ELSEVIER

Inorganica Chimica Acta 281 (1998) 90–94

Inorganica Chimica Acta

Gold(III) compounds as potential antitumor agents: cytotoxicity and DNA binding properties of some selected polyamine–gold(III) complexes

Stefania Carotti [a], Annalisa Guerri [b], Teresita Mazzei [a], Luigi Messori [b], Enrico Mini [a], Pierluigi Orioli [b,*]

[a] Department of Pharmacology, University of Florence, viale Morgagni 65, 50134 Florence, Italy
[b] Department of Chemistry, University of Florence, via Gino Capponi 7, 50121 Florence, Italy

Received 30 December 1997; received in revised form 5 February 1998; accepted 23 February 1998

Abstract

Three gold(III)–polyamine complexes of different structure — ([Au(en)$_2$]Cl$_3$, [AuCl(dien)]Cl$_2$ and [Au(cyclam)](ClO$_4$)$_2$Cl) — were synthesized and tested for cytotoxic properties against a human tumor cell line (A2780) either sensitive (A2780/S) or resistant (A2780/R) to cisplatin. Significant cytotoxic activities were found for both [AuCl(dien)]Cl$_2$ and [Au(en)$_2$]Cl$_3$, whereas [Au(cyclam)](ClO$_4$)$_2$Cl was poorly cytotoxic. The stability of these gold(III) complexes under physiological conditions was investigated through absorption spectroscopy; their ability to bind DNA, the presumed final target for the cytotoxic action, and modify its conformation was studied through atomic absorption and circular dichroism spectroscopies. Attempts are made to define preliminary structure–function relationships for cytotoxic gold(III) complexes.

Keywords: Gold complexes; Polyamine complexes; Cytotoxicity

1. Introduction

Over the last two decades, several studies have been devoted to the evaluation of gold compounds as potential antitumor agents, much of the work focusing on gold(I) phosphine complexes [1–3]. Also, a few gold(III) complexes have been shown to display significant antitumor properties [2]. The Au(III) coordination center is isoelectronic with Pt(II) and both give rise to square planar geometries; the main difference between gold(III) and platinum(II) compounds is that ligand substitution reactions are usually much faster in the case of gold(III) compounds [4]. The strict relationship to platinum(II) compounds makes gold(III) complexes good candidates for development and testing as anticancer drugs, although the relatively high kinetic lability and the usually high redox potentials have largely hindered such investigations. Some recent studies reporting that novel gold(III) compounds show favorable antitumor properties both in vitro and in vivo have raised new interest in this research area [5–7]. For instance, Buckley et al. [5] reported that some newly synthesized organogold(III) compounds (more precisely dimethylaminomethylphenyl gold(III) complexes) exhibit significant cytotoxic properties in vitro and are active towards established human tumors implanted in mice; the cytotoxicity and antitumor properties of these organogold(III) compounds are apparently the consequence of direct DNA damage. The gold(III) compounds AuCl$_2$(esal) and AuCl$_3$(Hpm), studied in our laboratory, have also shown important in vitro cytotoxicity versus a panel of cisplatin-sensitive tumor cell lines [6,7]; remarkably, the latter compounds are able to overcome nearly completely resistance to platinum. By analogy with the mechanism of action of cisplatin [8,9], and on the grounds of previous experimental evidence [10,11], it has been proposed that the presumed final target of AuCl$_2$(esal) and AuCl$_3$(Hpm) is DNA, even though other targets cannot be excluded a priori [7].

