Gold(III) Complexes with Bipyridyl Ligands: Solution Chemistry, Cytotoxicity, and DNA Binding Properties

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Gold(III) compounds generally exhibit significant cytotoxic effects on cancer cell lines and are of potential interest as antitumor drugs. We report here on the solution chemistry, the cytotoxicity, and the DNA binding properties of two new bipyridyl gold(III) compounds: [Au(bipy)$(OH)_2$][PF_6] (**1**) and the organometallic compound [Au(bipy^c-H)(OH)][PF_6] (**2**) (bipy^c = 6-(1,1-dimethylbenzyl)-2,2′-bipyridine). Both compounds are sufficiently soluble, and stable for hours, within a physiological buffer at 37 °C; [Au(bipy)$(OH)_2$][PF_6], at variance with [Au(bipy^c-H)(OH)][PF_6], is quickly and quantitatively reduced by ascorbate. Both compounds showed relevant cytotoxic effects toward the A2780S, A2780R, and SKOV3 tumor cell lines; lower effects were detected on the CCRF-CEM/S and CCRF-CEM/R lines. In most cases the mechanisms of resistance to CDDP are only marginally effective against these gold(III) complexes. The interactions of [Au(bipy)$(OH)_2$][PF_6] and [Au(bipy^c-H)(OH)][PF_6] with calf thymus DNA were investigated in vitro by various techniques to establish whether DNA represents a primary target for these compounds. Addition of saturating amounts of DNA did not affect appreciably the visible spectra of these gold(III) complexes. Some slight modifications of the CD spectra of calf thymus DNA and of the DNA melting parameters were observed; in any case, ultrafiltration experiments showed that binding of these gold(III) complexes to DNA is weak and reversible. The mechanistic implications of these findings are discussed.

Introduction

In recent years new interest has been focused on gold(III) complexes as potential cytotoxic and antitumor drugs.[1] Buckley et al. first reported on some organogold(III) complexes endowed with significant cytotoxic and antitumor properties.[2] We recently showed that some simple mononuclear gold(III) complexes are sufficiently stable within a physiological environment and display relevant cell killing properties toward selected human tumor cell lines.[3−6] Within this frame we are now considering two gold(III) complexes prepared in the laboratory of Prof. Minghetti. These complexes are characterized by the presence of a bipyridyl ligand within a square planar arrangement of the gold(III) center.[7−9] In [Au(bipy)$(OH)_2$][PF_6], two coordination positions of the square planar environment are occupied by two nitrogens of the bipyridyl ligand, the remaining positions being occupied by two hydroxide groups. [Au(bipy^c-H)(OH)][PF_6] is an organogold(III) complex (bipy^c = 6-(1,1-dimethylbenzyl)-2,2′-bipyridine): donors to the square planar gold(III) center are the two nitrogens of the bipyridyl moiety, the C_2 carbon of the phenyl group, and the oxygen of a hydroxide group.

In this paper we report on the solution chemistry and the cytotoxic activity of these compounds; the favorable biological properties observed in the preliminary tests in vitro led us to investigate the interactions of these compounds with calf thymus DNA, one of the probable biological targets.

Structural Features of the Investigated Gold(III) Complexes

The structures of complexes [Au(bipy)$(OH)_2$][PF_6] (**1**) and [Au(bipy^c-H)(OH)][PF_6] (**2**), although not available, are assumed to match those of [Au(bipy)$(OMe)_2$][PF_6] (**3**)[7] and of [Au(bipy^c-H)Cl][$AuCl_4$] (**4**),[8,9] respectively (Figures 1 and 2).

Notably, these complexes are characterized by the classical square-planar arrangement of the gold(III) chromophore. Modest deviations from square-planar geometry are found in the bipyridyl complexes whereas these are quite large in the cyclometalated derivatives due to limited flexibility of the N,N,C ligand. In all cases, the bipyridyl moiety acts as a bidentate ligand; in compound **1** there are two oxygen atoms directly bound to the gold(III) center whereas in the cyclometalated derivatives tetracoordination is completed by a carbon atom and by a chlorine or an oxygen atom. Notably, in the cyclometalated derivatives the six-membered metallacycle is in boat conformation, and one of the hydrogen atoms of the methyl groups in pseudo-axial position is rather close to the gold atom.

