Antitumor Properties of Some 2-[(Dimethylamino)methyl]phenylgold(III) Complexes

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Received February 26, 1996⊗

Four analogues of the gold(III) complex [$AuCl_2$(damp)] (**1**) (damp = 2-[(dimethylamino)methyl]-phenyl) have been evaluated for antitumor activity. The compounds have structural features in common with cisplatin which was included as a comparison in the study. *In vitro*, against a panel of cell lines established from tumors of different tissue types, the gold complexes showed broadly similar growth inhibitory properties with some selectivity to the HT1376 bladder cell line. In a panel of human ovarian carcinoma cell lines, non-cross-resistance to cisplatin was observed, for the complexes, in an acquired cisplatin-resistant line. *In vivo*, using subcutaneously implanted xenografts derived from the HT1376 bladder and CH1 ovarian cell lines, [Au(acetato)$_2$(damp)] (**3**) and [Au(malonato)(damp)] (**5**) (administered intraperitoneally) gave significant tumor inhibition. Mechanistic studies performed with compound **3** showed marked differences to cisplatin. Thus, much higher concentrations of the gold compound were required to affect Col E1 plasmid mobility, and an alkaline elution study showed that **3** did not cause interstrand DNA cross-links in SK-OV-3 cells. Exposure of SK-OV-3 cells to **3** induced only relatively minor changes in cell cycle distribution. Furthermore **3** was only marginally active *in vivo* against the cisplatin-sensitive murine ADJ/PC6 plasmacytoma. In summary, the gold(III) complexes **3** and **5** exhibited selective cytotoxicity *in vitro* and showed *in vivo* antitumor activity against human carcinoma xenografts. Also, although **3** has some structural similarity to cisplatin, its mode of action appears to be different.

Introduction

Gold compounds have a long history of use as medicinal agents with antiarthritic drugs such as myocrysin and auranofin as more recent examples.[1,2] There have been several studies to evaluate gold compounds for potential antitumor properties with much of the work involving gold(I)−phosphine complexes.[3,4] The bis(diphenylphosphino)ethane (dppe) complexes [ClAu(μ-dppe)AuCl] and [Au(dppe)$_2$]Cl demonstrated activity against a range of murine tumors,[5,6] and auranofin was found active against intraperitoneal (ip) P388 leukemia.[7] A few gold(III) complexes have also displayed antitumor activity. Thus [Cl_3Au(μ-dppe)$AuCl_3$][5] and also the dimethylgold complexes [$AsPh_4$][$AuCl_2Me_2$] and [Me_2Au(μ-SCN)$_2AuMe_2$] inhibited ip P388.[8]

The Au(III) coordination center is isoelectronic with Pt(II), and both can form square-planar complexes. Therefore some gold(III) compounds can at least bear a formal resemblance to platinum antitumor agents such as cisplatin and carboplatin, although ligand substitution reactions are usually faster for gold compounds.[9,10] Evidence suggests that cisplatin exerts its antitumor effect through binding to DNA,[11] and studies have shown that gold(III) complexes are also capable of interacting with DNA.[12,13]

The gold(III) complex [$AuCl_2$(damp)] (damp = 2-[(dimethylamino)methyl]phenyl) has been described previously.[14,15] The damp ligand, in this complex, forms part of a five-membered chelate ring in which the nitrogen atom of the amine group and a carbon of the aryl ring bond to the metal (Figure 1). In substitution reactions the cis-oriented chloride ligands resemble those of cisplatin (*cis*-[$PtCl_2(NH_3)_2$]) in that they alone tend to be displaced. The damp moiety remains chelated to the gold. In a previous study [$AuCl_2$(damp)] (compound **1**) was tested for antitumor activity[15] and, against a panel of human tumor cell lines, exhibited a similar cytotoxicity profile to that of cisplatin. The compound was therefore evaluated against one of the sensitive lines (ZR-75-1 breast carcinoma) grown as a tumor xenograft in nude mice. The activity found, although not marked, suggested that analogues of **1** should be studied, particularly those having a higher aqueous solubility. For similar platinum complexes, solubility is very dependent on the nature of the anionic ligand,[16] and we therefore decided to identify more soluble analogues of **1**, in which other anionic moieties are substituted for the chloride groups. One such analogue is the previously reported diacetato complex **3**.[14] This compound readily dissolves in water, although results from a recent study suggest that it hydrolyzes to yield cationic Au(III)-damp species.[17] This study also reported that **3** reacted with biological ligands such as adenine, guanosine, cysteine, and glutathione. These reactions involved displacement of the acetate groups and were not accompanied by reduction, although many other gold(III) complexes are readily reduced in biological media to yield gold(I) species or metallic gold.[3] This paper outlines the further evaluation of **1** and four of its analogues for antitumor properties.

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⊗ Abstract published in *Advance ACS Abstracts,* November 15, 1996.

S0022-2623(96)00156-2 CCC: $12.00

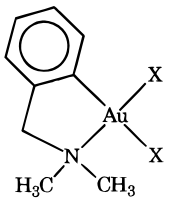

Figure 1. Gold(III) complexes: **1**, X = Cl; **2**, X = SCN; **3**, X = acetato; **4**, X_2 = oxalato; **5**, X_2 = malonato.

Table 1. Solubility of Gold Complexes at 25 °C in Water and 1-Octanol

	solubility (mM)	
compd	water	1-octanol
1	0.25	0.92
2	0.13	0.78
3	>5.23	>5.28
4	0.43	0.17
5	3.58	3.57

Results

Synthesis. Compound **1** was first reported by Vicente[14] and is the precursor to the other compounds discussed in this paper. Two alternative routes to **1** were described. One was the reaction of chloro[2-[(dimethylamino)methyl]phenyl]mercury(II) [HgCl(damp)] with [$AuCl_3$(tht)] (tht = tetrahydrothiophene) and the other used $Me_4N[AuCl_4]$ as the gold precursor. However, experimental details were only given for the reaction with [$AuCl_3$(tht)]. We found that using this method the product was difficult to purify and yields were low. As an alternative method we investigated the reaction of [HgCl(damp)] with $Na[AuCl_4]$ in aqueous acetonitrile. This was found to be a very facile reaction, with the desired product being formed in less than 10 min in greater than 80% yield.

Compound **2** (Figure 1) was formed by direct metathesis of **1** with NaSCN.[15] Compound **3** was prepared by the reaction of **1** with silver acetate as described by Vicente.[14] This method was adapted to prepare the novel compounds **4** and **5**.

Solubilities. The solubilities of compounds **1**−**5** are given in Table 1. In both water and 1-octanol **3** and **5** exhibited the highest solubilties.