Here, we report on the cytotoxic properties of three more gold(III) compounds (namely [Au(en)$_2$]Cl$_3$, [AuCl(dien)]Cl$_2$ and [Au(cyclam)](ClO$_4$)$_2$Cl), possessing remarkably different chemical structures, towards a representative human tumor cell line (A2780), either sensitive or resistant to cisplatin. This study, in connection with

* Corresponding author. Tel.: +39-55-275 7554; fax: +39-55-275 7555; e-mail: orioli@risc1.lrm.fi.cnr.it

0020-1693/98/$ - see front matter
PII S0020-1693(98)00148-0

the results obtained earlier on $AuCl_3$(esal) and $AuCl_3$(Hpm) on the same cell line, represents the first attempt to define structure–function relationships for cytotoxic gold(III) compounds. In particular, we aim to elucidate whether the presence of direct gold(III)–chloride bonds is an essential feature for cytotoxicity; at the same time, we aim to establish whether kinetic or thermodynamic stabilization of the 3+ oxidation state of gold, achieved through appropriate selection of the ligands, results in an enhancement (or a decrease) in the biological activity. Moreover, the ability of these compounds to bind DNA and modify its conformation was investigated through atomic absorption and circular dichroism (CD) spectroscopies.

2. Experimental

2.1. Synthesis of the compounds

The gold(III) complexes $[Au(en)_2]Cl_3$, $[AuCl(dien)]Cl_2$ and $[Au(cyclam)](ClO_4)_2Cl$ were synthesized according to the reported procedures. $[Au(en)_2]Cl_3$ was prepared according to Ref. [12]: upon adding a solution of 1,2-ethylenediamine monohydrate in diethyl ether to a solution of $HAuCl_4$ in ether, a gummy yellow precipitate formed. A white precipitate then formed as ethanol was added to the orange water solution of the previous compound. $[AuCl(dien)]Cl_2$ was prepared as described previously [13]: a solution of diethylenetriamine 3HCl in water was added slowly and with stirring to a solution of $HAuCl_4$ (20% wt./vol.). A yellow precipitate formed immediately. Then, a solution of Na(OH) 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 cyclam (1,4,8,11-tetraazacyclotetradecane) in CH_3CN for 1 h yielded the 'Au(III)-in' complex $(ClO_4)_2Cl$.

2.2. Electronic spectra

The absorption spectra in the UV–Vis region were recorded on a Cary 5 spectrophotometer operating at room temperature. The present gold(III) complexes are sufficiently soluble in water; electronic spectra were recorded on freshly prepared solutions. The hydrolysis experiments were performed by adding small amounts of freshly prepared, concentrated solutions of the various gold(III) complexes to water or buffer solutions, and by monitoring the electronic spectra of the resulting mixtures for 60–180 min after mixing.

2.3. Cytotoxicity studies

For cytotoxicity studies, the representative cisplatin-sensitive ovarian carcinoma A2780/S human cell line was used. The platinum-resistant A2780/R cell line was produced by repeated 1 h weekly exposure to 50 μM of the sensitive parental cell line [15]. 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 conducted in 96-well plates using RPMI 1640 medium + 5% FBS, according to the procedure described by Skekan et al. [16].

2.4. Atomic absorption measurements

Atomic absorption measurements were carried out with a Varian instrument. Calibration curves were built up for all the individual complexes. Samples for atomic absorption spectroscopy were prepared as follows: aliquots of the various gold(III) complex solutions were added to buffered calf thymus DNA solutions in such a way to obtain r values of ~0.1. Samples were left to stand overnight at 25°C. DNA was then precipitated by addition of 70% ethanol and the pellet separated by centrifugation and resuspended in the buffer. Then, the gold content was measured both in the supernatant and in the resuspended pellet through AAS.

2.5. Circular dichroism spectra

The CD spectra of calf thymus DNA reacted with increasing amounts of the various gold(III) complexes, in a standard physiological buffer, were recorded with a Jasco J500C dichrograph. Spectra were taken immediately after mixing and were monitored for 30–60 min afterwards. Calf thymus DNA was purchased from Sigma Chemical Co. The concentrations of calf thymus DNA were ~60 μg ml^{-1}, in phosphate buffer (50 mM, pH 7.4) and 4 mM NaCl. The concentration of the gold(III) complex is expressed as the number of moles of gold added per mole of base pair (r) with r values usually ranging between 0.1 and 0.5.