Solution Chemistry

Absorption Spectra. The complexes [Au(bipy)$(OH)_2$][PF_6] (**1**) and [Au(bipy^c-H)(OH)][PF_6] (**2**) are sufficiently soluble in water and within the reference physiological buffer (50 mM phosphate, NaCl 4 mM, pH 7.4 at 25 °C); bright yellow aqueous solutions of either complex are

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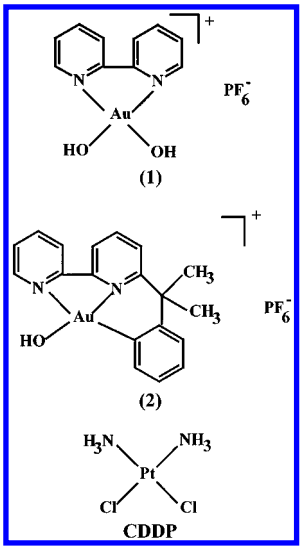

Figure 1. Chemical drawing of the two gold(III) compounds $[Au(bipy)(OH)_2][PF_6]$ (**1**) and $[Au(bipy^c\text{-}H)(OH)][PF_6]$ (**2**); the structure of cisplatin is reported for comparison.

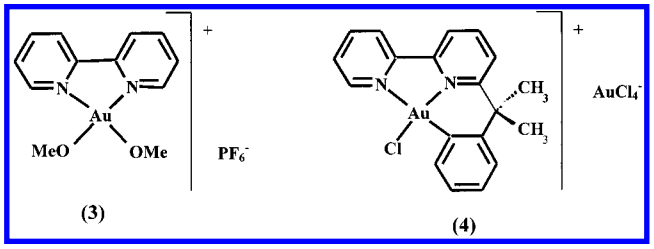

Figure 2. Chemical drawing of the two gold(III) compounds $[Au(bipy)(OMe)_2][PF_6]$ (**3**) and $[Au(bipy^c\text{-}H)Cl][AuCl_4]$ (**4**).

easily prepared. Spectra are shown in Figure 3. Visible bands of these complexes are LMCT in nature and are diagnostic of the presence of gold in the oxidation state +3. The spectrum of $[Au(bipy)(OH)_2][PF_6]$ is characterized by two partially overlapping bands at 310 and 325 nm, with a shoulder around 300 nm; the spectrum of $[Au(bipy^c\text{-}H)(OH)][PF_6]$ exhibits a broad band around 320 nm with a shoulder at 330 nm. Freshly prepared solutions of the two complexes were spectrophotometrically monitored for 72 h: it turned out that the visible spectra of $[Au(bipy)(OH)_2][PF_6]$ and $[Au(bipy^c\text{-}H)(OH)][PF_6]$ are stable for all the time at 25 °C; only a very small decrease in spectral intensity has been detected (less than 2% after 72 h). Notably, the visible spectra of either complex in water and in the reference buffer are almost identical.

It can be anticipated that the dominant species in solution, at physiological pH, will contain gold(III)-coordinated hydroxo groups, as previously found for similar gold(III) complexes. In fact, the gold(III) center is strongly acidic and drastically lowers the pK_a of coordinated waters. In nice agreement with this view, we observed that the visible spectra of either compound are pH independent within the pH range 2−7. Notably, both gold(III) complexes showed high stability in the RPMI, a commonly used medium for cell cultures, despite the presence of a number of reducing agents.

We also checked whether these complexes may be reduced by ascorbate: addition of sodium ascorbate causes immediate disappearance of the main visible band of the $[Au(bipy)(OH)_2]^+$ chromophore but does not

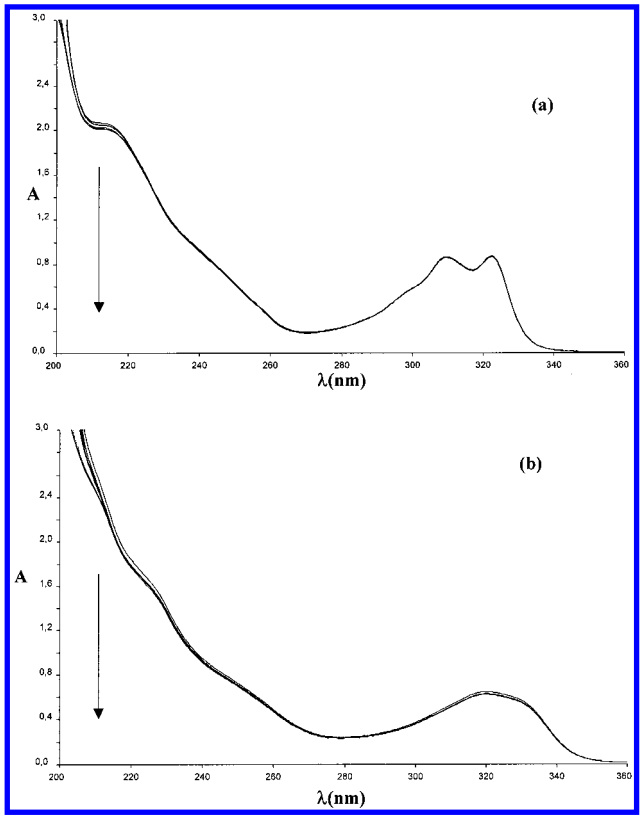

Figure 3. Electronic spectra of the two gold(III) complexes $[Au(bipy)(OH)_2][PF_6]$ (a) and $[Au(bipy^c\text{-}H)(OH)][PF_6]$ (b) in 4 mM NaCl buffer followed during time (from $t = 0$ to $t = 5$ h).

