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*Author's Review*

# Structure and Cytotoxic Properties of Some Selected Gold(III) Complexes*

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Gold(III) complexes, isostructural and isoelectronic with platinum(II) complexes, are of potential interest as antitumor drugs. We prepared four representative square planar gold(III) complexes – $[AuCl_3(Hpm)]$, $[AuCl_2(esal)]$, $[AuCl(dien)]Cl_2$ and $[Au(en)_2]Cl_3$ – and characterized them both in the solid state and in solution. Thereafter, the cytotoxicity of these compounds was evaluated *in vitro* against the A2780 human ovarian tumor cell line that was used as the reference cell line. Remarkably, all these gold(III) complexes showed significant cytotoxic effects, $[AuCl_2(esal)]$ showing a potency comparable to cisplatin. The present gold(III) complexes were also tested on the corresponding cisplatin-resistant line and revealed they were able to overcome resistance to cisplatin to a large extent. The implications of these findings for the development of new gold(III) complexes to be tested as antitumor agents are discussed.

*Key words:* gold complexes, X-ray structure, antitumoral compounds, cytotoxicity.

## INTRODUCTION

The success of cisplatin in anticancer chemotherapy has raised great interest in the study of metal complexes as possible antitumor agents. The main goals of the intense research activity carried out in the field are the

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* Dedicated to Professor Boris Kamenar on the occasion of his 70th birthday.

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following: i) search for new metal based anticancer drugs; ii) understanding of their mechanism of action.

Within this frame we have synthesized in our laboratory a number of gold(III) complexes to be tested for antitumor activity *in vitro*. The choice of gold(III) derives from the fact that this ion gives rise to complexes that are isoelectronic and isostructural with those of platinum(II), whose antitumor activity is well established.[1] Surprisingly, in spite of the strict chemical similarity, only very few literature data exist on the use of gold(III) complexes as anticancer agents. The present knowledge of the antitumor properties of gold(III) complexes was summarized by Sadler.[2] From the few data available, and by analogy with the case of platinum(II) complexes, it is hypothesized that the biological action of gold(III) compounds is mediated by a direct interaction with DNA.[2] For instance, trichloropyridine gold(III) was reported to react with a number of different conformations of pBR322 DNA and to produce interstrand cross links and single strand breaks;[3] $Et_3PAuBr_3$ is known to bind Hind III/Ncil, a 139 base pair restriction fragment from pBR322.[4]

For a preliminary evaluation of the cytotoxic properties of these compounds, we prepared four representative gold(III) complexes – *viz.* trichloro(2-pyridylmethanol) gold(III), $[AuCl_3(Hpm)]$,[5] dichloro(*N*-ethylsalicylaldiminato) gold(III), $[AuCl_2(esal)]$,[5] trichlorodiethylendiamine gold(III), $[AuCl(dien)]Cl_2$[6] and trichlorobisethylendiamine gold(III), $[Au(en_2)]Cl_3$,[7] – characterized their