3. Results

3.1. Chemistry

A schematic drawing of the structures of the various gold(III) complexes tested in this study is shown in Fig. 1. The three complexes — $[AuCl(dien)]Cl_2$ (d), $[Au(en)_2]Cl_3$ (e), and $[Au(cyclam)](ClO_4)_2Cl$ (f) — essentially exhibit a square planar geometry, with nitrogen atoms as donors, as revealed by crystallographic studies [13,14, 17,18]; in the case of $[AuCl(dien)]Cl_2$, one of the four coordination positions around the gold(III) center is occupied by a chloride ion, which behaves as a good leaving group. For comparison purposes, schematic drawings of $AuCl_4^-$ (a), $AuCl_3$(Hpm) (b), and $AuCl_2$(esal) (c), studied previously in our laboratory, are also shown.

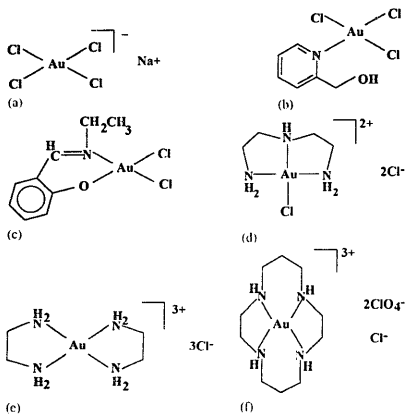

Fig. 1. Schematic drawings of $AuCl_4^-$ (a), $AuCl_3(Hpm)$ (b), $AuCl_2(esal)$ (c), $[AuCl(dien)]Cl_2$ (d), $[Au(en)_2]Cl_3$ (e) and $[Au(cyclam)](ClO_4)_2Cl$ (f).

At variance with $AuCl_2(esal)$ and $AuCl_3(Hpm)$, $[Au(en)_2]Cl_3$, $[AuCl(dien)]Cl_2$ and $[Au(cyclam)](ClO_4)_2Cl$ are, in some degree, soluble in water; as expected, dissolution in water results in strongly acidic hydrolysis. $[Au(en)_2]Cl_3$ and $[Au(cyclam)](ClO_4)_2Cl$ do not show electronic transitions in the 300–600 nm range; intense transitions are instead observed in the near-UV region, which are assigned tentatively as nitrogen to gold(III) charge transfer transitions. In contrast, $[AuCl(dien)]Cl_2$ shows a quite intense transition at 320 nm, which is assigned as a Cl to gold(III) charge transfer band.

The spectra of $[Au(en)_2]Cl_3$, $[AuCl(dien)]Cl_2$ and $[Au(cyclam)](ClO_4)_2Cl$ in water, under acidic conditions, are stable for several hours. In all cases, an increase in the pH to neutrality, or to slightly alkaline values, causes deprotonation of one of the gold coordinated amine groups and the appearance of an intense visible band between 320 and 370 nm that has previously been assigned as an N^- to gold(III) charge transfer transition [14]. The pK_a value for this deprotonation has previously been shown to be ~5.4 for $Au(cyclam)](ClO_4)_2Cl$ [14] and ~6.3 for $[Au(en)_2]Cl_3$ [19], revealing that the deprotonated species is predominant at physiological pH and, therefore, the species formally responsible for the observed biological activity. The position of the charge transfer band for the three compounds, dissolved in a physiological buffer containing 0.1 M phosphate, 4 mM NaCl, pH 7.4, is 330 nm for $[Au(en)_2]Cl_3$, 365 nm for $[AuCl(dien)]Cl_2$ and 360 nm for $[Au(cyclam)](ClO_4)_2Cl$. Remarkably, these spectra do not undergo any significant change over a time period of several hours, implying that the three complexes are sufficiently stable within a physiological environment.