alter the visible spectrum of the $[Au(bipy^c\text{-}H)(OH)]^+$ species. This means that the oxidation state +3 is more stable in the case of the organogold(III) complex compared to $[Au(bipy)(OH)_2][PF_6]$, in line with previous indications from electrochemical studies.

The compound $[Au(bipy)(OH)Cl][PF_6]$ exhibits a visible spectrum similar but not identical to that of $[Au(bipy)(OH)_2][PF_6]$ (data not shown). Remarkably the visible spectrum slightly changes with time; the final spectrum is virtually superimposable to that of $[Au(bipy)(OH)_2][PF_6]$. In our interpretation the chromophore $[Au(bipy)(OH)Cl]^+$ transforms into $[Au(bipy)(OH)_2]^+$ through slow hydrolysis of the chloride group. The intense bands at 310 and 325 nm must be assigned as N to gold(III) charge transfer transitions.

^{1}H NMR Spectra. The behavior in solution of these compounds was further analyzed by ^{1}H NMR spectroscopy. Notably, when dissolved in water or in the reference buffer, these compounds produce ^{1}H NMR spectra corresponding to a single, dominant species. In nice agreement with the spectrophotometric data, the ^{1}H NMR spectra are stable for hours. Only in the case of $[Au(bipy)(OH)_2][PF_6]$ dissolved in the buffer, a new set of signals from a secondary species slowly appears with time (data not shown). After 2 h, the signals at low field (9.24 and 9.01 ppm) exhibit an intensity that is about one-third of the intensity of the major species; no further increase in intensity is observed in the following 2 h. The nature of the secondary species has not been determined until now; notably this species is not observed in D_2O. Formation of a dimeric species cannot be ruled out.

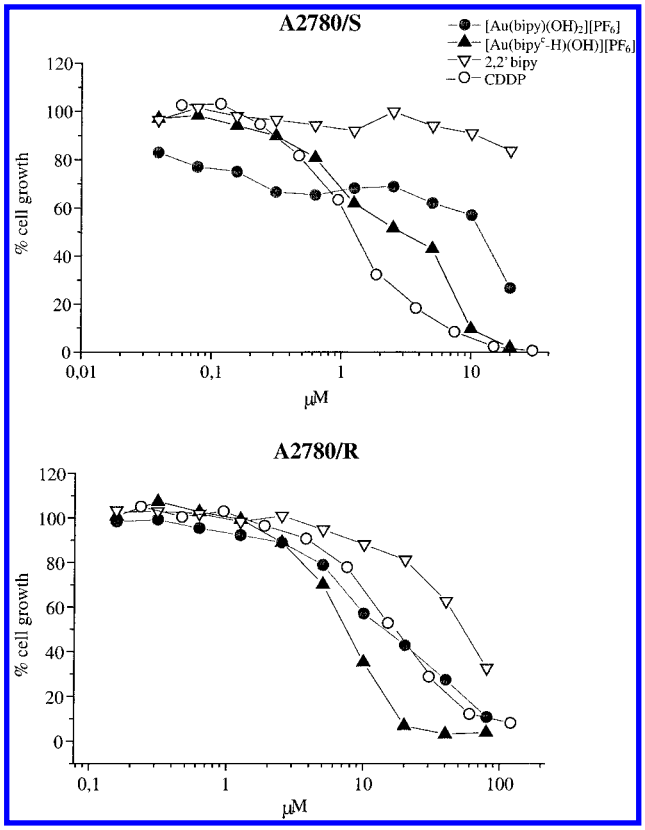

Figure 4. Drug sensitivity profiles of cisplatin-sensitive and -resistant human ovarian carcinoma cell lines (A2780/S and A2780/R) toward [Au(bipy)$(OH)_2$][PF_6] and [Au(bipy^c-H)(OH)]-[PF_6]. Graphs show the percentage of growth with respect to the control upon incubation with increasing amounts of the gold(III) complex. For comparison purposes, the curves obtained with CDDP are reported**.** The values reported in these graphs are the average of at least four independent experiments.

Thus, ^{1}H NMR spectroscopy provides evidence of the existence for hours of a dominant species in solution that is most likely responsible for the observed biological effects.