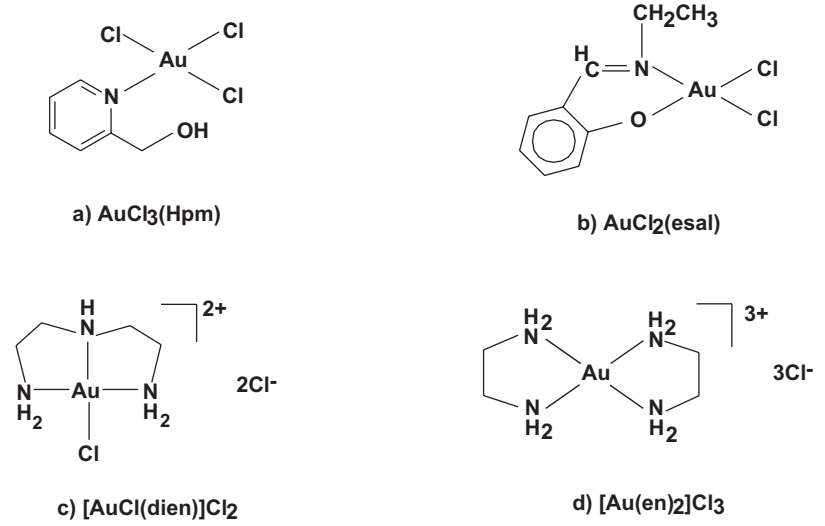


Figure 1. Schematic drawings of the complexes: a) $[AuCl_3(Hpm)]$, b) $[AuCl_2(esal)]$, c) $[AuCl(dien)]Cl_2$, and d) $[Au(en)_2]Cl_3$.

chemical properties in the solid state and in solution and evaluated their cytotoxic activity *in vitro* towards the A2780 human ovarian carcinoma cell line.

All the mentioned complexes essentially correspond to a square planar geometry but have different sets of donor atoms (Figure 1). The $[AuCl_3(Hpm)]$ contains three chlorides coordinated to the gold(III) center; $[AuCl_2(esal)]$ contains two coordinated chlorides, $[AuCl(dien)]Cl_2$ a single coordinated chloride and $[Au(en_2)]Cl_3$ no coordinated chlorides. In all cases, the remaining donors are nitrogen atoms – except for $[AuCl_2(esal)]$, which has a nitrogen and an oxygen – belonging either to amine groups or to heterocycles. Thus, one of the main goals of the present research is to establish whether any direct correlation exists between the nature of the gold(III) ligands and the cytotoxic properties of these compounds.

## EXPERIMENTAL

### *Synthesis of the Compounds*

The gold(III) complex $[AuCl_3(Hpm)]$ was synthesized according to the reported procedure:[5] a solution of 2-pyridylmethanol in water was added dropwise to a stirred solution of $NaAuCl_4$ and sodium chloride in water at 0 °C. A yellow solid, $[AuCl_3(Hpm)]$, separated immediately; the stirring went on for 45 minutes. The purity of the resulting products was tested through elemental analysis. The $[AuCl_2(esal)]$ was synthesized according to the reported procedure as well:[5] a solution of $NaAuCl_4$ in water was cooled to about –5 °C. To this solution, *N*-ethylsalicylaldimine dissolved in methanol was added dropwise under stirring during 5–10 min. The mixture became cloudy mustard-brown and was allowed to stand in the refrigerator for a week. The upper aqueous layer was decanted from the dark oil which had formed. Chlorobenzene was added to the oil, much of which dissolved to give a purple solution. This solution was evaporated in vacuum to a small volume and then addition of diethyl ether gave a green-blue precipitate of the complex $[AuCl_2(esal)]$. The $[AuCl(dien)]Cl_2$ was prepared according to Ref. 6; the $[Au(en_2)]Cl_3$ was prepared according to Ref. 7.

### *X-ray Crystallography*

All data for $[AuCl_3(Hpm)]$ and $[AuCl_2(esal)]$ were collected on an Enraf-Nonius CAD4 diffractometer with graphite-monochromated Cu-K$\alpha$ radiation ($\lambda$ = 1.54180 Å) at room temperature. Cell parameters were obtained from 25 well-centered reflections by using least-square refinement. The absorption correction was done with the program PSISCAN included in the Enraf-Nonius Structure Determination Package.[8] The structures were solved using direct methods and successive difference Fourier synthesis. All non-hydrogen atoms were refined anisotropically and hydrogen atoms were placed in the calculated position and refined isotropically. The calculations were carried on with the SIR92[9] and SHELXL93[10] programs.

