amount of the original gold(III) species is found unreacted at the end of the process.

When Auoxo6 was reacted with the same model proteins, somewhat similar spectral patterns were found. Again, the progressive decay of the characteristic LMCT bands of the gold(III) chromophore was observed accompanied by the appearance of the bands at ~280 nm assigned to the free ligand. However, in the case of hSA and cyt c, these redox processes were found to be much faster than for Auoxo1. The rates of these reactions were, on the average, 5−10 times faster than in the case of Auoxo1. At the end of the process most of the added gold was found associated with the protein.

For both Auoxo1 and Auoxo6, independent ICP-OES determinations were carried out on the final solutions, following 24 h of incubation at 37 °C and extensive ultrafiltration against the buffer, working at a metallodrug/protein ratio of 1:1. ICP results point out that a very large percentage of total gold (>80%) remains in the upper fractions, tightly associated with either hSA or cyt c. At variance, in the case of ubiquitin, the percentage of gold that is found associated with the protein is only ~50%.

Additional, specific information on the nature of gold/protein adducts was finally obtained in the case of cyt c by ESI mass spectrometry. ESI MS profiles were collected after reacting cyt c with either Auoxo1 or Auoxo6 (Figure 8), working at 1:1 Auoxo/cyt c ratios. After 12 h of incubation, cyt c was extensively ultrafiltered against the ammonium carbonate buffer and the ESI MS spectra of the upper fractions were recorded. In both cases the final deconvoluted ESI MS spectra provided clear evidence of adduct formation. Remarkably, a number of peaks were observed corresponding to formal binding of a number of Au^{+} ions (ranging from 1 to 4) to the protein. A similar ESI MS behavior was previously reported by Sadler and co-workers when analyzing the $(Au(PEt_3)Cl)$/cyclophilin system.[28] It is remarkable that no sign of the bipiridyl ligand coordinated to gold is found anymore, implying that the reduction process causes complete disruption of the starting dinuclear compound with cleavage of the oxo bridges, release of the bipyridyl ligand, and protein binding of the isolated gold ions.

Discussion

During the past decade, a number of gold(III) complexes and organogold(III) compounds have been shown to manifest interesting antiproliferative properties, in vitro, against cultured

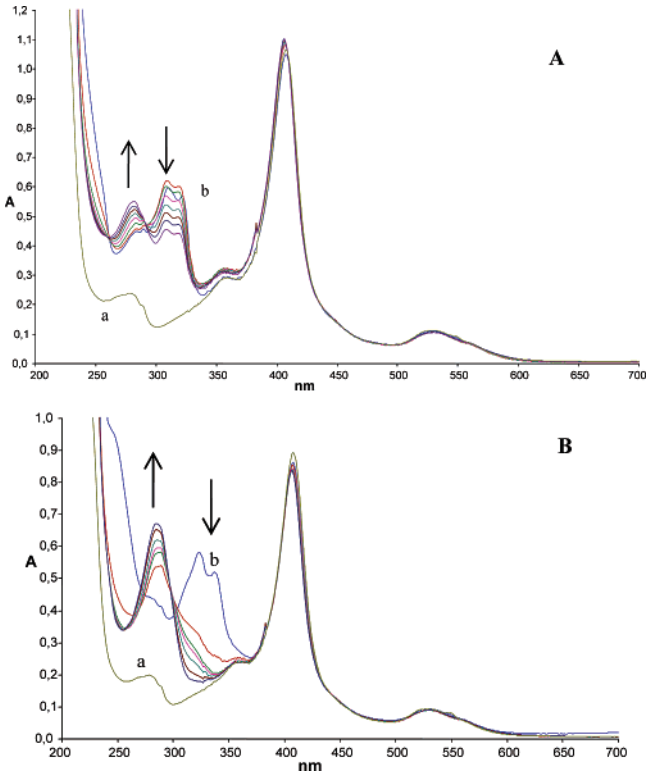


Figure 6. Time-dependent spectral profiles of dinuclear gold(III) compounds/cytochrome *c* adducts. Spectra correspond to 10^{-5} M cytochrome *c* before (a) and after (b) the addition of Auoxo1 ratio (A) and Auoxo6 (B) in a 1:1 ratio. The further evolution of the various systems over time is reported for up to 24 h of incubation at 37 °C. The arrows indicate the changes of the major bands during this period. The buffer contains 25 mM $(NH_4)_2CO_3$, pH 7.4.