Cytotoxic Properties

The in vitro cytotoxic properties of these gold(III) complexes were first evaluated toward the human ovarian carcinoma cell line A2780 either sensitive (A2780/S) or resistant (A2780/R) to cisplatin. This cell line has been extensively used in our laboratory as the reference line to evaluate the cytotoxic potency of several gold(III) complexes.[3] For comparison purposes the cytotoxicity of cisplatin (CDDP) was evaluated under the same conditions. Results are shown in Figure 4 and Table 1. Both bipyridyl gold(III) complexes show important cell killing effects with IC_{50} values falling in the micromolar range. [Au(bipy^c-H)(OH)][PF_6] is the most active with a cytotoxic activity 2 times higher than CDDP in the A2780/R cell line (Table 1). Later on, the cytotoxic properties of these complexes were evaluated on the human ovarian cell line SKOV3 (inherently resistant to cisplatin) and on the CCRF-CEM leukemic cell line either sensitive (CCRF-CEM/S) or resistant (CCRF-CEM/R) to cisplatin. In these cell lines both gold(III) complexes produced smaller inhibition of cell growth with respect to the A2780 line; again, [Au(bipy^c-H)(OH)][PF_6] is more active. Notably, these gold(III) complexes retain to a large extent their cytotoxic activity toward the cisplatin-resistant A2780/R and CCRF-CEM/R lines. The resistance index (ratio of IC_{50} of cisplatin-resistant cell line and IC_{50} of parental sensitive cell line) is ≈ 12 and ≈14 for CDDP in A2780 and CCRF-CEM, respectively, while it varies from ≈1 to ≈4 in the case of the bipyridyl gold(III) compounds (Table 1). This means that the mechanisms of resistance to CDDP—most likely intracellular detoxification and increased repair of DNA damage—are only marginally effective toward these gold(III) complexes, in line with previous findings on other gold(III) compounds.[4]

Table 1. Inhibitory Effects of Gold(III) Complexes on the Growth of Some Cisplatin-Sensitive (A2780/S, CCRF-CEM/S) and -Resistant (A2780/R, SKOV3, CCRF-CEM/R) Human Tumor Cell Lines

	IC_{50}[a] (μM), mean ± SE			
cell lines	[Au(bipy)-$(OH)_2$][PF_6]	[Au(bipy^c-H)-(OH)][PF_6]	2,2′ bipy	CDDP
A2780/S	8.8 ± 3.9 *n* = 4	3.3 ± 1.4 *n* = 5	>20	1.3 ± 0.2 *n* = 5
A2780/R	24.1 ± 8.7 *n* = 6 (2.7)	8.2 ± 1.5 *n* = 7 (2.5)	44.8 ± 10.5 *n* = 4	15.3 ± 1.9 *n* = 5 (11.7)
SKOV3	34.4 ± 4.7 *n* = 5	13.3 ± 1.6 *n* = 5	41.3 ± 11.4 *n* = 5	21.6 ± 4.1 *n* = 5
CCRF-CEM/S	52.9 ± 11.6 *n* = 5	11.9 ± 2.1 *n* = 5	51.9 ± 3.4 *n* = 5	1.0 ± 0.3 *n* = 5
CCRF-CEM/R	58.6 ± 0.9 *n* = 2 (1.1)	51.2 ± 5.6 *n* = 3 (4.3)	61.7± 6.2 *n* = 3 (1.2)	14.1 ± 8.2 *n* = 2 (14.1)

[a] IC_{50} is defined as the concentration of drug required to inhibit cell growth by 50% compared to control. *n* = number of determinations. Values in parentheses indicate the ratio of IC_{50} of cisplatin-resistant cell line and IC_{50} of parental sensitive cell line.

The cytotoxic properties of the free 2,2′ bipyridyl ligand were also tested; this ligand is virtually devoid of toxicity toward the A2780/S cell line while it shows some cytotoxicity at high concentrations in the other cell lines (Table 1).

DNA Binding Properties

Prompted by the favorable results of the biological tests, we investigated whether the cytotoxic effects of these gold(III) compounds might be a consequence of a direct interaction with nuclear DNA. DNA is commonly believed to represent the major target of antitumor metal complexes. Thus, the interactions in vitro of bipyridyl gold(III) complexes with purified calf thymus DNA were analyzed by various physicochemical methods including spectrophotometry, circular dichroism, DNA melting, and ultradialysis.

(i) Spectrophotometry. The visible spectra of both gold(III) complexes were recorded before and after addition of saturating amounts of calf thymus DNA as shown in Figure 5. From inspection of the spectra it is evident that in both cases addition of DNA neither produces quick reduction of the gold(III) center nor modifies the gold(III) chromophore. No significant time dependent spectral changes are observed.