Evaluation against Cell Line Panels. The compounds were initially tested against a "primary panel" of cell lines established from a range of human tumors. The results (Table 2) show some similarities between the cytotoxicity profiles of the five gold compounds. Thus whereas the SW620 and SW1116 lines were the least sensitive to the compounds, the HT1376 line was consistently one of the most sensitive. The results for cisplatin were similar. The compounds were also tested in a disease-oriented screen utilizing a panel of human ovarian tumor cell lines including two sublines possessing acquired resistance to cisplatin (CH1-R and A2780-R) (Table 2). The cytotoxicity profile was similar for all of the gold complexes, with IC_{50} values for the most sensitive lines (CH1 and A2780) in the low-micromolar range. Notably, non-cross-resistance was observed in the cisplatin-resistant CH1-R line. Cross-resistance to cisplatin was observed in the A2780-R line.

***In Vivo* Antitumor Activity.** Compound **3** was evaluated against the murine ADJ/PC6 plasmacytoma where the best result achieved was inhibition of the tumor by 24%, obtained at the maximum tolerated dose (25 mg/kg). In this model antitumor activity is usually expressed as a "therapeutic index" (TI) which is given by TI = LD_{50}/ED_{90} (ED_{90} = the dose required to reduce the tumor mass by 90%).[18] However, due to the modest inhibition observed, a TI value could not be calculated.

In the HT1376 bladder tumor xenograft model, **3** and **5** both demonstrated antitumor activity. Animals given the highest dose levels of these compounds showed relative tumor volumes (RTVs) that were lower than those of the nondosed controls at all assessment times (Table 3). For **3** the difference between the RTVs of the dosed and control groups was significant ($p \leq 0.05$) at 29 days, and for **5** the difference was significant at both 14 and 21 days. Cisplatin showed a similar level of activity. Animals dosed with **1** had RTVs that were lower than those of the controls at all times, but at no time did the difference become significant. Compound **4** did not exhibit activity against this tumor. Compound **3** was also active against the PXN/109/TC (CH1) ovarian tumor (Table 3). In this model the RTVs of the highest dosed animals were lower than those of the controls at all times, and at 14 and 21 days the difference was significant.

Mechanistic Studies. a. Plasmid Mobility. The Col E1 plasmid exists in two forms which appear on an electrophoresis gel as two separate bands: the faster-running closed circular superhelical form (RF II) and the slower single-stranded nicked relaxed closed circular form (RF I).[19] Shifts in the mobility of either form indicate interaction of the compounds with the plasmid DNA. Cisplatin produced the expected reduction in mobility of RF II (Figure 2, top). This is associated with the induction of inter- and intrastrand cross-links inducing superwinding, unwinding, and rewinding of superhelical DNA. The mobility of RF I increased due to shortening of the DNA by similar types of binding. For the gold(III) complexes an effect was seen only at the highest concentration used (1500 μM). At this concentration, the mobility of RF II was reduced (Figure 2, bottom).

b. Alkaline Filter Elution. DNA cross-link or single-strand break formation was investigated in SK-OV-3 cells using alkaline elution.[20] The rate of elution of DNA extracted from untreated cells, or cells treated with **3** or cisplatin (both at 100 μM), was measured using a ^{14}C-label. A ^{3}H-label was used for internal standard cells. The fraction of each label retained on a filter was plotted on a log/log graph, and representative plots are given in Figure 3.

Figure 3A shows results from the study to determine interstrand cross-link formation. In this, following treatment, the cells were irradiated (prior to the alkaline elution procedure). DNA from cells exposed to **3** eluted at a similar rate to DNA from untreated, irradiated cells but faster than DNA from cells treated with cisplatin. This indicates that, in contrast to cisplatin, there was no induction of DNA interstrand cross-links following exposure to **3**.

Figure 3B shows results from the study to determine DNA strand breaks. With the exception of one of the untreated controls, the cells were not irradiated. The rate of elution of DNA from cells after treatment with **3** was marginally faster than that from untreated, unir-

Table 2. *In Vitro* Growth Inhibition (IC_{50}, μM) of Tumor Cell Lines

	1[a]	**2**	**3**	**4**	**5**	cisplatin
			Primary Panel[b]			
SW620	124	51	281	205	67	167
SW1116	119	47	238	215	80	163
ZR-75-1	27	34	45	41	36	27
HT29/219	55	25	67	19	36	17
HT1376	30	6.7	13	10	11	23
SK-OV-3	45	20	13	10	11	23
range[c]	4.6	7.6	21.6	21.5	7.3	9.8
			Ovarian Carcinoma Cell Line Panel[d]			
HX62	57	31	34	30	27	18
SK-OV-3	109	39	107	42	30	5.2
CH1	13	10	11	11	2.7	0.12
CH1-R[e]	22 (1.7)	11 (1.1)	12 (1.1)	13 (1.2)	3.3 (1.2)	0.56 (4.7)
A2780	8.2	2.0	3.5	3.7	2.7	1.2
A2780-R[e]	47 (5.7)	26 (13)	35 (10)	35 (9.5)	16 (5.9)	10 (8.3)
range[c]	13.3	19.5	31	11.4	11.1	150

[a] Primary panel values for **1** are from ref 15. [b] 4 h drug exposure. [c] Range = highest IC_{50}/lowest IC_{50} for particular compound. [d] 96 h drug exposure. [e] Values in parentheses are resistance factors (RF = IC_{50}(resistant line)/IC_{50}(parent line)).