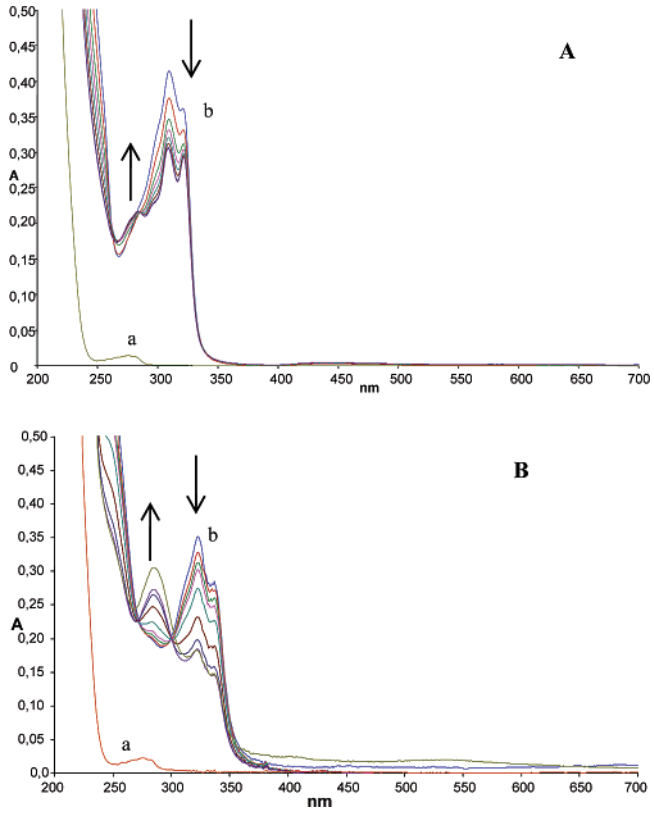


Figure 7. Time-dependent spectral profiles of dinuclear gold(III) compounds/ubiquitin adducts. Spectra corresponds to 10^{-5} M ubiquitin before (a) and after (b) the addition of Auoxo1 (A) and Auoxo6 (B) in a 1:1 ratio. The further evolution of the various systems over time is reported for up to 24 h of incubation at 37 °C. The arrows indicate the changes of the major bands during this period. The buffer was 10 mM phosphate, pH 7.4, 20 mM NaCl.

human tumor cell lines. Some of them are today promising candidates for further development as cytotoxic and/or antitumor agents. Apart from their relevant antiproliferative properties, interest in these compounds strongly relies on the fact that their mechanism of action seems to be very different from that of classical platinum(II) compounds, possibly implying a direct mitochondrial damage. Hopefully, this might lead to a greatly different spectrum of anticancer activities and to a substantial overcoming of resistance toward platinum drugs.

To enlarge the "arsenal" of gold(III) compounds tested so far as potential anticancer agents, we report here, for the first time, on a series of structurally related dinuclear gold(III) compounds. These compounds are characterized by the presence of an extended planar system consisting of a common "Au_2O_2 diamond core" and of bidentate bipyridyl ligands bearing a variety of substituents. Established procedures, depending on the position of the substituent(s), have been employed for their syntheses.[18−20] The resulting molecular framework is capable of finely tuning the chemical properties of the reactive metal centers, as evidenced, inter alia, by an extensive study concerning the oxygen atom transfer reaction to organic substrates.[20,22,23]