(ii) Circular Dichroism. While visible spectroscopy mainly monitors the gold(III) chromophore during its reaction with DNA, CD is the appropriate technique to monitor the conformational variations of DNA in solution. A series of CD spectra recorded following addition of increasing amounts of [Au(bipy)$(OH)_2$][PF_6] to calf

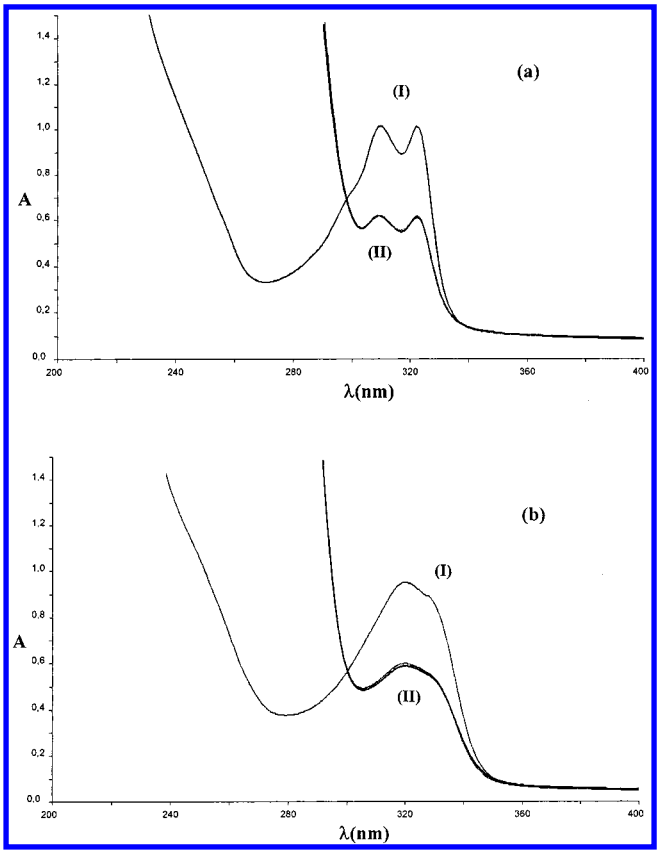

Figure 5. Electronic spectra of [Au(bipy)$(OH)_2$][PF_6] (a) and [Au(bipy^c-H)(OH)][PF_6] (b) before (I) and after (II) addition of calf thymus DNA at the ratio $r = 0.1$, followed during 1 h. The change in intensity is completely ascribed to dilution.

thymus DNA are shown in Figure 6. The gold(III)/DNA bp ratio ranged from $r = 0$ to $r = 1$. Small CD changes are detected as r is increased, supporting the view that this gold(III) complex interacts somehow with DNA; a similar CD spectral pattern was found for [Au(bipy^c-H)(OH)][PF_6].

(iii) Ultradialysis. Ultradialysis experiments were carried out on solutions containing either gold(III) complex mixed with calf thymus DNA to monitor the stability and the reversibility of the interaction. In the first series of experiments, samples were ultradialyzed down to half volume and the spectra of the upper and lower fractions recorded. Comparable amounts of the complex were detected in both fractions whereas calf thymus DNA completely remained in the upper fraction. Since the visible spectra of the individual complexes are not significantly perturbed by DNA addition, it is possible to make rough estimates of the bound and free complex concentrations from the absorbance values in the two phases. Apparently, in both cases, only a minor fraction of total complex is bound to DNA; in any case, the DNA bound fraction for [Au(bipy^c-H)(OH)][PF_6] is significantly larger than for [Au(bipy)$(OH)_2$][PF_6] (29.8% and 6.8%, respectively). Extensive ultradialysis against the buffer resulted in complete removal of either complex from the upper solution. This means that the binding is weak and fully reversible in accord with an interaction mode mainly electrostatic in nature.

(iv) Melting Experiments. The interactions of bipyridyl gold(III) complexes with calf thymus DNA were further monitored by recording DNA thermal denaturation profiles. This technique provides direct information on stabilization (or destabilization) effects induced on the DNA double helix by ligand binding. Comparative analysis of the melting parameters in the presence of increasing amounts of the drug provides valuable information on the nature of the interaction.

