Chlorogold(I) and Gold(III) Complexes of 6-Amino-1,3-dimethyl-5-arylazouracil Derivatives: IR Spectroscopy, Growth Inhibition of HeLa Cells and X-ray Crystal Structure of 6-Amino-1,3-dimethyl-5-phenylazoniumuracil Dichloroaurate(I) Sesquihydrate

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Abstract

The preparation and X-ray crystal structure of the complex formed between 6-amino-1,3-dimethyl-5-phenylazouracil (DZH) and gold(I) chloride, $[DZH_2][AuCl_2]\cdot 1.5H_2O$, are described. The complex crystallizes in the monoclinic system: space group $C2/c$, $a = 17.865(4)$, $b = 16.333(3)$, $c = 14.014(2)$ Å, $\beta = 118.59(1)^\circ$, $Z = 8$, $R = 0.049$. The structure is ionic and consists of $AuCl_2^-$ anions, DZH^+ cations and crystal water molecules bonded together by hydrogen bonds. The anion $AuCl_2^-$ is almost linear [Cl–Au–Cl = 179.1(1)°], with Au–Cl distances of 2.245(3) and 2.255(4) Å. The cation is essentially co-planar and the protonation takes place at the nitrogen atom N(10) of the azo group bonded to the phenyl ring.

Likewise, three gold(III) complexes of type $AuLCl_2$ were prepared from $NaAuCl_4\cdot 2H_2O$ and 6-amino-1,3-dimethyl-5-arylazouracil derivatives (LH). A *cis*-square-planar structure is proposed for these complexes on the basis of IR data. Tests of the isolated gold(I) and gold(III) complexes for their growth inhibition of HeLa cells showed considerable inhibition for the compounds $[DZH_2][AuCl_2]\cdot 1.5H_2O$, $Au(DZ)Cl_2$ and $Au(DZC)Cl_2$.

Introduction

Interest in gold coordination chemistry has been growing in recent years with the successful use of gold(I) phosphine complexes such as 'Auranofin' [(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyrosinato-*S*)(triethylphosphine) gold(I)] and triethyl phosphinechlorogold(I), in oral administration, for the treatment of rheumatoid arthritis [1, 2]. After extensive clinical trials [3] Auranofin was approved for clinical use in 1985 under the trade name 'Ridaura'.

Auranofin has also shown evidence of anticancer activity in *in vivo* studies of P388 leukaemia in mice [4, 5]. These results then prompted further efforts, directed toward the preparation of gold(I) complexes analogous to Auranofin [6–12] which might exhibit a broader spectrum of anticancer activity. Likewise, the interaction of gold(I) complexes with DNA has been investigated in an attempt to explain their mechanism of action. Absorption and circular dichroism spectroscopy and gel electrophoresis studies have revealed that gold complexes can bind to DNA *in vivo* apparently via the guanine and cytosine bases [13, 14].

The anticancer activity of Pt(II) complexes containing nitrogen ligands is well documented, but relatively little attention has been paid to the isoelectronic and isostructural gold(III) complexes, even though some gold(III) complexes exhibit anticancer activity [15], and evidence obtained by viscosimetry, microdensitometry and UV spectroscopy suggests that gold(III) complexes bind to DNA *in vitro* [16, 17]. Au(III) is a strong oxidant, however, and generally is easily reduced, especially *in vivo* (probably by free thiol groups) [18].

In the current work we report on the isolation and characterization of stable gold(I) and gold(III) complexes of 6-amino-1,3-dimethyl-5-arylazouracil

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derivatives. Since both some 5-arylazopyrimidines [19] and some gold complexes have been shown to have potential antitumour activity, the gold complexes reported below were tested for growth inhibition of HeLa cells.

Experimental

6-Amino-1,3-dimethyl-5-arylazouracil derivatives, LH (Fig. 1), were prepared as described earlier [20]. $NaAuCl_4 \cdot 2H_2O$ was purchased from Aldrich Chemicals.

Microanalyses of C, H and N were performed with a Perkin-Elmer 240C analyzer. Gold was determined thermogravimetrically with a Mettler TG-50 thermobalance. Infrared spectra were recorded in the 4000–180 cm^{-1} range on a Perkin-Elmer 983G spectrophotometer, using KBr and polyethylene pellets. The equivalent conductance of the compounds was recorded at 20 °C in methanol solution $\approx 3.9\ 10^{-3}$ M. Measurements were taken within 2 min

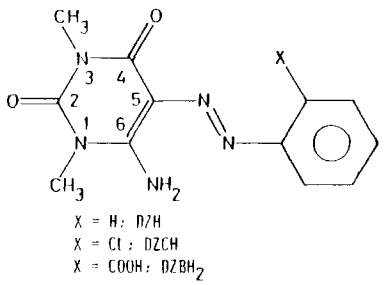

Fig. 1. 6-Amino-1,3-dimethyl-5-arylazouracil derivatives (LH).

of the preparation of the solution using a Radiometer CDM2f conductimeter. Analyses and conductivity data are presented in Table 1.