Remarkably, these dinuclear gold(III) compounds exhibit a high solubility in DMSO and a reasonable solubility within a 99:1 water/DMSO environment. The solution chemistry of the six compounds that are considered in this study was evaluated within the reference phosphate buffer at pH 7.4, mainly by UV−visible absorption spectroscopy. It emerges that all compounds display an acceptable stability profile of the oxidation state +3. The dimetallic species are the dominant ones in solution for several hours. Some small spectral changes that are observed over 24 h of continuous spectrophotometric monitoring are attributed to occurrence of limited aquation and/or oligomerization processes.

Remarkably, addition of biologically relevant reducing agents such as ascorbic acid or glutathione, presented in a slight molar excess, causes relatively fast and complete reduction of the gold(III) centers. These observations suggest that all tested compounds retain significant oxidizing properties and thus may undergo important redox-driven transformations within a reducing biological environment.

The cytotoxic properties of these compounds were evaluated in vitro on the reference human ovarian carcinoma A2780 cell line, either sensitive or resistant to cisplatin. Five out of six tested dinuclear gold(III) complexes were found to manifest moderate antiproliferative properties with IC_{50} values falling in the 10−30 μM range. However, one of them (Auoxo6) turned out to be significantly more cytotoxic than the other compounds (∼5−15 times more active) and is thus a good candidate for further pharmacological testing. No relevant cross-resistance phenomena were observed in the cisplatin resistant line, implying that the biochemical mechanisms involved in resistance to platinum compounds are only marginally effective against these gold(III) species.

To gain more information on the mechanistic aspects of these dinuclear gold(III) complexes, the reactivity toward model biomolecules of two representative compounds of this series, i.e., Auoxo1 and Auoxo6, was analyzed in detail according to established experimental protocols of our laboratory. In particular, we analyzed comparatively their reactivity toward calf thymus DNA and the model proteins serum albumin, cytochrome *c*, and ubiquitin. Remarkably, significant differences

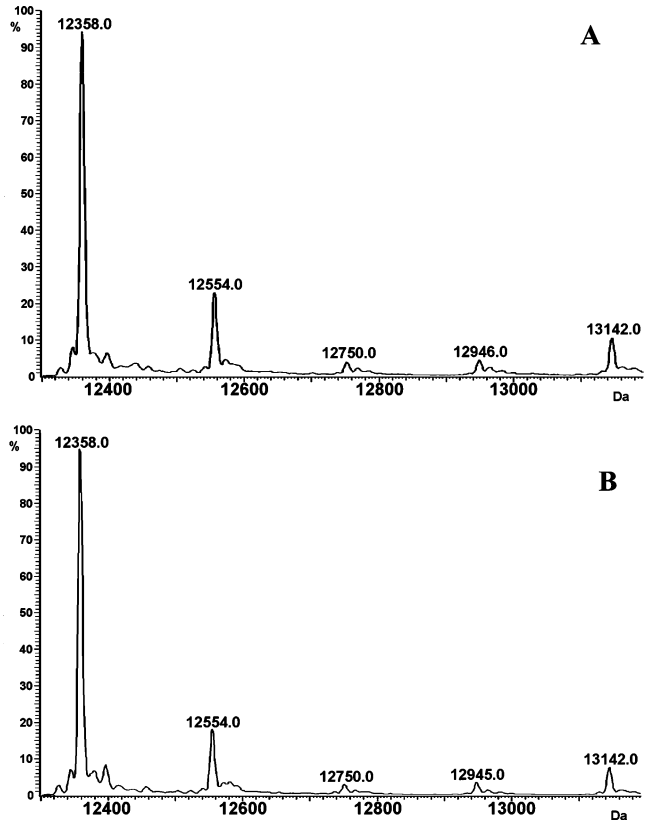


Figure 8. Deconvoluted ESI MS spectral profiles for cytochrome *c* adducts with Auoxo1 (A) and Auoxo6 (B).