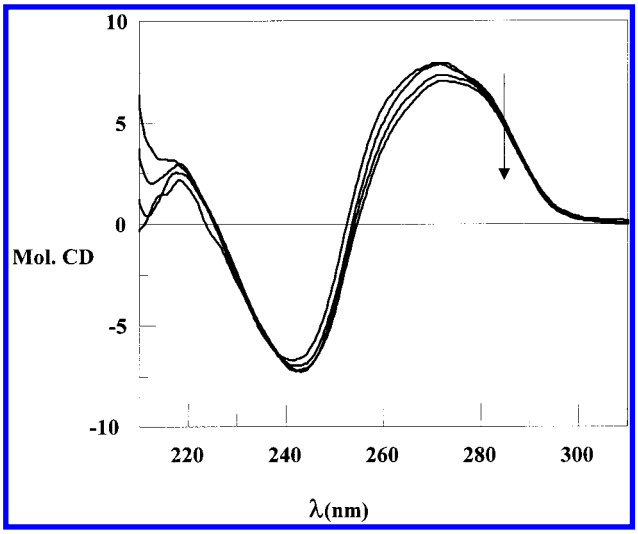

Figure 6. CD spectra of [Au(bipy)$(OH)_2$][PF_6] after addition of calf thymus DNA at the ratio $r = 0.0$, 0.2, 0.4, 1.0 (from the top to the bottom).

Characteristic melting profiles were recorded at $r = 0.1$; in both cases the gold(III) complexes bring about some stabilization of the double helix (T_m values of 71.3 °C and 70.0 °C were determined for the DNA adducts with [Au(bipy)$(OH)_2$][PF_6] and [Au(bipy^c-H)(OH)][PF_6] to be compared with the value of 68.9 °C of free DNA). Overall, the interaction is weak, and the conformational effects on DNA are relatively modest.

Discussion

Gold(III) complexes generally exhibit favorable cytotoxic properties and are good candidates for extensive evaluation as antitumor agents; indeed, encouraging results in vitro were recently reported for a number of simple gold(III) complexes. It is known that a strict requirement for antitumor drug candidates is a sufficient stability under physiological conditions; in the case of gold(III) complexes such requirement can be met by coordination to strong bidentate or polydentate ligands with nitrogen donors. In this paper we have analyzed the chemical and biological properties of two gold(III) complexes characterized by the presence of a bipyridyl ligand. Notably the two compounds differ in that [Au(bipy)$(OH)_2$][PF_6] is a simple gold(III) complex whereas [Au(bipy^c-H)(OH)][PF_6] is an organometallic compound with a carbon−gold bond. The final goal of the present investigation is to enlarge the repertoire of gold(III) complexes of potential interest as antitumor complexes, through the detailed characterization of their chemical properties and the preliminary evaluation of the cytotoxic effects.

Chemical Characterization. Spectrophotometric results point out that both complexes are highly stable within a phosphate buffer at pH 7.4. This view is nicely supported by ^{1}H NMR data pointing out a substantial stability of both compounds either in water or in the reference phosphate buffer. We proposed that the

respective monopositive cations, $[Au(bipy)(OH)_2]^+$ and $[Au(bipy^c\text{-}H)(OH)]^+$, are the dominant species existing in solution. Notably $[Au(bipy^c\text{-}H)(OH)]^+$ is resistant toward reduction by sodium ascorbate whereas $[Au(bipy)(OH)_2]^+$ is not. This finding suggests that the former species is more resistant toward physiologically occurring reducing agents when administered in vivo. In any case we found that both complexes are quite stable when dissolved in the standard RPMI medium, thus meeting the basic requirements for further biological testing. In view of these results, an extensive screening of the cytotoxic properties toward selected human tumor cell lines was carried out.

Biological Properties. In vitro cytotoxicity screening represents a widespread method for the rapid selection of antitumor drug candidates. Since the early 1990s, a cytotoxicity assay based on a panel of 60 human tumor cell lines represents the standard protocol employed by NCI for cancer drug screening.

Thus both bipyridyl gold(III) compounds were preliminarly tested against a small panel of human tumor cell lines comprising A2780, SKOV3, and CCRF-CEM. Specifically, the A2780 has been used extensively by our group as the reference line to test the cell killing properties of gold(III) complexes.

Complexes 1 and 2 turned out to produce important cell killing effects on this line. The IC_{50} values of 8.8 μM and 3.3 μM match those of other gold(III) complexes, determined under identical conditions.[4] On the grounds of these results, $[Au(bipy^c\text{-}H)(OH)][PF_6]$ qualifies as one of the most active gold(III) complexes on the A2780 line. Lower but still important cytotoxic effects were detected when both complexes were tested on the SKOV3 and CCRF-CEM cell lines. Remarkably both compounds are able to overcome, to a large extent, resistance to cisplatin as witnessed by the relatively low resistance index values. These encouraging results prompted us to perform additional mechanistic studies on both compounds.