### *Cytotoxicity*

For cytotoxicity studies, the human ovarian carcinoma A2780/S cell line was used. The cisplatin resistant A2780/R cell line was produced by exposure of the sensitive parental cell lines to incremental concentrations of cisplatin and was maintained in culture with 10 and 50 μM CDDP, respectively. Cell lines were maintained in RPMI 1640 medium supplemented with fetal bovine serum and antibiotics at 37 °C in a 5% $CO_2$ atmosphere and subcultured twice weekly. Experiments were conducted on exponentially growing cells. The SRB assay was conducted in 96-well plates using RPMI 1640 + 5% FBS. The SRB assay was performed according to the procedure described by Skehan *et al.*[11]

## RESULTS

### *Structural Description of the Investigated Gold(III) Complexes*

The crystal structures of $[AuCl(dien)]Cl_2$ and $[Au(en)_2]Cl_3$ were previously solved by other groups and are available in the literature.[6,7] The crystal structures of $[AuCl_3(Hpm)]$ and $[AuCl_2(esal)]$ have been solved in our laboratory and published separately.[12,13] ORTEP views of these latter complexes are shown in Figure 2. All gold(III) complexes considered in this study essentially exhibit a square planar geometry with small distortions from regularity. Au–Cl and Au–N distances fall in the expected range [Au–Cl (av) = 2.274 Å; Au–N (av) 2.031 Å]. In $[AuCl_3(Hpm)]$ the coordination plane makes an angle of 73.0° with the pyridine ring, a position that minimizes the repulsion energy. The coordination plane in $[AuCl_2(esal)]$ is not coplanar

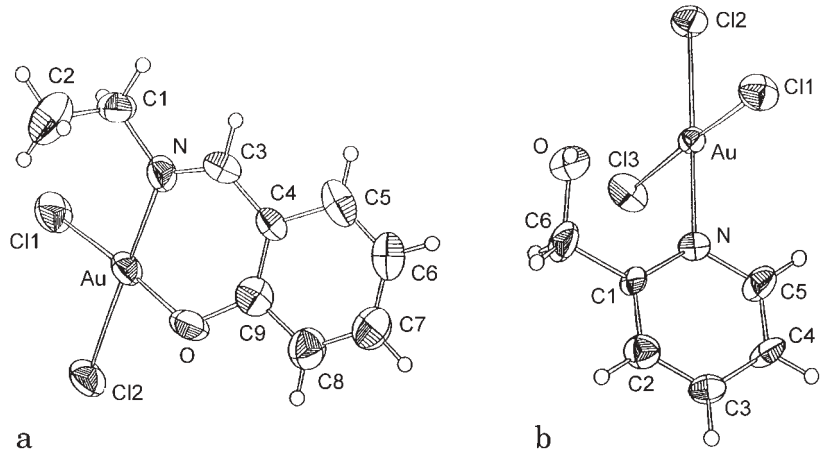


Figure 2. ORTEP diagrams of the complexes: a) $[AuCl_2(esal)]$ and b) $[AuCl_3(Hpm)]$. The ellipsoids show 50% of probability.

with the aromatic moiety of the salicylaldiminate ligand either, the angle being 152°. Interestingly enough, in $[AuCl(dien)]Cl_2$ gold(III) coordination is completed by two chlorides at 3.12 and 3.18 Å.

### *Solution Properties*

The $[Au(en)_2]Cl_3$ and $[AuCl(dien)]Cl_2$ are fairly soluble in water; in contrast, $[AuCl_3(Hpm)]$ and $[AuCl_2(esal)]$ are poorly soluble in water but dissolve easily in DMSO. The stability of the four complexes in a physiological buffer was investigated spectrophotometrically. In fact, all complexes exhibit characteristic ligand to metal charge transfer bands in the visible region, which allow direct monitoring of the gold(III) chromophore. It is ob-