were observed in the reactions of Auoxo1 and Auoxo6 with calf thymus DNA. While Auoxo1 interacts only weakly with calf thymus DNA, most likely through purely electrostatic interactions, Auoxo6 gives rise to specific redox processes and binds firmly to the DNA double helix. Remarkably, a characteristic "induced CD" feature has been detected for the latter adduct that is diagnostic of a tight interaction.[29] It is possible that such evident differences in their reactivity with DNA might account for the higher biological activity and cytotoxicity of Auoxo6 compared to Auoxo1.

On the other hand, an interesting reactivity of these two dinuclear gold(III) compounds with the mentioned model proteins was revealed. The general spectral patterns of reactivity toward model proteins are similar for both Auoxo1 and Auoxo6 being characterized by the progressive decrease in intensity of the characteristic bands of the gold(III) centers and by the concomitant appearance of the UV band of the respective free bipyridyl ligands. Such behavior may be interpreted in terms of a redox reactivity between the gold(III) centers and some accessible side chain groups of these proteins. Notably, in the cases of hSA and cyt c, reduction of Auoxo6 is much faster than reduction of Auoxo1 (the process is about 5 times faster). As a consequence of such redox chemistry, the dinuclear gold(III) compounds break down and release their bipyridyl ligands. The resulting metallic species (possibly in the form of isolated gold(I) ions) remain attached to the protein as suggested by ICP measurements and by the characteristic ESI MS features of gold(III)-treated cyt c. A somewhat similar kind of reactivity was previously observed when reacting dinuclear gold(III) Auoxo compounds with triphenylposphine. In fact, this latter reaction leads to formation of $OPPh_3$ and $[Au(PPh_3)_2]^+$, with release of the free bipyridyl ligand.[19,20] Analogously, rather similar spectral profiles were seen when reacting Auterpy, a gold(III) terpyridyl compound with bovine serum albumin.[26]

In conclusion, the present study has highlighted several interesting chemical and biological features of a series of novel dinuclear gold(III) complexes, manifesting promising pharmacological properties. One of them, Auoxo6, shows indeed very encouraging antiproliferative properties and will be the subject of further pharmacological investigations. Specific redox mechanisms of direct damage to DNA or to potential protein targets have been well documented that might be mechanistically relevant and might account for the significant antitumor effects observed in vitro.

Experimental Section

1. Synthesis of the Gold(III) Complexes. The title compounds were synthesized according to procedures reported in ref 18 (Auoxo3−Auoxo6) and in ref 19 (Auoxo1 and Auoxo2). The analytical data obtained for all six compounds were in agreement with published data.

2. Cytotoxicity Assay. Sterile tissue-culture plates and other tissue-culture plastic wares were purchased from Corning. Dimethyl sulfoxide (DMSO) and cisplatin (CDDP) were from Sigma-Aldrich. Human ovarian carcinoma sensitive cell line (A2780/S) and its cisplatin-resistant clone (A2780/R) were cultured in DMEM (Euroclone Ltd., U.K.) supplemented with 10% fetal calf serum (FCS, Euroclone Ltd, U.K.). Cell lines were grown in humidified 5% CO_2 at 37 °C; cells were split twice a week by trypsinization (0.05% trypsin/0.02% EDTA in PBS, from Euroclone Ltd., U.K.). The stock solutions (10^{-2} M) of compounds were prepared by dissolving the compounds in 1 mL of DMSO. Then the stock solutions were diluted, from 1:100 to higher dilutions, in PBS buffer before the addition to the cell culture. Inhibition of cell growth was determined after a 72 h drug exposure by the sulforhodamine B (SRB) assay.[25]

3. Solution Chemistry. UV−Visible Absorption Spectra. The absorption spectra of the gold(III) compounds in the UV−visible region were recorded on a Perkin-Elmer Lambda 20 Bio spectrophotometer. The hydrolysis experiments were carried out by adding small amounts of freshly prepared, concentrated DMSO solutions of Auoxo1−Auoxo6 to the reference buffer (10 mM phosphate, pH 7.4) at 37 °C and monitoring the electronic spectra of the resulting mixtures over 24 h. Moreover, we checked the reactivity of the gold(III) complexes in the presence of sodium ascorbate in excess.