Table 3. *In Vivo* Antitumor Activity of the Gold(III) Complexes and Cisplatin

compd	dose (mg/kg)	measurement time (days)	group size	RTV[a] ± SE	T/C[b]
		HT1376 Xenograft			
3	6.25	7	6/6	379 ± 43	1.37
		13	6/6	724 ± 140	1.43
		21	6/6	953 ± 275	1.07
		29	6/6	1671 ± 452	1.16
	12.5	7	6/6	212 ± 33	0.77
		13	6/6	498 ± 156	0.98
		21	6/6	682 ± 131	0.77
		29	6/6	1078 ± 229	0.75
	25	7	6/6	258 ± 43	0.93
		13	6/6	454 ± 114	0.90
		21	6/6	671 ± 194	0.76
		29	6/6	870 ± 151	0.60*
5	8.75	7	6/6	264 ± 44	1.00
		14	6/6	513 ± 81	1.00
		21	6/6	894 ± 138	1.06
		28	6/6	1233 ± 204	1.17
	17.5	7	3/6	266 ± 52	1.01
		14	3/6	451 ± 104	0.88
		21	3/6	699 ± 119	0.83
		28	3/6	802 ± 137	0.76
	35	7	6/6	210 ± 32	0.80
		14	6/6	292 ± 49	0.57*
		21	6/6	488 ± 75	0.58*
		28	6/6	816 ± 108	0.77
cisplatin	4.5	7	6/6	245 ± 20	0.88
		13	6/6	364 ± 36	0.72
		21	6/6	544 ± 115	0.61*
		29	6/6	1051 ± 291	0.73
		PXN/109/TC Xenograft			
3	5	7	6/6	160 ± 18	0.69
		14	6/6	282 ± 43	0.80
		21	6/6	423 ± 54	0.84
		28	6/6	673 ± 126	1.08
	10	7	6/6	173 ± 19	0.76
		14	6/6	316 ± 53	0.90
		21	6/6	515 ± 106	1.03
		28	6/6	833 ± 167	1.34
	20	7	6/6	173 ± 25	0.74
		14	6/6	200 ± 16	0.57*
		21	6/6	258 ± 43	0.51*
		28	6/6	481 ± 75	0.77

[a] RTV = 100(mean volume of tumors at assessment time)/(mean start volume of tumors). [b] T/C = (RTV of treated group)/(RTV of nontreated {control} group). * Denotes that the difference between the test and control RTV values is significant at the 5% level (*t*-test).

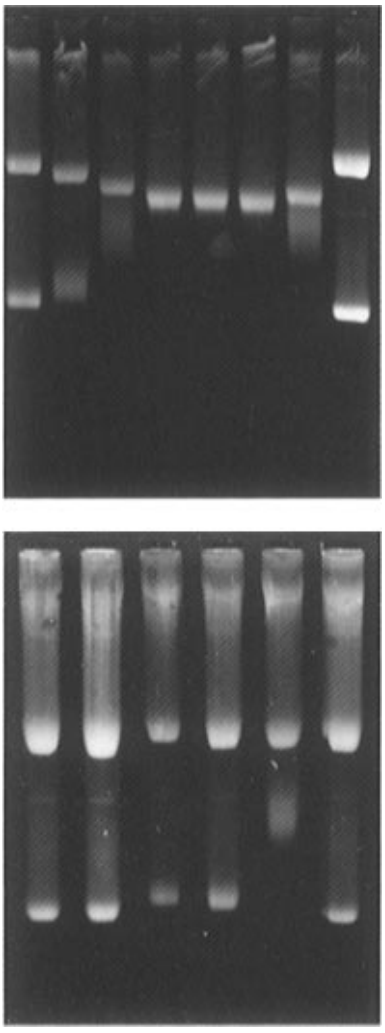

Figure 2. Photographs of electrophoresis gels showing the changes in mobility of the RF I (upper band) and RF II (lower band) forms of the Col E1 plasmid after treatment with cisplatin (top) and **3** (bottom). Top: from left to right; cisplatin at 6.25, 9.4, 18.8, 37.5, 50, 75, and 100 μM and plasmid control. Bottom: from left to right; **3** at 75, 150, 300, 600, and 1500 μM and plasmid control.

radiated cells. A faster elution rate was observed for DNA from untreated, irradiated cells (positive control for strand break formation) and a slower rate for DNA from cisplatin-treated cells. These results may indicate the induction of small numbers of single-strand breaks by **3**.

c. Flow Cytometry. The effects of **3** on the cell cycle, following exposure of SK-OV-3 cells to 30 or 100

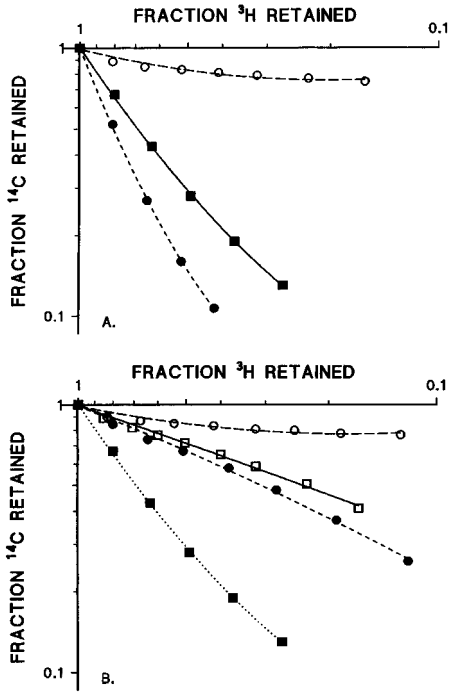

Figure 3. Representative alkaline elution experiments in SK-OV-3 cells showing formation of (A) interstrand cross-links and (B) DNA single-strand breaks after a 6 h exposure to **3** at 100 μM (●) and cisplatin at 100 μM (○). Control (untreated) irradiated cells (■) and control (untreated) unirradiated cells (□) are also included.

μM for 2 h, were relatively minor. For example, at 24 h postexposure the percent of cells in G1, S, and G2 were 43.3%, 41.6%, and 15.1%, respectively, for control cells; 34.4%, 51.8%, and 14.0%, respectively, for cells exposed to 30 μM; and 24.4%, 53.8%, and 21.9%, respectively, for cells exposed to 100 μM. Hence, there was some dose-related decrease in G1 and buildup in S/G2.

Discussion

In previous studies, gold compounds have often been tested in ip/ip murine tumor models.[6,7] These systems bear little relation to the clinical situation where the tumor is both histologically and anatomically different. In these models both tumor cells and the test compound are introduced into the peritoneal cavity of the host animal. In reality, the tumor is frequently distal to the site of drug administration, and factors such as the drug solubility, distribution, and metabolism are crucial. The importance of these differences is reflected in the fact that compounds showing activity against sensitive ascitic tumors, such as the P388 murine leukemia, were often found to be inactive against distal site tumors.[7,21] As predictors of clinical activity, these ip/ip murine models have been of some value in identifying agents effective in the treatment of human leukemias and lymphomas. However they have been much less effective in identifying agents with activity against human solid tumors.[22,23]

In order to overcome the limitations of such *in vivo* murine tumor models, we adopted a different approach. Initially the gold(III)-damp complexes were tested *in vitro* against two panels of human tumor cell lines. The first panel consisted of cells from tumors representative of different tissues types which exhibit a differential response to cisplatin. Differential cytotoxicity was used as an indicator of potential antitumor activity.[24] The gold compounds exhibited a cytotoxicity profile similar to that of cisplatin against these cells, with the HT1376 (bladder) line being consistently one of the most sensitive. The second panel was disease-oriented and consisted of cell lines established from human ovarian tumors. In this panel were two pairs of cisplatin-sensitive and cisplatin-resistant lines, with defined mechanisms of resistance (CH1/CH1-R, A2780/A2780-R), and also two inherently cisplatin-resistant lines (HX62, SK-OV-3).[25] This panel can identify novel agents which circumvent cisplatin resistance. Notably the gold complexes exhibited similar cytotoxicity against both cell lines of the CH1-sensitive/resistant pair where the mechanism of resistance is increased repair of platinum−DNA lesions.[26] Resistance was seen for the A2780-R line however. For this line, studies indicate that the mechanism of resistance to cisplatin is multifactorial involving increased DNA repair, decreased drug uptake, and elevated glutathione levels.[27,28] The fact that **3** has been shown to react with glutathione[17] might thus be relevant.