3.2. Cytotoxicity

The in vitro cytotoxic properties of $[Au(en)_2]Cl_3$, $[AuCl(dien)]Cl_2$ and $[Au(cyclam)](ClO_4)_2Cl$ were evaluated on the human ovarian cell line A2780 either sensitive (A2780/S) or resistant (A2780/R) to cisplatin. Results are shown in Fig. 2 and Table 1; for comparison purposes, the IC_{50} values of $AuCl_4^-$, $AuCl_2(esal)$, and $AuCl_3(Hpm)$, tested previously in our laboratory on the same cell lines, are reported. It is apparent that the three compounds tested in this study exhibit markedly different cytotoxicity profiles; whereas the $[Au(cyclam)](ClO_4)_2Cl$ complex is virtually inactive, both $[Au(en)_2]Cl_3$ and $[AuCl(dien)]Cl_2$ are fairly cytotoxic with IC_{50} values falling in the 5–20 μM range. Noticeably, $[Au(en)_2]Cl_3$ and $[AuCl(dien)]Cl_2$ retain almost completely their cytotoxicity versus the cisplatin-resistant A2780 line. In fact, whereas the sensitivity to cisplatin in A2780/R decreases by more than a factor of 8 as compared with A2780/S, the sensitivity to $[Au(en)_2]Cl_3$ and $[AuCl(dien)]Cl_2$ decreases only by a factor of ~2, on average. These results suggest that the mechanisms of resistance to platinum are only marginally effective towards the present gold(III) compounds; results of the same kind had earlier been reported for $AuCl_2(esal)$ and $AuCl_3(Hpm)$ [6,7].

The fact that $[Au(cyclam)](ClO_4)_2Cl$ is virtually inactive on the A2780/S cell line suggests that the macrocycle encap-

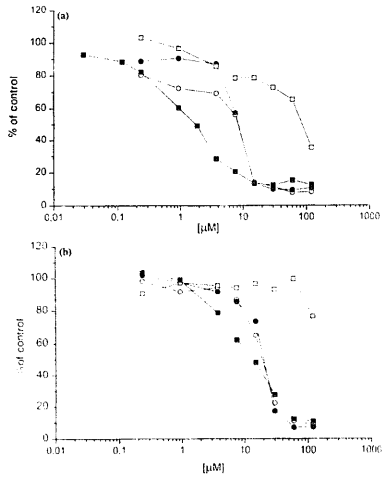

Fig. 2. Drug sensitivity profiles of cisplatin sensitive and resistant human tumor cell lines (A2780/S (a) and A2780/R (b)) towards polyamine gold(III) complexes. Graphs show the percentage of growth with respect to control upon incubation of increasing amounts of $[Au(en)_2]Cl_3$ (–●–), $[AuCl(dien)]Cl_2$ (–○–), and $[Au(cyclam)](ClO_4)_2Cl$ (–□–). The effects of cisplatin (–■–) are also shown.

Table 1
IC_{50} values (μM) of various gold(III) complexes against the human ovarian carcinoma A2780 cell line either sensitive or resistant to cisplatin

Cell line	AuCl(dien)	$AuCl_3(en)$	Aucyclam	$AuCl_3$(Hpm)	Auesal	$NaAuCl_4$	CDDP
A2780/S	8.2 ± 1.3	8.4 ± 1.1	99	10.1 ± 1.0	2.1 ± 0.7	11.0 ± 2.0	2.26 ± 1.3
A2780/R	18.7 ± 3.0	17 ± 6	n.d.	21.0	3.8 ± 1.4	17.7	19.3 ± 10

Standard deviation values are reported.

sulating the gold(III) ion largely blocks its reactions with target biomolecules and neutralizes its biological activity. Surprisingly, $[Au(en)_2]Cl_3$ exhibits a rather relevant cytotoxicity (IC_{50} = 8 μM), comparable with that shown by $[AuCl(dien)]Cl_2$, indicating that the presence of a relatively stable bidentate ligand such as ethylenediamine does not lead to inactivation of the biological properties of the coordinated gold(III) center. However, at the present state of knowledge, it is difficult to establish whether the cytotoxicity of $[Au(en)_2]Cl_3$ depends directly on the biological action of the complex as such or is the consequence of ligand exchange reactions and of the formation of active metabolites.