Preparation of $[DZH_2][AuCl_2] \cdot 1.5H_2O$

To a stirred solution of $NaAuCl_4 \cdot 2H_2O$ (0.4 g, 1 mmol) in 100 ml of water containing NaCl (0.6 g, 10 mmol) was added over 15 min an equivalent amount of Na_2SO_3 (0.13 g, 1 mmol). After 30 min the reduction was complete. Addition of DZH (0.26 g, 1 mmol) immediately gave a yellow precipitate, which was filtered off and dried under vacuum. The product was recrystallized from ethanol/water (20:1). Crystals suitable for X-ray analysis were obtained by slow evaporation of a solution of the compound in ethanol/water.

Preparation of $AuLCl_2$ (LH = DZH, DZCH and $DZBH_2$)

The general procedure for the synthesis of $AuLCl_2$ was as follows. To a heated, stirred solution of $NaAuCl_2 \cdot 2H_2O$ (0.4 g, 1 mmol) in ethanol/water (20:1, 100 ml) was added over 15 min the 6-amino-1,3-dimethyl-5-arylazouracil derivative (1 mmol), and the mixture was refluxed for 1 h. The resulting red solution was allowed to stand at room temperature for three days, whereupon the complex precipitated. The complex was filtered off, washed with ethanol and dried with diethyl ether.

X-ray Structure Determination of $[DZH_2][AuCl_2] \cdot 1.5H_2O$

Single-crystal data collection was performed at ambient temperature with a Nicolet P3F diffractometer using graphite monochromatized Mo Kα

TABLE 1. Analytical Data and Physical Properties

	$[DZH_2][AuCl_2] \cdot 1.5H_2O$	$Au(DZ)Cl_2$	$Au(DZC)Cl_2$	$Au(DZBH)Cl_2$
C(%)				
calculated	25.96	27.40	25.71	27.39
found	26.07	27.57	25.97	27.69
H(%)				
calculated	3.09	2.30	1.98	2.12
found	2.98	2.49	2.07	2.34
N(%)				
calculated	12.62	13.31	12.49	12.28
found	12.70	13.16	12.47	12.65
Au(%)				
calculated	35.47	37.44	35.14	34.55
found	34.79	36.67	34.94	35.08
Yield (%)	58	74	61	67
Λ ($cm^2\ \Omega^{-1}\ mol^{-1}$)	113	5	6	4
Concentration (10^{-3} M)	3.95	3.92	3.88	3.90
Description	orange crystals	yellow powder	red crystals	red powder

radiation (λ = 0.71069 Å). The unit cell parameters for the orange crystal selected were obtained from least-squares refinement of 25 well-centred reflections ($18° < 2\theta < 27°$). The crystal data are: specimen 0.25 × 0.25 × 0.40 mm, monoclinic, space group $C2/c$, a = 17.865(4), b = 16.333(3), c = 14.014(2) Å, β = 118.59(1)°, V = 3591(1) Å^3, Z = 8, D_c = 2.05 g/cm^3, formula weight = 555.2, $F(000)$ = 2120. A total of 4410 reflections were collected by ω-scan technique ($3° < 2\theta < 55°$) at variable scan speed of 2.0–20.0° min^{-1}. Of these, 2442 were considered as observed ($|F_o| > 6\sigma|F_o|$). Intensities of three check reflections measured after every 57 reflections showed only statistical variation. The data were corrected for Lorentz and polarization effects and for dispersion. Φ-scan of six reflections showed an empirical absorption corrrection (μ = 87.5 cm^{-1}) to be unnecessary.