Most of these human tumor cells can be grown as solid tumor xenografts in nude mice, allowing the *in vivo* evaluation of compounds against tumor types found to be sensitive *in vitro*. All of the gold(III) complexes, with the exception of **2**, were evaluated against the HT1376 xenograft, and **3** was also evaluated against the PXN/109/TC (CH1) xenograft. Compound **2** was found to have a low solubility, and as its *in vitro* cytotoxicity profile indicated no advantage over the other complexes, it was not tested further. Both **3** and **5** were active against the HT1376 tumor *in vivo*. The degree of activity was similar to that of cisplatin. **3** was also active against the cisplatin-sensitive PXN/109/TC tumor, although this was less than that reported for cisplatin.[29] The lower activity of **1** and the inactivity of **4** might, at least in part, be related to the fact that they have much lower solubilities in water and 1-octanol than **3** and **5** (Table 1). This relatively poor solubility could well have a detrimental effect on their absorption at the injection site and their subsequent transport to the tumor.

It is known that **3** is hydrolyzed in water,[17] most likely yielding species such as $[Au(OAc)(damp)(H_2O)]^+$ and $[Au(damp)(H_2O)_2]^{2+}$. Thus, at least for **3**, the species interacting at the biological target to produce the antitumor effect is not likely to be the original complex. It is noteworthy therefore that a similar (although slower) hydrolysis occurs with cisplatin, and it is thought that the interaction of a product such as *cis*-$[PtCl(NH_3)_2(H_2O)]^+$, with DNA, is responsible for the antitumor effect.[11]

A series of mechanistic studies were conducted on **3**. In these, cisplatin was included as a comparison to see whether the mode of action of the gold compound resembled that of the structurally similar platinum drugs. The results of the studies indicated no such resemblance. Cisplatin produces characteristic lesions in cellular DNA (intrastrand and interstrand cross-linking),[30] and following incubation with SK-OV-3 cells, interstrand cross-linking was detected using alkaline elution. A similar effect was not detected with **3**. Also flow cytometry studies demonstrated that, unlike cisplatin, **3** is not cell cycle specific in its mode of action.

Plasmid mobility results indicate that **3** and **5** can interact with DNA. However, the concentrations of the compounds required to elicit an observable effect ($\geq$1500 μM) would not be pharmacologically relevant, being much higher than the range of IC_{50} values determined in the primary cell line panel (Table 2). In contrast, cisplatin altered plasmid mobility at a concentration of $<$10 μM which was much lower than its IC_{50} value range. It is noteworthy that in a previous study involving [$AuCl_3$(py)], much higher concentrations of the gold complex, compared to cisplatin, were required to alter the electrophoretic mobility of DNA.[12] The observation that **3** exhibits only marginal activity against the ADJ/PC6 tumor is further evidence that the compound does not act in the same way as cisplatin. This tumor is highly sensitive to cisplatin and other drugs capable of producing cross-links in DNA.[31]

In conclusion, the square-planar gold(III) complexes **3** and **5** displayed antitumor activity in human tumor xenograft models. However, although these compounds share structural features in common with platinum antitumor drugs, *in vitro* studies and results from the ADJ/PC6 tumor model indicate that the mechanism of action of **3** (and by inference **5**) is different. These complexes might therefore represent a novel class of metal-containing antitumor agent with a spectrum of activity different from that of cisplatin.

Experimental Section

Na[$AuCl_4$] and the silver salts used were obtained from Johnson Matthey, Royston, Herts, U.K. Chloro[2-[(dimethylamino)methyl]phenyl]mercury(II) ([HgCl(damp)])[32] and bis(acetato)[2-[(dimethylamino)methyl]phenyl-*C,N*]gold(III) (**3**)[14] were prepared according to the literature. All other reagents were purchased from Aldrich Chemical Co., Gillingham, Dorset, U.K. NMR measurements were made on a Bruker AC300 spectrometer at 300 MHz (^{1}H) and 75 MHz (^{13}C).

Dichloro[2-[(dimethylamino)methyl]phenyl-*C,N*]gold(III) (1). Sodium tetrachloroaurate(III) dihydrate (11.9 g, 30 mmol) was dissolved in water (200 mL) and [HgCl(damp)] (11.1 g, 30 mmol) in acetonitrile (250 mL) added. The dark solution was stirred for 5 min, treated with charcoal, and filtered. The volume of the yellow filtrate was reduced on a rotary evaporator until crystallization was observed. The mixture was cooled in an ice bath and the yellow product collected by filtration, washed with water, and dried *in vacuo*. The yield was 9.7 g (81%): ^{1}H NMR ($CDCl_3$) δ 7.65−7.00 (m, 4H, C_6H_4), 4.35 (s, 2, CH_2), 3.20 (s, 6, $N(CH_3)_2$); ^{13}C{^{1}H} NMR ($CDCl_3$) 123.2, 128.1, 129.0, 131.2, 143.6, 148.0 (phenyl), 75.8 (CH_2), 53.7 ($N(CH_3)_2$). Anal. ($C_9H_{12}NAuCl_2$) C, H, N; Au: calcd, 49.0; found, 48.5.