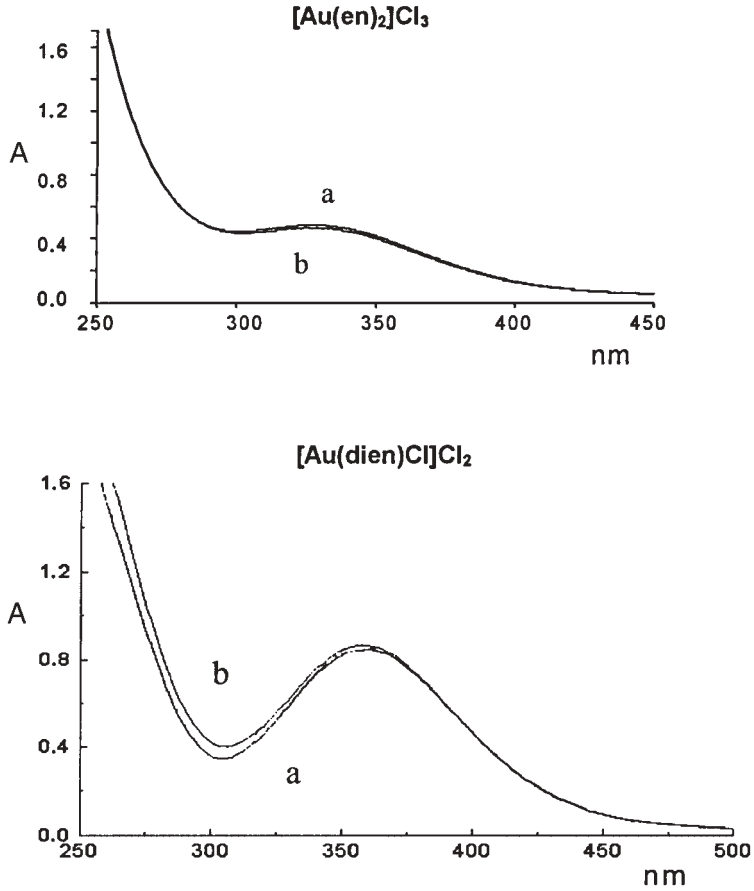


Figure 3. UV spectra of $[Au(en)_2]Cl_3$ and $[AuCl(dien)]Cl_2$ in physiological buffer a) at mixing, and b) after one hour.

served that $[Au(en)_2]Cl_3$ and $[AuCl(dien)]Cl_2$ are stable for several hours when dissolved within a physiological buffer (Figure 3); in contrast, $[AuCl_3(Hpm)]$ and $[AuCl_2(esal)]$, which are quite stable in DMSO, undergo quick transformation and degradation when dissolved in the same buffer.[14]

### *Cytotoxic Properties*

The cytotoxic properties of the four complexes were analyzed with respect to the A2780 human ovarian carcinoma cell line, either sensitive or resistant to cisplatin. The results are shown in Figure 4 and Table I. All complexes exhibit significant cytotoxic properties with $IC_{50}$ values falling in the 5–15 micromolar range. The order of potency shown by the four complexes is the following: $[AuCl_2(esal)] > [Au(en)_2]Cl_3 > [AuCl(dien)]Cl_2 > [AuCl_3(Hpm)]$.

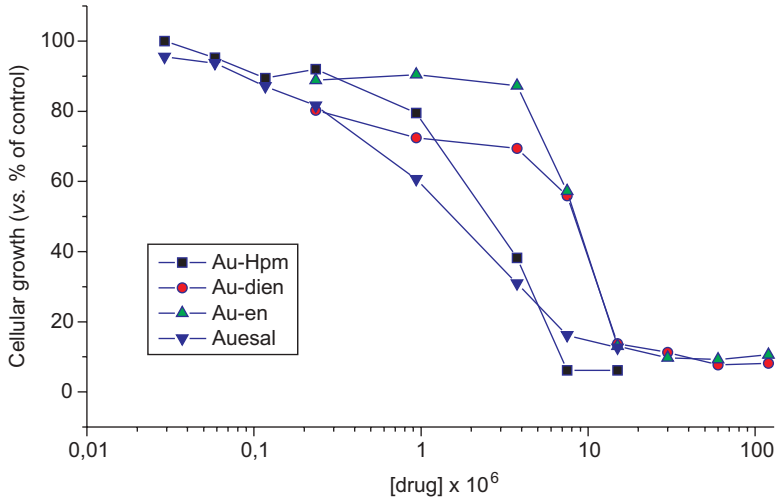


Figure 4. Cytotoxicity curves of the various gold(III) complexes.