Mechanistic Studies: DNA Binding Properties. It is generally believed that the cytotoxic effects of metal complexes are the consequence of a direct damage to nuclear DNA although experimental evidence is often lacking. Whereas this statement is generally true for platinum complexes, it is still largely questionable for antitumor metal complexes containing ruthenium, tin or gold. Controversial data are present in the literature.[12,13]

To further elucidate this issue, the interactions of the present bipyridyl gold(III) complexes with calf thymus DNA were analyzed by a variety of techniques including spectrophotometry, melting, circular dichroism, and ultrafiltration. Although some small effects on DNA conformation and stability were detected, overall our results suggest that the gold(III) chromophore is not significantly modified and that binding of these gold(III) complexes to calf thymus DNA is relatively weak. DNA conformation is not largely affected as well. Thus, one can state that DNA damage is relatively modest and very unlikely to account for the observed cytotoxic properties; other DNA independent mechanisms are probably operative that lead to the observed biological effects. Results recently obtained in our laboratory point out that binding of these complexes to model proteins is tight and might represent the molecular basis of the biological action of these gold(III) complexes.

Conclusions

Two bipyridyl gold(III) complexes, pretty stable under physiological conditions, were shown to exhibit important cytotoxic properties toward a panel of human tumor cell lines. In particular, the $[Au(bipy^c\text{-}H)(OH)][PF_6]$ complex turned out to be highly cytotoxic toward the A2780 cell line. The mechanisms of resistance to CDDP of the CCRF-CEM and A2780 lines are only marginally effective toward these gold(III) complexes. The interactions of both compounds with DNA were analyzed in detail through various independent techniques. It was found that the interactions with the DNA double helix are weak, reversible, and predominantly electrostatic in nature, suggesting that DNA is not the primary target for the cytotoxic effects of these complexes.

Experimental Section

Chemicals. Common chemical reagents were purchased from SIGMA Chemical Co. (Milano, Italy) and Pharmacia; cisplatin was purchased from Teva Pharma Italia (Milano, Italy). Fetal calf serum (FCS), antibiotics, and RPMI-1640 medium were obtained from Gibco Life Technologies Italia (Milano, Italy).

Synthesis of $[Au(bipy)(OH)_2][PF_6]$ and $[Au(bipy^c\text{-}H)(OH)][PF_6]$ $[Au(bipy)(OH)_2][PF_6]$ (1). The title compound was prepared according to ref 7. An aqueous suspension of Ag_2O was added to a solution of $[Au(bipy)Cl_2][PF_6]$ in acetone. The mixture was stirred for 24 h at room temperature. AgCl was removed by filtration and the solution evaporated to dryness under reduced pressure. The residue was extracted with acetonitrile and filtered over Celite. The pale-yellow filtrate was concentrated to a small volume, and diethyl ether was added to give a white precipitate of $[Au(bipy)(OH)_2][PF_6]$. The obtained product was checked by elemental analysis; the purity of the compound was higher than 98%. Further evidence for the correct identification of the obtained compound is provided by the electronic spectra.

$[Au(bipy^c\text{-}H)(OH)][PF_6]$ (2). An aqueous solution of KOH (33 mg, 0.59 mmol) was added to an aqueous suspension of $[Au(bipy^c\text{-}H)Cl][PF_6]$[8,9] (179 mg, 0.27 mmol). The mixture was refluxed for 1 h under stirring and filtered. The volume of the colorless filtrate was reduced on a rotary evaporator until crystallization was observed. The white product was collected by filtration and dried in vacuo. The yield was 128 mg (75%): ^{1}H NMR (acetone-d_6) δ 9.21 (dd, 1H, H$^{6'}$), 8.92−7.18 (m, 10H, other aromatics), 4.58 (s, 1H, OH), 2.11 (s, 6H, CH_3). IR (Nujol, cm^{-1}) ν_{max}: 3360 (m, broad) OH. FAB$^+$-MS *m*/*z* (%): 487(100) [M$^+$], 470(30) [M$^+$ − OH], 393(10)[M$^+$ − OH − C_6H_5]. The spectra in water and in the buffer are similar to those in acetone except for some changes in the chemical shifts of the aromatic signals. Anal. ($C_{19}H_{18}AuF_6N_2OP$) C, H; N: calcd 4.43; found, 4.33.

Solution Chemistry. Electronic Spectra. The absorption spectra in the UV−visible region were recorded on a Perkin-Elmer Bio 20 spectrophotometer operating at room temperature. The gold(III) complex $[Au(bipy)(OH)_2][PF_6]$ and $[Au(bipy^c\text{-}H)(OH)][PF_6]$ are quite soluble in water (10^{-3} M solutions can be obtained); electronic spectra were recorded on freshly prepared buffered solutions at room temperature. The hydrolysis experiments were carried out by adding small amounts of freshly prepared, concentrated water solutions of $[Au(bipy)(OH)_2][PF_6]$ and $[Au(bipy^c\text{-}H)(OH)][PF_6]$ to the reference buffer (50 mM phosphate, pH 7.4 at 25 °C, 4 or 100 mM NaCl) and monitoring the electronic spectra of the resulting mixtures over 5 h. The stability of the chromophore in water and RPMI medium was also investigated. Moreover we checked the reactivity of the two gold(III) complexes in the presence of sodium ascorbate in excess.