Bis(thiocyanato)[2-[(dimethylamino)methyl]phenyl-*C,N*]gold(III) (2). 1 (1.00 g, 2.5 mmol) was dissolved in dichloromethane (100 mL). Sodium thiocyanate (1.00 g, 12 mmol) in water (50 mL) was added to the solution, and the mixture was stirred in the dark for 5 h. After filtering, the layers were separated, and the yellow organic solution was dried over magnesium sulfate. The solution was filtered, reduced to about 10 mL under reduced pressure, and treated with diethyl ether to induce crystallization. Crystallization was completed by cooling in a freezer. The yellow product was collected by filtration, washed with diethyl ether, and dried *in vacuo*. The yield was 0.3 g (27%): ^{1}H NMR (DMSO-d_6) δ 7.60−7.20 (m, 4H, C_6H_4), 4.65 (s, 2, CH_2), 3.25 (s, 6, $N(CH_3)_2$); ^{13}C{^{1}H} NMR (DMSO-d_6) 128.5, 131.7, 132.8, 132.9, 133.0, 133.8 (phenyl), 76.8 (CH_2), 56.4 ($N(CH_3)_2$), 102.1, 115.6 (SCN). Anal. ($C_{11}H_{12}N_3AuCl_2S_2$) C, H, N, Au.

Oxalato[2-[(dimethylamino)methyl]phenyl-*C,N*]gold(III) Hemihydrate (4). 1 (1.00 g, 2.5 mmol) was dissolved in acetone (50 mL) and silver nitrate (0.84 g, 4.9 mmol) added. The reaction mixture was stirred in the dark for 1 h and the silver chloride filtered off. The filtrate was treated with potassium oxalate (1.0 g, 6.0 mmol) in a mixture of water (10 mL) and acetone (5 mL). The initial precipitate was removed, and the reaction mixture was stirred for 15 min. Water (200 mL) was added to precipitate the white product which was collected by filtration, washed with water, and dried *in vacuo*. The yield was 0.56 g (54%): ^{13}C{^{1}H} NMR (acetone-d_6) 123.9, 124.3, 128.0, 128.3, 129.1, 130.7 (phenyl), 98.7 (CH_2), 54.3 ($N(CH_3)_2$), 143.2, 134.5 (oxalato). Anal. ($C_{11}H_{13}NAuO_{4.5}$) C, H, N, Au.

Malonato[2-[(dimethylamino)methyl]phenyl-*C,N*]gold(III) (5). 1 (5.72 g, 14 mmol) was dissolved in acetone (200 mL) and the solution purged with argon. Silver malonate (4.50 g, 14 mmol) was added, and the reaction mixture was stirred in the dark for 48 h. The mixture was filtered, and the colorless filtrate was reduced in volume under reduced pressure until crystallization was just observed. Crystallization of the product was completed by cooling in an ice bath. The white product was collected by filtration and dried *in vacuo*. The yield was was 0.90 g (14%): ^{1}H NMR (DMSO-d_6) δ 7.40−7.10 (m, 4H, C_6H_4), 4.55 (s, 2, CH_2), 3.50 (s, 2, $O_2CCH_2CO_2$), 3.15 (s, 6, $N(CH_3)_2$); ^{13}C{^{1}H} NMR (DMSO-d_6) 123.1, 126.2, 126.6, 128.8, 138.0, 145.8 (phenyl), 73.5 (CH_2), 52.5 ($N(CH_3)_2$), 171.6, 170.4 (malonato CO_2), 49.7 (malonato CH_2). Anal. ($C_{12}H_{14}NAuO_4$) C, H, N, Au.

Aqueous Solubilities. The aqueous solubility of each compound was determined by measuring the concentration of gold in solution. Water (5.0 mL) was added to a known amount of sample (5−10 mg) in a vial and the lid secured using water-proof tape. The suspension was tumbled at 25 °C for 24 h and the solution filtered through Whatman 541 filter paper into a clean vial. Samples were diluted as necessary, and the final solution contained 1 ppm indium as an internal standard. Gold content was determined by ICP-MS on a VG Plasmaquad PQII+ instrument using matrix-matched standards prepared from a 10 000 ppm gold standard in 10% aqua regia. All samples were analyzed in peak-jumping mode using ^{115}In and ^{197}Au isotopes under standard ICP-MS operating conditions.

Solubilities in 1-Octanol. A 1-octanol solution of each compound was prepared as described above for water. After tumbling for 24 h the solution was filtered through Whatman 1PS filter paper. A known quantity of solution was removed and evaporated to near dryness on a hotplate. The residue was dissolved in a known volume of dilute aqua regia and the gold content determined in a similar manner to the aqueous samples.

Primary *in Vitro* Antitumor Screen. Primary screening for antitumor activity was conducted against an *in vitro* panel of seven human tumor cell lines consisting of SW403, SW620, and SW1116 colon carcinoma,[33] HT29/219 colorectal carcinoma,[34] ZR-75-1 breast carcinoma,[35] HT1376 bladder carcinoma,[36] and SK-OV-3 ovarian carcinoma.[37] All lines were obtained from the European Collection of Animal Cell Cultures. Details of the use of this panel for chemosensitivity testing are described elsewhere.[24]

The SW403, SW620, and SW1116 cells were maintained in Leibovitz L15, ZR-75-1 was maintained in RPMI 1640 containing 1 mM sodium pyruvate, and HT29/219, HT1376, and SK-OV-3 were maintained in Eagles minimal essential medium. All media were supplemented with 10% fetal bovine serum, penicillin (100 IU/mL), and streptomycin (100 μg/mL). All media and supplements were obtained from Imperial Laboratories (Andover, Hants, U.K.).

The compounds were dissolved in 10% dimethylacetamide (DMA) in phosphate-buffered saline at a concentration of 2 mg/mL. The cells were seeded onto 96-well microtire plates at a concentration of 5×10^4−10^5 cells/mL. After a 24 h preincubation period, the cells were treated with test compound for 4 h at concentrations of 0−200 μg/mL. The compound-containing medium was then replaced with fresh medium, and the cells were incubated for a further 72 h. Cell growth was assayed using sulforhodamine B (SRB).[38] Cell survival (%) was calculated relative to untreated control cells. Dose/survival curves were constructed from these data, and

the IC_{50} (concentration of compound giving 50% survival) was calculated. Cisplatin was included as a reference in all tests.

Disease-Oriented *in Vitro* Assay of Antitumor Activity. Six human ovarian carcinoma cell lines, HX62, SK-OV-3, CH1, CH1cisR, A2780, and A2780cisR, were used. SK-OV-3 was obtained from the American Type Culture Collection and A2780 and A2780cisR were kindly provided by Dr. T. Hamilton (Fox Chase Cancer Center, Philadelphia, PA). Establishment details and platinum drug chemosensitivity properties of these lines have been described previously.[39] CH1cisR and A2780cisR represent lines derived from their respective parent lines but possessing acquired resistance (approximately 6- and 12-fold, respectively) to the platinum-containing anticancer drug cisplatin.