The structure was solved by Patterson methods using the SHELXS program system and subsequent ΔF synthesis [21]. All non-hydrogen atoms were refined anisotropically. The hydrogen atom maxima of the amino group and the hydrogen atom maximum near N(10) were found from an ΔF map, but only some of the phenyl and methyl hydrogen atom positions were found, and none of the hydrogen atom poistions of the crystal water molecules. The phenyl hydrogen atoms were therefore placed at calculated positions (C–H = 1.0 Å), while the four remaining methyl hydrogen atoms were placed according to the strongest hydrogen atom maximum of either group. The hydrogen atoms were not refined. The function minimized was $\Sigma w(\Delta F)^2$, where $w = 1/\sigma(F)^2$, resulting in the final R value 0.049 (R_w = 0.050). The final difference Fourier map showed large maxima in the vicinity of the $AuCl_2^-$ ion. The greatest maximum (2.1 e Å^{-3}) was situated in the vicinity of Au and the maxima of 1.5 e Å^{-3} in the vicinity of Cl atoms. Scattering factors were those included in the program and anomalous dispersion corrections were applied [22]. All calculations were done on a VAX 8650 computer and the refinements and all subsequent calculations with XTAL system programs [23].

Viability Assay

HeLa cells were seeded into Petri dishes containing DMEM supplemented with 10% calf serum, at a density of 1 × 10^5 cells per dish. The gold(I) compound was added to the culture medium 24 h later and the cells were cultured for a further 24 h. Dead cells were washed out and viable one were trypsinized and counted to determine if the gold(I) compound was toxic. No toxic concentrations of drug were used in the subsequent growth inhibition assay. Each compound was tested at least three times at each concentration. Results were expressed as the total number of cells recovered from the drug treatment. Since gold(I) compounds were dissolved in DMSO, control experiments with DMSO at the same solution as the gold(I) compounds (2 and 10 μl per 2 ml of medium), and without DMSO, were carried out in parallel.

Growth Inhibition Assay

Fifty drug-treated cells were seeded into 30 mm Petri dishes containing DMEM plus 10% calf serum and allowed to form isolated clones during a period of 12 days. At the end of this time, cells were fixed and stained with crystal violet. Clones obtained were classified into three types, according to size: >1 mm (big), <1 mm (small) and isolated cells that were not able to proliferate to form clones. The amounts of size-classed clones were expressed as a percentage of the total amount of clones per dish.

Results and Discussion

$[DZH_2][AuCl_2]\cdot 1.5H_2O$

This complex was obtained in good yield by use of Na_2SO_3 as reducing agent:

$$NaAuCl_4 + Na_2SO_3 \rightleftharpoons Na_2SO_4 + NaAuCl_2$$

$$+ 2HCl \overset{DZH}{\rightleftharpoons} [DZH_2][AuCl_2]$$

The conductivity measurements indicate that the compound is a 1:1-type electrolyte in methanolic solution. In the IR spectrum, the N=N stretching band of DZH occurring at 1522 cm^{-1} is shifted to 1450 cm^{-1}, indicating protonation of DZH at a nitrogen atom of the azo group [24]. Further supporting this position is the broad band due to N(azo)–H stretching in the region 3100–2800 cm^{-1}. The Au–Cl stretching vibration appears at 350 cm^{-1}, which is similar to the frequencies reported for related compounds containing the linear $AuCl_2^-$ anion [25].

The results of the crystal structure determination confirm the ionic structure, which consists of $AuCl_2^-$ anions, DZH_2 cations and crystal water molecules bonded together by hydrogen bonds (Figs. 2 and 3).

Figure 2 shows the asymmetric unit of the structure with the numbering system and Fig. 3 illustrates the packing of the molecules. Atomic coordinates are listed in Table 2 and selected bond lengths and angles are given in Table 3.

The dichlorogold(I) anion is almost linear with a Cl–Au–Cl angle of 179.1(1)°, and the Au–Cl distances of 2.245(4) and 2.255(4) Å are practically equal. Besides the two Au–Cl bonds the central atom does not participate in other short interatomic contacts; there are no contacts shorter than 3.40 Å between gold and other non-hydrogen atoms. The Au–Cl distances are very similar to those found for the $AuCl_2^-$ anion in the simple salt [n-Bu_4N][$AuCl_2$] (2.257(4) Å [26]) and shorter than that in the

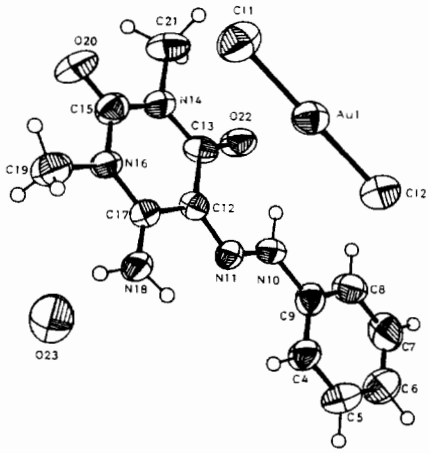

Fig. 2. Asymmetric unit of the structure with labelling.

mixed valence complexes $Cs_2[AuCl_2][AuCl_4]$ (2.281(2) Å [27]) and $Rb_3[AuCl_2][AuCl_4]$ (2.26(4) and 2.27(4) Å [28]). As has been pointed-out [26], counterion effects and packing forces must be the most important factors affecting Au–Cl distances and the Cl–Au–Cl angle.