TABLE I

$IC_{50}$ values for the listed complexes: average data of 3 experiments

| | [Au(Hpm)] | [Au(esal)] | [Au(dien)] | $[Au(en)_2]$ | CDDP |
|---|---|---|---|---|---|
| A2780/S | 10.1 ± 1.0 | 2.1 ± 0.7 | 8.2 ± 1.3 | 8.4 ± 1.1 | 1.6 ± 1.3 |
| A2780/R | 21.0[a] | 3.8 ± 1.4 | 18.7 ± 3.0 | 17.0 ± 6.0 | 16.1 ± 8.7 |
| Ratio R/S | 2.08 | 1.81 | 2.28 | 2.02 | 10.06 |

[a] 2 experiments only.

Remarkably, all complexes exhibit favorable cytotoxic properties toward the cisplatin resistant cell line. The decrease in sensitivity to gold(III) complexes when passing from the A2780S to the A2780R line is on average less than a factor two whereas sensitivity to cisplatin decreases by more than one order of magnitude.

## DISCUSSION AND CONCLUSIONS

Gold(III) complexes, isostructural and isoelectronic with Pt(II) compounds, are potentially attractive as antitumor agents. Given the strict similarity to cisplatin, gold(III) complexes are of interest for two main reasons: on the one side, they may constitute a new class of anticancer compounds with a novel profile of antitumor activity; on the other side, they represent a further attempt to elucidate the mechanism of action of antitumor $d^8$ square planar metal complexes, which still remains largely unknown. In this context, it is of fundamental importance to determine to what extent gold(III) compounds are cytotoxic and to define their structure-function relationships. Previous investigations had already revealed favorable cytotoxic properties for some gold(III) complexes; however, the data present in the literature are still very scarce, probably as a consequence of the high reactivity of gold(III) complexes.

In any case, the evaluation of the cytotoxic properties of a series of gold(III) compounds synthesized in our laboratory has provided satisfactory and even surprising results. Indeed, analysis of the activity profiles suggests that in some cases the intrinsic cytotoxicity of the present compounds is comparable to or only slightly lower than that of cisplatin ($IC_{50}$ values in the 1–10 μM range). In some cases, these compounds exhibit a cytotoxic action that even exceeds that of cisplatin itself. Since all the investigated complexes exhibit $IC_{50}$ values roughly falling within a single order of magnitude, it may be ruled out that there is a direct correlation between the number of gold-coordinated chloride groups and the cytotoxic effects. In fact, either [AuCl(dien)]$Cl_2$ or [Au$(en)_2$]$Cl_3$ bearing only a single or no gold coordinated chloride exhibit cytotoxic properties comparable to those of [$AuCl_3$(Hpm)] and [$AuCl_2$(esal)] with two or three gold(III) coordinated chlorides. Such a finding allows us to state that the presence of one or more gold(III) coordinated halides is not an essential requirement for cytotoxcity in gold(III) complexes. Apparently, more complex effects are responsible for the observed biological acitivity. In addition, it has been recently reported[14] that the gold(III) complex [Au(cyclam)]$(ClO_4)_2Cl$ containing four nitrogen donor atoms is virtually devoid of cytotoxicity toward the same tumor cell line. Even the favorable cytotoxic properties of [$AuCl_2$(esal)] are a surprise if one considers the high chemical reactivity of this compound. Indeed, un-

der physiological conditions, [$AuCl_2$(esal)] readily hydrolyzes and partially transforms into a reduced gold(I) species. The fact that the measured cytotoxicity is nevertheless relevant may imply that this complex either remains in part as a gold(III) species and is quickly taken up by cells as such or that one of its metabolites is highly cytotoxic.