The cell lines grew as monolayer cultures in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Life Technologies Ltd., Paisley, U.K.) supplemented with gentamicin (50 μg/mL), amphotericin B (2.5 μg/mL), insulin (10 μg/mL), hydrocortisone (0.5 μg/mL), and L-glutamine (2 mM) in a 6% CO_2/air atmosphere. All lines were free of *Mycoplasma* during the course of this study.

The gold complexes were dissolved in either DMA (at 25 mg/mL), for **1**, **2**, and **4**, or water (at 500 μg/mL), for **3** and **5**, immediately before use. Growth inhibition was then assessed using a 96 h drug exposure and SRB assay as described previously.[39] Cells were seeded at $3-5 \times 10^3$/well in 96-well microtiter plates and allowed to attach overnight before drug addition in quadruplicate wells. Following drug exposure, the remaining cells were fixed in 10% ice-cold trichloroacetic acid and quantified using 0.4% SRB dissolved in 1% acetic acid.

Plasmid Mobility. The circular superhelical plasmid DNA Col E1 (Sigma, Poole, U.K.), containing approximately 80% and 20% of forms I and II, respectively, was used to investigate conformational changes in plasmid DNA produced by drug–DNA interactions. The plasmid (1 μg) was incubated for 4 h at 37 °C with increasing concentrations of the gold(III) complexes **3**–**5** (0–1500 μM) and cisplatin (0–100 μM) in TE buffer (10 mM Tris and 1 mM EDTA, pH 7.5). Cisplatin was included as a positive control. To aid solubility **4** and cisplatin were dissolved in DMA and then diluted with TE buffer to a final concentration of 3.75% DMA. Appropriate DMA/plasmid controls were included.

Submarine electrophoresis was performed on a 0.8% agarose gel in TBE buffer (89 mM Tris, 89 mM boric acid, and 2 mM EDTA, pH 8). The unit was setup for anodal migration and run overnight at a potential of 40 V and 20 mA at room temperature. Gels were visualized by staining with ethidium bromide in TBE buffer (1.0 μg mL^{-1}) for 30 min before viewing at 302 nm using an ultraviolet transilluminator (TW40, UVP Inc.). Photographs of the gels were taken using an orange filter and a 5–10 s exposure at *f*5.6.[40]

Alkaline Filter Elution. Approximately 5×10^5 SK-OV-3 cells were seeded into a series of 25 cm^2 tissue culture flasks, allowed to attach overnight, and then grown in the presence of 50 nCi/mL [^{14}C]thymidine (specific activity 53 mCi/mmol; Amersham International) for 24 h to randomly label the DNA. In addition, a parallel flask of cells (to be used as an internal standard) was labeled with 0.17 μCi/mL [*methyl*-^{3}H]thymidine (specific activity 5 Ci/mmol) plus 10^{-5} M unlabeled thymidine. Cells (^{14}C-labeled) were exposed to either **3** or cisplatin at 100 μM for 6 h. Cisplatin, previously shown to induce interstrand cross-links in the SK-OV-3 cell line,[41] was included as a positive control. A control flask of untreated cells (^{14}C-labeled) was also included. Cells were then washed with ice-cold phosphate-buffered saline (PBS) to remove unbound drug, harvested by gentle trypsinization, and resuspended in ice-cold PBS. Cell viability was then determined by staining with lissamine green. From each flask of ^{14}C-labeled cells, 1×10^5 cells were added to two tubes containing 5 mL of ice-cold PBS. One set (for interstrand cross-link determination) was irradiated on ice with 5 Gy of ^{60}Co γ rays from a 2000 Ci source (dose rate of 2 Gy/min). The remaining set (for single-strand break determination) was not irradiated. Irradiated internal standard [^{3}H]thymidine-labeled cells (1×10^5) were then added to all tubes. Test and internal standard cells were then gently added to 2 μm pore size 25 mm polycarbonate filters (Nucleopore Corp., Pleasanton, CA) and lyzed by the addition of 2 × 10 mL of lysis buffer (0.1 M glycine, 0.025 M disodium EDTA, and 2% sodium dodecyl sulfate, pH 10), the first addition containing 0.5 mg/mL proteinase K. DNA was then eluted over 15 h in 10 mL of elution buffer (0.1 M tetrapropylammonium hydroxide, 0.02 M EDTA, and 0.1% sodium dodecyl sulfate, pH 12.2) (elution rate of 0.011 mL/min; fractions collected every 90 min). Radioactivity (^{14}C and ^{3}H) in each fraction was then determined by liquid scintillation counting (Wallac 1410, Pharmacia, Milton Keynes, U.K.).

Flow Cytometry. Approximately 1×10^6 SK-OV-3 cells were exposed to **3** for 2 h in 25 cm^2 flasks at concentrations approximating to IC_{50} (13.7 μg/mL; 30 μM) or $3 \times IC_{50}$ (100 μM). Drug was then washed off, and cells were harvested by trypsinization at 0, 8, 16, 24, 36, and 48 h postexposure and fixed in ice-cold 70% ethanol. After washing, cells were resuspended in 800 μL of PBS, 100 μL of propidium iodide (100 μg/mL), and 100 μL of RNAase solution (1 mg/mL) and incubated for 2 h at 37 °C. Cell cycle analysis was then determined from DNA histograms as described previously[42] using an Ortho Cytofluorograf 50H instrument using a Spectra-Physics argon ion laser tuned to produce 200 mW at 488 nm.

***In Vivo* Antitumor Activity. a. ADJ/PC6.** This was performed as described previously.[31,39] Briefly, at 20 days postsubcutaneous implantation of 1 mm^3 tumor fragments, drugs were administered (at halving doses) by a single intraperitoneal injection as sonicated suspensions in arachis oil. Ten days later, tumors were dissected out and the weights of control and treated groups compared.

b. Human Tumor Xenografts. Tumor fragments were implanted subcutaneously into the flanks of nude mice. Animals bearing established, palpable tumors were randomized into treatment groups of 6 mice and a control group of 10 mice. For the evaluations utilizing the HT1376 tumor, the test compounds were injected as suspensions in 0.5% carboxymethylcellulose in 0.9% saline. For the evaluation utilizing the PXN/109/TC tumor, **3** was suspended in arachis oil. All of the test compounds were administered intraperitoneally. Cisplatin and **1** were administered at their previously determined maximum tolerated doses, while the other compounds were given at their maximum tolerated doses and at two 2-fold dilutions. Unless specified otherwise, doses were given on days 0, 7, 14, and 21, and tumor dimensions were taken on days 0, 7, 14, 21, and 28. Relative tumor volumes were calculated from RTV = 100(mean volume of tumors at assessment time)/(mean start volume of tumors).