4. Interactions with DNA.

4.1. Electronic Spectra. Electronic spectra of Auoxo1−Auoxo6 were recorded before and after the addition of 10^{-4} M calf thymus DNA (Sigma D3664) at a stoichiometric ratio (metal/DNA base pairs) $r = 0.1$ over 24 h at 37 °C in buffer consisting of 10 mM phosphate and 20 mM NaCl, pH 7.4. After 24 h of incubation, samples were extensively ultrafiltered using Centricon YM-10 (Amicon Bioseparations, Millipore Corporation).

4.2. Circular Dichroism Spectra. CD spectra were recorded on a Jasco 600 dichrograph operating at room temperature, interfaced with a PC, and analyzed through the standard Jasco software package. The samples were prepared mixing various amounts of Auoxo complexes to 10^{-5} M calf thymus DNA to yield the metal/DNA base pairs ratios $r = 0.1$, 0.05, and 0.01. Samples were allowed to incubate at 37 °C for 24 h and then extensively ultrafiltered using Centricon YM-10 (Amicon Bioseparations, Millipore Corporation).

5. Interactions with Proteins. UV−Visible Absorption Spectra. Electronic spectra of each selected protein (human serum albumin, horse heart cytochrome *c*, bovine ubiquitin (Sigma codes A3782, C7752, and U-6253, respectively) at 10^{-5} M were recorded before and after the addition of the Auoxo complexes at a stoichiometric ratio of 1:1 over 24 h at 37°C in buffer consisting of 10 mM phosphate and 20 mM NaCl, pH 7.4 Samples were allowed to incubate at 37 °C for 24 h and then extensively ultrafiltered using Centricon YM-10 or YM-3 depending on the molecular weight of the selected protein (Amicon Bioseparations, Millipore Corporation).

6. ESI Mass Spectrometry. Metal complexes/cyt c adducts were prepared in 25 M ammonium carbonate buffer, pH 7.4, with a protein concentration of 5×10^{-4} M and a gold-to-protein ratio of 1:1. The reaction mixtures were incubated for 24 h at 37 °C. Samples were extensively ultrafiltered using Centricon YM-3 (Amicon Bioseparations, Millipore Corporation) in order to remove the unbound gold complex. After a 100-fold dilution with MilliQ water, ESI MS spectra were recorded by direct introduction at 3 μL/min flow rate into an LTQ linear ion trap (Thermo, San Jose, CA) equipped with a conventional ESI source. The specific conditions used for these experiments were as follows: the spray voltage was 3.5 kV, the capillary voltage was 40 V, and the capillary temperature was kept at 353 K. Sheath gas was set at 13 (arbitrary units), whereas sweep gas and auxiliary gas were kept at 0 (arbitrary units). ESI spectra were acquired using Xcalibur software (Thermo), and deconvolution was obtained using Bioworks software (Thermo). The mass step size in deconvolution calculation was 0.25 Da, and the spectrum range considered was *m*/*z* 1100−2000. The same experiments were repeated with varying capillary temperature (from 90 to 180 °C), but the peak patterns and relative abundances were not influenced (data not shown).

7. ICP Analysis. ICP-OES spectra of the samples were recorded using an Optima 2000 instrument (Perkin-Elmer, Europe).

Acknowledgment. MIUR and Ente Cassa di Risparmio di Firenze are gratefully acknowledged for financial support. We thank Dr. Guido Mastrobuoni from CISM (University of Florence) for recording the ESI MS spectra.

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JM060436A