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## REFERENCES

1. R. J. Puddephatt, *The Chemistry of Gold*, Elsevier, Amsterdam, 1978.
2. P. J. Sadler and O. M. Ni Dhubhghaill, in: B. K. Keppler (Ed.), *Metal Complexes in Cancer Chemotherapy*, VCH, New York, 1993, pp. 221–228.
3. P. J. Sadler, M. Nasr, and V. L. Narayanan, in: E. B. Douple and I. H. Krakhoff (Eds.), *Platinum Coordination Complexes in Cancer Chemotherapy*, Nijhoff Publishers, Boston, 1984, pp. 290–304.
4. B. Ward and J. C. Dabrowiak, *J. Am. Chem. Soc.* **109** (1987) 3810–3811.
5. A. Dar, K. Moss, S. M. Cottrill, R. V. Parish, C. A. McAuliffe, R. G. Pritchard, B. Beagley, and J. Sandbank, *J. Chem. Soc., Dalton Trans*. (1992) 1907–1913.
6. B. P. Block and J. C. Bailar, *J. Am. Chem. Soc.* **73** (1951) 4722–4725.
7. G. Nardin, L. Randaccio, G. Annibale, G. Natile, and B. Pitteri, *J. Chem. Soc., Dalton Trans.* (1979) 220–222.
8. B. A. Frenz, *Enraf-Nonius Structure Determination Package*; Enraf-Nonius Delft, Holland, 1985.
9. M. C. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, G. Polidori, R. Spagna, and D. Viterbo, *J. Appl. Crystallogr.* **22** (1989) 389–394.
10. G. M. Sheldrick, SHELXL 93. A Program for Structure Refinement, University of Göttingen, Germany, 1993.
11. P. Skehan, R. Stroreng, D. Scudiero, A. Monks, J. Mcmahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, and M. R. Boyd, *J. Natl. Cancer Inst*. **82** (1996) 1107–1112.
12. B. Bruni, M. Ferraroni, P. Orioli, and G. P. Speroni, *Acta Crystallogr., Sect. C* **52** (1996) 1423–1424.
13. P. Calamai, A. Guerri, L. Messori, P. Orioli, and G. P. Speroni, *Inorg. Chim. Acta* **285/2** (1999) 309–312.
14. S. Carotti, A. Guerri, T. Mazzei, L. Messori, E. Mini, and P. Orioli, *Inorg. Chim. Acta* **281** (1998) 90–94.

## SAŽETAK

### Struktura i citotoksična svojstva nekih izabranih zlatovih(III) kompleksa

*Bruno Bruni, Annalisa Guerri, Giordana Marcon, Luigi Messori i Pierluigi Orioli*

Zlatovi(III) kompleksi koji su izostrukturni i izoelektronski s platinskim(II) kompleksima potencijalni su antitumorni lijekovi. Priređena su četiri tipična kompleksa trovalentnog zlata kvadratne koordinacije – $[AuCl_3(Hpm)]$, $[AuCl_2(esal)]$, $[AuCl(dien)]Cl_2$ and $[Au(en)_2]Cl_3$ – i potanko proučena, kako u kristalnom stanju tako i u otopini. Nadalje, citotoksičnost tih spojeva procijenjena je *in vitro* na ljudskim tumornim stanicama jajnika A2780 koje su bile korištene kao referentna skupina stanica. Znakovito je da su svi ti zlatovi(III) kompleksi pokazivali znatne citotoksične učinke od kojih je $[AuCl_2(esal)]$ pokazivao djelovanje usporedljivo onom cisplatina. Kada su zlatovi(III) kompleksi bili ispitani na odgovarajućoj skupini stanica koje su bile rezistentne na cisplatin, rezultati su znatno premašivali otpornost prema cisplatinu. Raspravlja se o značenju rezultata ovih istraživanja na razvoj novih kompleksa trovalentnog zlata koji bi se mogli uporabiti kao antitumorni agensi.