The protonation of DZH seems to take place at the nitrogen atom N(10) of the azo group, in agreement with the IR data. This same nitrogen atom has been shown to be the protonation site of DZH in the complex $[DZH_2]_2[AuBr_2]Br\cdot H_2O$ [24]. The

TABLE 2. Fractional Atomic Coordinates and Temperature Factors[a] for $[DZH_2][AuCl_2]\cdot 1.5H_2O$[b]

Atom	x	y	z	U_{eq} (Å^2)
Au	0.74608(3)	0.52428(4)	0.08923(4)	0.0584(3)
Cl(1)	0.8505(2)	0.4332(3)	0.1840(3)	0.084(3)
Cl(2)	0.6411(2)	0.6135(2)	−0.0071(3)	0.066(2)
C(4)	0.4299(7)	0.6238(8)	0.0689(10)	0.054(8)
C(5)	0.3639(8)	0.6787(9)	0.0178(11)	0.070(10)
C(6)	0.3779(9)	0.7608(9)	0.0291(12)	0.075(10)
C(7)	0.4579(9)	0.7920(8)	0.0923(12)	0.081(11)
C(8)	0.5241(8)	0.7393(8)	0.1447(10)	0.061(9)
C(9)	0.5096(7)	0.6556(7)	0.1313(9)	0.052(7)
N(10)	0.5821(6)	0.6038(6)	0.1935(7)	0.049(6)
N(11)	0.5768(5)	0.5257(6)	0.1905(7)	0.044(5)
C(12)	0.6470(6)	0.4833(7)	0.2519(8)	0.042(6)
C(13)	0.7302(6)	0.5172(8)	0.3218(9)	0.047(7)
N(14)	0.7955(5)	0.4625(6)	0.3376(7)	0.047(6)
C(15)	0.7847(7)	0.3801(8)	0.3719(9)	0.053(8)
N(16)	0.7043(5)	0.3479(6)	0.3030(7)	0.044(6)
C(17)	0.6362(6)	0.3967(7)	0.2459(8)	0.040(7)
N(18)	0.5609(6)	0.3625(6)	0.1834(8)	0.052(8)
C(19)	0.6953(8)	0.2579(8)	0.3006(11)	0.067(9)
O(20)	0.8443(5)	0.3343(6)	0.4229(7)	0.068(6)
C(21)	0.8822(7)	0.4964(8)	0.4449(11)	0.061(9)
O(22)	0.7428(4)	0.5912(5)	0.3314(6)	0.054(5)
O(23)	0.4939(6)	0.2054(6)	0.1261(7)	0.079(7)
O(24)	0.0000	0.5663(9)	0.2500	0.089(11)

[a]Temperature factors calculated by $U_{eq} = 1/3(U_{11} + U_{22} + U_{33})$. [b]e.s.d.s. given in parentheses.