3.3. DNA binding properties: circular dichroism and atomic absorption measurements

It is commonly believed that the biological activity of anticancer metal complexes is strictly connected to their ability to bind DNA, damage its structure and impair its function [20]. Impairment of DNA function results in inhibition of replication and transcription processes and, eventually, if the DNA lesions are not rapidly and properly repaired, in cell death. A mechanism of this kind is probably operative for platinum compounds and is likely to be operative also in the case of gold(III) complexes closely related to platinum(II) complexes. Some experimental evidence already exists in the literature supporting this view [10]. In order to verify this hypothesis we followed, in vitro, through CD spectroscopy, the reaction of our investigational gold(III) complexes with calf thymus DNA. CD spectroscopy is indeed a valuable tool for analysis of ligand-induced changes of DNA conformation [21]. CD spectra of calf thymus DNA samples upon addition of increasing amounts of the various gold(III) complexes were recorded in the 0.1–0.5 metal-to-base pair concentration range. It emerges that within the investigated concentration range, the various gold(III) complexes do not modify appreciably the classical B-type conformation of calf thymus DNA; only slight spectral changes are detected at the very high metal-to-DNA ratios. The largest changes in the CD spectra were observed upon the addition of $[Au(cyclam)]^{3+}$, as shown in Fig. 3; the spectral changes consist mainly of a significant decrease in the intensity both of the positive band at 280 nm and of the negative band at 240 nm and are suggestive of partial loss of helicity. Conversely, atomic absorption data indicate that substantial amounts of added gold(III) ions are found associated to DNA after 24 h incubation (~60–50% for $Au(en)_2$; ~45–35% for $[Au(cyclam)](ClO_4)_2Cl$; ~35–25% for $[AuCl(dien)]Cl_2$), demonstrating that under the employed solution conditions, gold binds DNA to a significant extent.

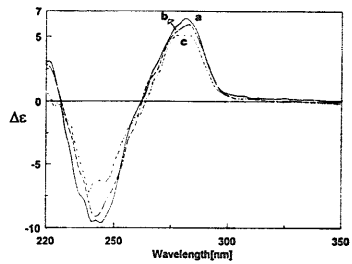

Fig. 3. CD spectra of calf thymus DNA alone (a) and after addition of $Au(cyclam)](ClO_4)_2Cl$ at metal-to-bp ratios of 0.1 (b) and 0.5 (c). Conditions: calf thymus DNA (60 μg ml^{-1}), pH 7.4, 50 mM phosphate buffer, 0.1 M NaCl.

4. Discussion

Gold(III) complexes, isoelectronic and isostructural with platinum(II) complexes, display relevant cytotoxic properties in vitro on various human tumor cell lines and are therefore good candidates for further evaluation and development as anticancer drugs. Prompted by recent results on the favorable cytotoxic properties of some novel gold(III) complexes, we have started a larger research program aimed at defining structure–function relationships for gold(III) compounds.

In this context, we report here on the chemistry, the cytotoxicity and the DNA binding properties of three representative square planar gold(III) complexes with polyamine ligands — ($[Au(en)_2]Cl_3$, $[AuCl(dien)]Cl_2$ and $[Au(cyclam)](ClO_4)_2Cl$) — possessing markedly different chemical structures. One of them, $[AuCl(dien)]Cl_2$, contains a labile gold(III)–chloride bond and is potentially able to form covalent bonds with biomolecules after chloride loss, whereas in the remaining two complexes the gold(III) center is tightly coordinated to four nitrogen ligands. All three complexes are water soluble, and are stable in buffered solutions for hours. In all cases, the presence of several nitrogen donors leads to a significant stabilization of the 3+ oxidation number of gold in comparison with previously tested gold(III) complexes. The strongly acidic properties of the gold(III) center

induce deprotonation of a gold coordinated amine group with pK_a values around 5.5–6.5.