Acknowledgment. We thank Mr. B. C. Moore and Mr. I. Briggs at Johnson Matthey for their NMR and ICP-MS work, respectively, Mr. P. Scobie-Trumper at the Experimental Biology Unit (University of Surrey) for his collaboration in the xenograft studies, and the SERC for a CASE Award (to B.P.H.). We also acknowledge the contributions made by Mr. M. Jones, Mrs. P. Goddard, and the late Mr. G. Abel at the Institute of Cancer Research for *in vitro* and *in vivo* technical assistance. Studies at the Institute of Cancer Research were supported by the Cancer Research Campaign (U.K.).

References

(1) Parish, R. V. Gold in medicine - chrysotherapy. *Interdisc. Sci. Rev.* **1992**, *17*, 221–228.
(2) Sadler, P. J. Inorganic chemistry and drug design. In *Advances in Inorganic Chemistry*; Sykes, A. G., Ed.; Academic Press: San Diego, 1991; Vol. 36, pp 1–48.
(3) Shaw, C. F. Gold. In *Metal Compounds in Cancer Therapy*; Fricker, S. P., Ed.; Chapman and Hall: London, 1994; pp 46–64.
(4) Ni Dhubhghaill, O. M.; Sadler, P. J. Gold complexes in cancer chemotherapy. In *Metal Complexes in Cancer Chemotherapy*; Keppler, B. K., Ed.; VCH: Weinheim, 1993; pp 221–248.
(5) Mirabelli, C. K.; Hill, D. T.; Faucette, L. F.; McCabe, F. L.; Girade, G. R.; Bryan, D. B.; Sutton, B. M.; O'Leary Bartus, J.; Crooke, S. T.; Johnson, R. K. Antitumor activity of bis(diphenylphosphino)alkanes, their gold(I) coordination complexes, and related compounds. *J. Med. Chem.* **1987**, *30*, 2181–2190.