^{1}H NMR Studies. Solution ^{1}H NMR spectra of [Au(bipy)(OH)$_2$][PF$_6$] and [Au(bipy^c-H)(OH)][PF$_6$] were recorded on a Bruker Avance spectrometer operating at 300 MHz. The 8×10^{-4} M solutions were prepared in D_2O and in 50 mM PO_4^{3-}, 4 mM NaCl, pH 7.4 buffer. The spectra were recorded immediately after dissolution and over a period of 1−3 h.

Cell Culture and Cytotoxicity Assay. For cytotoxicity studies, two ovarian carcinoma (A2780/S and SKOV3) and one T-lymphoblastoid leukemia (CCRF-CEM/S) human cell lines were used. The cisplatin-resistant A2780/R cell line was produced by repeated 1 h weekly exposure to 50 μM of the sensitive parental cell line.[14] The CCRF-CEM/R cell line was selected by continuous exposure of the sensitive parental cells to increasing cisplatin concentrations and was maintained in culture with 10 μM cisplatin. Cell lines were cultured in RPMI-1640 medium (GIBCO Life technologies Product catalog, RPMI 1640 medium, Cat. No. 11817) supplemented with 10% FCS and antibiotics (streptomycin 100 μg/mL and penicillin 100U/mL) at 37 °C in a 5% CO_2 atmosphere and subcultured twice weekly. Experiments were conducted on exponentially growing cells. Drugs were dissolved in sterile bidistilled water. Inhibition of cell growth was determined after a 72 h drug exposure through the Sulforhodamine B (SRB) assay[15] for the ovarian carcinoma cell lines or cell number counting (model D Coulter counter, Coulter electronics, Ltd., Luton, Bedfordshire, England) for the leukemic cell lines.

Interactions with DNA. Electronic Spectra. Electronic spectra of [Au(bipy)(OH)$_2$][PF$_6$] and [Au(bipy^c-H)(OH)][PF$_6$], 3.6×10^{-4} M, were recorded before and after addition of calf thymus DNA ($r = 0.0$, 0.1, 0.2, 0.4, 0.8, 1.0 where r is the gold/basepair ratio) in the phosphate 50 mM, NaCl 4 mM, pH 7.4 buffer. The interaction between the gold(III) compounds and calf thymus DNA ($r = 0.1$) was also investigated by recording spectra during 3 h.

CD Spectra. CD spectra were recorded at increasing [Au(bipy)(OH)$_2$][PF$_6$]/calf thymus DNA and [Au(bipy^c-H)(OH)][PF$_6$]/calf thymus DNA ratios ($r = 0.0$, 0.2, 0.4, 1.0), immediately after mixing, on a Jasco J600 dichrograph operating at room temperature, interfaced with a PC, and analyzed through the standard Jasco software package.[16]

Ultrafiltration Experiments. The gold(III) complexes/calf thymus DNA samples, characterized by $r = 0.1$, were dialyzed after 24 h incubation at room temperature. The spectra of the upper portion of the solution containing calf thymus DNA and of the lower portion of the solution, containing only the gold(III) complex, were recorded.

Melting Profiles. All melting measurements were carried out in the 10^{-2} M $NaClO_4$ and 10^{-3} M NaCl buffer. Calf thymus DNA was dissolved in the buffer and DNA concentration determined by absorption measurements at 260 nm. DNA concentration was equal to 3.6×10^{-5} M [nucleotide]. DNA was treated with [Au(bipy)(OH)$_2$][PF$_6$] and [Au(bipy^c-H)(OH)][PF$_6$] at different mol/bp ratios ($r = 0.01$, 0.1, 1.0), and each sample was incubated for 24 h at room temperature.

Thermal denaturation experiments were performed in quartz cuvettes with a Perkin-Elmer Lamba 20 Bio spectrophotometer equipped with a thermostated cell as described in reference 17. Samples were continuously heated with a rate of temperature increase of 0.5 °C/min while monitoring the absorbance changes at 260 nm. The investigated interval of temperature ranged from 50 to 90 °C. Upon reaching 90 °C, samples were cooled back to 40 °C in order to follow the renaturation process. Values for melting temperatures (T_m) and for the melting interval (ΔT) were determined according to the reported procedures.[18]

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Supporting Information Available: ^{1}H NMR spectra of [Au(bipy^c-H)(OH)][PF$_6$]. This material is available free of charge via the Internet at http://pubs.acs.org.

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