The cytotoxic properties of the three compounds are markedly different: when tested on the A2780 line, both $[Au(en)_2]Cl_3$ and $[AuCl(dien)]Cl_2$ show an important cytotoxicity toward the A2780/S cell line (IC_{50} of the order of 5–10 μM), whereas $[Au(cyclam)](ClO_4)_2Cl$ is poorly cytotoxic (cytotoxicity is observed only for concentrations as high as 10^{-4} M). Remarkably, resistance to platinum does not result in an important decrease in the cytotoxicity of either $[AuCl(dien)]Cl_2$ or $[Au(en)_2]Cl_3$, implying that the mechanisms that reduce platinum cytotoxicity in the resistant cells are scarcely effective towards gold(III) compounds.

The present results, considered together with earlier data on gold(III) complexes, allow us to make some inferences on structure–function relationships within this family of compounds. The gold(III) compounds tested previously in our laboratory — $AuCl_2$(esal), $AuCl_3$(Hpm) and $AuCl_4^-$ — had been shown to be fairly cytotoxic with IC_{50} values in the 1–15 μM range; more precisely, $AuCl_2$(esal) was the most cytotoxic with IC_{50} values of the order of 1–4 μM, whereas $AuCl_3$(Hpm) and $AuCl_4^-$ were shown to be almost equally cytotoxic with IC_{50} values in the 8–15 μM range. However, if one considers that these compounds undergo rapid transformation within a physiological environment, it may be inferred that their cytotoxicity is not the direct effect of the reactivity of the original gold(III) center but is probably mediated by some active metabolite, possibly in a reduced form. At variance, the present compounds — $[AuCl(dien)]Cl_2$, $[Au(en)_2]Cl_3$ and $[Au(cyclam)](ClO_4)_2Cl$ — owing to the lower number (or lack) of hydrolyzable chloride groups and to stabilization of the 3+ state brought about by the nitrogen donors, are far more stable than the former compounds in a physiological environment; thus, cytotoxicity may be quite safely ascribed to a gold(III) species, more precisely to an amine-deprotonated gold(III) species. The poor cytotoxicity observed for the $[Au(cyclam)](ClO_4)_2Cl$ complex is probably the consequence of stabilization and inactivation of the gold(III) center by the macrocyclic ligand; conversely, the relevant cytotoxicity observed for $[AuCl(dien)]Cl_2$ fits rather well the view that this species behaves as a monodentate ligand, following chloride hydrolysis, and may damage crucial biomolecules (e.g. proteins, DNA). Observation of relevant cytotoxic properties also for $[Au(en)_2]Cl_3$ is more intriguing. In fact, given the absence of free coordination positions around the gold(III) center, one would predict for this compound a poor reactivity and a poor ability to damage biomolecules. Observation of cytotoxicity in the micromolar range, in contrast, suggests either that this complex is cytotoxic as such (for example, by direct binding to DNA, as shown for $[Pt(en)_2]Cl_2$ [22]) or that it undergoes ligand exchange reactions and transformation into yet unidentified active metabolites.

In conclusion, the present investigation of the cytotoxic properties of polyamine–gold(III) complexes has shown a different degree of biological activity for the various investigated complexes, in strict dependence on their chemical structure. In particular, two of them — $[Au(en)_2]Cl_3$ and $[AuCl(dien)]Cl_2$ — exhibit rather encouraging cytotoxic properties, being also able to overcome, to a large extent, resistance to cisplatin. At variance with some previously investigated gold(III) complexes, both $[AuCl(dien)]Cl_2$ and $[Au(en)_2]Cl_3$ are reasonably stable within a physiological environment; these circumstances make them good candidates for further biological evaluation. The similar activity profiles of $[Au(en)_2]Cl_3$ and $[AuCl(dien)]Cl_2$ suggest that the presence of a labile gold–chloride bond is not an essential feature for cytotoxicity; instead, excessive stabilization of the gold(III) center by a polydentate ligand such as cyclam leads to reduction or even loss of the biological activity.