(6) Berners-Price, S. J.; Mirabelli, C. K.; Johnson, R. K.; Mattern, M. R.; McCabe, F. L.; Faucette, L. F.; Sung, C.-M.; Mong, S.-M.; Sadler, P. J.; Crooke, S. T. *In vivo* antitumor activity and *in vitro* cytotoxic properties of bis[1,2-bis(diphenylphosphino)ethane]-gold(I) chloride. *Cancer Res.* **1986**, *46*, 5486−5493.
(7) Mirabelli, C. K.; Johnson, R. K.; Sung, C.-M.; Faucette, L. F.; Muirhead, K.; Crooke, S. T. Evaluation of the *in vivo* antitumor activity and *in vitro* cytotoxic properties of auranofin, a coordinated gold compound, in murine tumor models. *Cancer Res.* **1985**, *45*, 32−39.
(8) Sadler, P. J.; Nasr, M.; Narayanan, V. L. The design of metal complexes as anticancer drugs. In *Platinum Coordination Complexes in Cancer Chemotherapy*; Hacker, M. P., Douple, E. B., Krakoff, I. H., Eds.; Martinus Nijhoff Publishing: Boston, 1984; pp 290−304.
(9) Cattalini, L.; Orio, A.; Tobe, M. L. Nucleophilic reactivity in substitution reactions of square-planar metal complexes. II. A comparison of the kinetic behaviour of platinum(II) and gold-(III) complexes. *J. Am. Chem. Soc.* **1967**, *89*, 3130−3134.
(10) Pesek, J. J.; Mason, W. R. Cyanide exchange kinetics for planar tetracyanometalate complexes by carbon-13 NMR. *Inorg. Chem.* **1983**, *22*, 2958−2959.
(11) Lempers, E. L. M.; Reedijk, J. Interactions of platinum amine compounds with sulfur-containing biomolecules and DNA fragments. *Adv. Inorg. Chem.* **1991**, *37*, 175−217.
(12) Mirabelli, C. K.; Sung, C.-M.; Zimmerman, J. P.; Hill, D. T.; Mong, S.; Crooke, S. T. Interactions of gold coordination complexes with DNA. *Biochem. Pharmacol.* **1986**, *35*, 1427−1433.
(13) Mirabelli, C. K.; Zimmerman, J. P.; Bartus, H. R.; Sung, C.-M.; Crooke, S. T. Inter-strand cross-links and single strand breaks produced by gold(I) and gold(III) coordination complexes. *Biochem. Pharmacol.* **1986**, *35*, 1435−1443.
(14) Vicente, J.; Chicote, M. T.; Bermudez, M. D. 2-[(Dimethylamino)-methyl]phenylgold(III) complexes. *J. Organomet. Chem.* **1984**, *268*, 191−195.
(15) Parish, R. V.; Howe, B. P.; Wright, J. P.; Mack, J.; Pritchard, R. G.; Buckley, R. G.; Elsome, A. M.; Fricker, S. P. Chemical and biological studies of dichloro(2-((dimethylamino)methyl)phenyl)-gold(III). *Inorg. Chem.* **1996**, *35*, 1659−1666.
(16) Cleare, M. J.; Hydes, P. C.; Malerbi, B. W.; Watkins, D. M. Antitumour platinum complexes: relationships between chemical properties and activity. *Biochimie* **1978**, *60*, 835−850.
(17) Parish, R. V.; Mack, J.; Hargreaves, L.; Wright, J. P.; Buckley, R. G.; Elsome, A. M.; Fricker, S. P.; Theobald, B. R. C. Chemical and biological reactions of diacetato[2-(dimethylaminomethyl)-phenyl]gold(III), [Au(O_2CMe)$_2$(dmamp)]. *J. Chem. Soc., Dalton Trans.* **1996**, 69−74.
(18) Kelland, L. R.; Barnard, C. F. J.; Evans, I. G.; Murrer, B. A.; Theobald, B. R. C.; Wyer, S. B.; Goddard, P. M.; Jones, M.; Valenti, M.; Bryant, A.; Rogers, P. M.; Harrap, K. R. Synthesis and *in vitro* and *in vivo* antitumor activity of a series of *trans* platinum antitumor complexes. *J. Med. Chem.* **1995**, *38*, 3016−3024.
(19) Mong, S.; Strong, J. E.; Bush, J. A.; Crooke, S. T. Use of covalently closed circular deoxyribonucleic acid for prescreening for antitumour compounds. *Antimicrob. Agents Chemother.* **1979**, *16*, 398−405.
(20) Kohn, K. W.; Ewig, R. A. G.; Erickson, L. C.; Zwelling, L. A. Measurement of strand breaks and cross-links by alkaline elution. In *DNA Repair - A Laboratory Manual of Research Procedures*; Friedberg, E. C., Hanawalt, P. C., Eds.; Marcel Dekker: New York, 1981; Vol. 1, Part B, pp 379−401.
(21) Schurig, J. E.; Meinema, H. A.; Timmer, K.; Long, B. H.; Casazza, A. M. Antitumor activity of bis[bis(diphenylphosphino)-alkane and alkene] group VIII metal complexes. *Prog. Clin. Biochem.* **1989**, *10*, 205−216.
(22) Corbett, T. H.; Valeriote, F. A.; Baker, L. H. Is the P388 murine tumor no longer adequate as a drug discovery model? *Invest. New Drugs* **1987**, *5*, 3−20.
(23) Schwartsmann, G.; Workman, P. Anticancer drug screening and discovery in the 1990s: a European perspective. *Eur. J. Cancer* **1993**, *29A*, 3−14.
(24) Fricker, S. P. Screening for new metal anti-tumour agents. In *Metal Ions in Biology and Medicine*; Collery, Ph., Poirier, L. A., Manfait, M., Etienne, J.-C., Eds.; John Libbey Eurotext: Paris, 1990; pp 452−456.
(25) Hills, C. A.; Kelland, L. R.; Abel, G.; Siracky, J.; Wilson, A. P.; Harrap, K. R. Biological properties of a panel of ten human ovarian carcinoma cell lines: calibration against 4 platinum complexes. *Br. J. Cancer* **1989**, *59*, 527−534.
(26) O'Neill, C. F.; Orr, R. M.; Kelland, L. R.; Harrap, K. R. Comparison of platinum (Pt) binding to DNA, and removal of total Pt adducts and interstrand crosslinks in three human ovarian carcinoma cell lines sensitive and resistant to cisplatin. *Cell. Pharmacol.* **1995**, *2*, 1−7.
(27) Johnson, S. W.; Perez, R. P.; Godwin, A. K.; Yeung, A. T.; Handel, L. M.; Ozols, R. F.; Hamilton, T. C. Role of platinum-DNA adduct formation and removal in cisplatin resistance in human ovarian cancer cell lines. *Biochem. Pharmacol.* **1994**, *47*, 689−697.
(28) Kelland, L. R.; Barnard, C. F. J.; Mellish, K. J.; Jones, M.; Goddard, P. M.; Valenti, M.; Bryant, A.; Murrer, B. A.; Harrap, K. R. A novel *trans*-platinum coordination complex possessing *in vitro* and *in vivo* antitumor activity. *Cancer Res.* **1994**, *54*, 5618−5622.
(29) Kelland, L. R.; Jones, M.; Abel, G.; Valenti, M.; Gwynne, J.; Harrap, K. R. Human ovarian carcinoma cell lines and companion xenografts: a disease-oriented approach to new platinum anticancer drug discovery. *Cancer Chemother. Pharmacol.* **1992**, *30*, 43−50.
(30) Comess, K. M.; Lippard, S. J. Molecular aspects of platinum-DNA interactions. In *Molecular aspects of anticancer drug-DNA Interactions*; Neidle, S., Waring, M., Eds.; Macmillan Press: London, 1993; Vol. 1, pp 134−168.
(31) Goddard, P. M.; Valenti, M. R.; Harrap, K. R. The role of murine tumour models and their acquired platinum-resistant counterparts in the evaluation of novel platinum antitumour agents: a cautionary note. *Ann. Oncol.* **1991**, *2*, 535−540.
(32) van der Ploeg, A. F. M. J.; van der Kolk, C. E. M.; van Koten, G. Arylmercury(II) compounds involving intramolecular coordination via 2-Me_2NCH_2- and chiral (S)-2-Me_2NCHMe-ring substituents. *J. Organomet. Chem.* **1981**, *212*, 283−290.
(33) Leibovitz, A.; Stinson, J. C.; McCombs, W. B., III; McCoy, C. E.; Mazur, K. C.; Mabry, N. D. Classification of human colorectal adenocarcinoma cell lines. *Cancer Res.* **1976**, *36*, 4562−4569.
(34) Fogh, J.; Trempe, G. New human cell lines. In *Human Tumour Cells in Vitro*; Fogh, J., Ed.; Plenum Press: New York, 1975; pp 115−154.
(35) Engel, L. W.; Young, N. A.; Tralka, T. S.; Lippman, M. E.; O'Brien, S. J.; Joyce, M. J. Establishment and characterization of three new continuous cell lines derived from human breast carcinomas. *Cancer Res.* **1978**, *38*, 3352−3364.
(36) Rasheed, S.; Gardner, M. B.; Rongey, R. W.; Nelson-Rees, W. A.; Arnstein, P. Human bladder carcinoma: characterization of two new tumour cell lines and search for tumour viruses. *J. Natl. Cancer Inst.* **1977**, *58*, 881−890.
(37) Fogh, J.; Wright, W. C.; Loveless, J. D. Absence of HeLa cell contamination in 169 cell lines derived from human tumours. *J. Natl. Cancer Inst.* **1977**, *58*, 209−214.
(38) Higgins, J. D., III; Neely, L.; Fricker, S. P. Synthesis and cytotoxicity of some cyclometallated palladium complexes. *J. Inorg. Biochem.* **1993**, *49*, 149−156.
(39) Kelland, L. R.; Abel, G.; McKeage, M. J.; Jones, M.; Goddard, P. M.; Valenti, M.; Murrer, B. A.; Harrap, K. R. Preclinical antitumour evaluation of bis-acetato-ammine-dichloro-cyclohexylamine platinum(IV): an orally active platinum drug. *Cancer Res.* **1993**, *53*, 2581−2586.
(40) Sealey, P. G.; Southern, E. M. Gel Electrophoresis of DNA. In *Gel Electrophoresis of Nucleic Acids. A Practical Approach*, 2nd ed.; Rickwood, D., Hames, B. D., Eds.; IRL Press: Oxford, 1990; pp 51−100.
(41) Kelland, L. R.; Barnard, C. F. J.; Mellish, K. J.; Jones, M.; Goddard, P. M.; Valenti, M.; Bryant, A.; Murrer, B. A.; Harrap, K. R. A novel *trans*-platinum coordination complex possessing *in vitro* and *in vivo* antitumor activity. *Cancer Res.* **1994**, *54*, 5618−5622.
(42) Ormerod, M. G.; Orr, R. M.; Peacock, J. H. The role of apoptosis in cell killing by cisplatin: a flow cytometric study. *Br. J. Cancer* **1994**, *69*, 93−100.

JM9601563