Acknowledgements

We would like to thank Dr Maja Marussich for carefully recording some of the CD and UV–Vis spectra reported in this paper.

References

[1] O.M. Ni Dhubhghaill, P.J. Sadler, in B.K. Keppler (ed.), Metal Complexes in Cancer Chemotherapy, VCH, Weinheim, 1993, p. 221.
[2] P.J. Sadler, R.E. Sue, Metal-Based Drugs 1 (1994) 107.
[3] C.K. Mirabelli, R.K. Johnson, C.-M. Sung, L. Faucette, K. Muirhead, S.T. Crooke, Cancer Res. 45 (1985) 32.
[4] R.J. Puddephatt, The Chemistry of Gold, Elsevier, Amsterdam, 1978.
[5] R.G. Buckley, A.M. Elsome, S.P. Fricker, G.R. Henderson, B.R. Theobald, R.V. Parish, B.P. Howe, L.R. Kelland, J. Med. Chem. 39 (1996) 5208.
[6] P. Calamai, S. Carotti, A. Guerri, L. Messori, E. Mini, P. Orioli, G.P. Speroni, J. Inorg. Biochem. 66 (1997) 103.
[7] P. Calamai, S. Carotti, A. Guerri, T. Mazzei, L. Messori, E. Mini, P. Orioli, G.P. Speroni, Anticancer Drug Des., in press.
[8] M.J. Bloemink, J. Reedijk, Met. Ions Biol. Syst. 32 (1996) 641.
[9] J. Reedijk, Chem. Commun. (1996) 801.
[10] C.K. Mirabelli, C.-M. Sung, J.P. Zimmerman, D.T. Hill, S. Mong, S.T. Crooke, Biochem. Pharm. 35 (1986) 1427.
[11] B. Ward, J.C. Dabrowiak, J. Am. Chem. Soc. 109 (1987) 3810.
[12] B.P. Block, J.C. Bailar Jr., Inorg. Chem. 73 (1951) 4722.
[13] G. Nardin, L. Randaccio, G. Annibale, G. Natile, B. Pitteri, J. Chem. Soc., Dalton Trans. (1979) 220.
[14] E. Kimura, Y. Kurogi, T. Takahashi, Inorg. Chem. 30 (1991) 4117.
[15] Y. Lu, J. Han, K.J. Scanlon, J. Biol. Chem. 263 (1988) 4891.
[16] P. Skekan, R. Stroreng, D. Scudiero, A. Monks, J. Mcmahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, J. Natl. Cancer Inst. 82 (1990) 1107.
[17] L. Kh, A.S. Minacheva, V.G. Gladkays, G.M.D. Sakharova, R.N. Shchelokov, M.A. Porau-Kahits, Zh. Neorg. Khim. 33 (1988) 683.
[18] E. Kimura, Y. Kuroki, T. Koike, M. Shionoya, Y. Iitaka, J. Coord. Chem. 28 (1993) 33.
[19] W.H. Baddey, F. Basolo, H.B. Gray, C. Nolting, A.J. Poe, Inorg. Chem. 2 (1963) 921.
[20] B.K. Keppler, in B.K. Holler (ed.), Metal Complexes in Cancer Chemotherapy, VCH, Weinheim, 1993, p. 37.
[21] D.M. Gray, R.L. Ratliff, M.R. Vaughan, Methods Enzymol. 211 (1992) 389.
[22] C.A. Franklin, J.V. Fry, J.G. Collins, Inorg. Chem. 35 (1996) 7541.