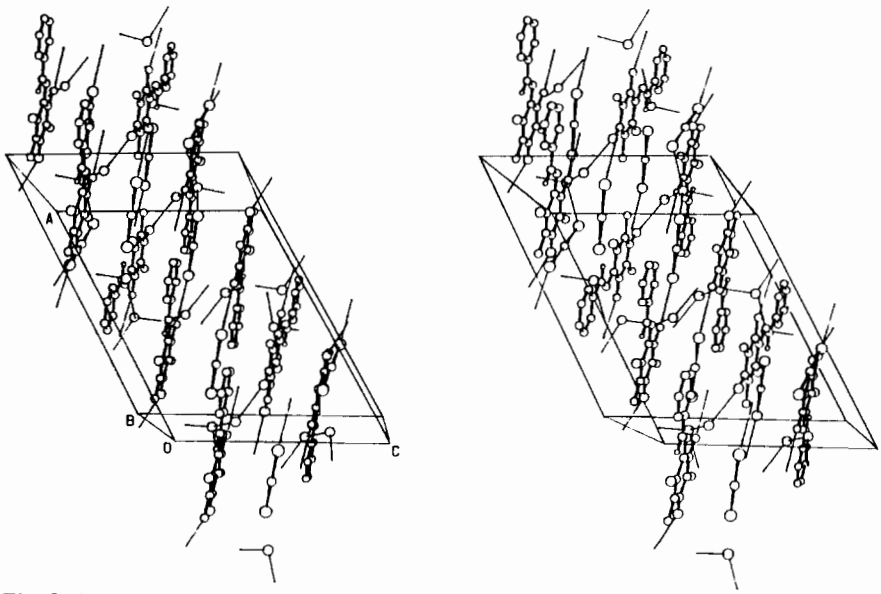

Fig. 3. Packing of the molecules within the unit cell. The possible hydrogen bonds are indicated by thin lines. The hydrogen atoms, except the hydrogen bonded to N(10), have been omitted for clarity.

TABLE 3. Interatomic Distances (Å) and Bond Angles (°)[a]

Au–Cl(1)	2.255(4)	C(12)–C(13)	1.44(1)
Au–Cl(2)	2.245(3)	C(12)–C(17)	1.42(2)
C(4)–C(5)	1.38(2)	C(13)–N(14)	1.38(1)
C(4)–C(9)	1.37(2)	C(13)–O(22)	1.22(1)
C(5)–C(6)	1.36(2)	N(14)–C(15)	1.36(2)
C(6)–C(7)	1.37(2)	N(14)–C(21)	1.48(1)
C(7)–C(8)	1.36(2)	C(15)–N(16)	1.39(1)
C(8)–C(9)	1.39(2)	C(15)–O(20)	1.21(1)
C(9)–N(10)	1.44(1)	N(16)–C(17)	1.35(1)
N(10)–N(11)	1.28(1)	N(16)–C(19)	1.48(2)
N(11)–C(12)	1.33(1)	C(17)–N(18)	1.33(1)
Cl(1)–Au–Cl(2)	179.1(1)	C(12)–C(13)–O(22)	122.0(9)
C(4)–C(5)–C(6)	121.1(11)	N(14)–C(13)–O(22)	121.0(9)
C(5)–C(6)–C(7)	121.3(12)	C(13)–N(14)–C(15)	123.5(9)
C(6)–C(7)–C(8)	118.8(12)	C(13)–N(14)–C(21)	117.6(9)
C(7)–C(8)–C(9)	119.6(11)	C(15)–N(14)–C(21)	118.9(9)
C(4)–C(9)–C(8)	122.1(11)	N(14)–C(15)–N(16)	119.1(9)
C(5)–C(4)–C(9)	117.1(11)	N(14)–C(15)–O(20)	121.1(10)
C(4)–C(9)–N(10)	121.6(10)	N(16)–C(15)–O(20)	119.8(11)
C(8)–C(9)–N(10)	116.1(9)	C(15)–N(16)–C(17)	121.6(10)
C(9)–N(10)–N(11)	122.3(8)	C(15)–N(16)–C(19)	117.2(9)
N(10)–N(11)–C(12)	117.8(8)	C(17)–N(16)–C(19)	121.1(8)
N(11)–C(12)–C(13)	126.0(11)	C(12)–C(17)–N(16)	119.5(8)
N(11)–C(12)–O(17)	114.8(9)	N(16)–C(17)–N(18)	118.9(10)
C(13)–C(12)–C(17)	119.3(9)	C(12)–C(17)–N(18)	118.9(10)
C(12)–C(13)–N(14)	117.0(10)		

[a]e.s.d.s given in parentheses.

TABLE 4. Deviations (Å) of Atoms from the Least-squares Planes. The Equations of the Planes are Expressed in Direct Space

I. Plane through the phenyl ring; $0.155x + 0.009y - 0.988z = 3.82$ Å					
C(4)[a]	C(5)[a]	C(6)[a]	C(7)[a]	C(8)[a]	C(9)[a]
0.00(2)	0.00(2)	0.00(2)	0.00(2)	−0.01(2)	0.01(2)
N(10)	N(11)	C(12)	N(18)	O(22)	
−0.07(2)	−0.10(2)	−0.20(3)	−0.20(4)	−0.24(3)	
II. Plane through the pyrimidine ring; $0.204x - 0.020y - 0.979z = 3.87$ Å					
C(12)[a]	C(13)[a]	N(14)[a]	C(15)[a]	N(16)[a]	C(17)[a]
0.00(1)	0.00(1)	0.00(1)	−0.02(2)	0.01(1)	−0.01(1)
C(4)	C(5)	C(6)	C(7)	C(8)	C(9)
−0.04(3)	−0.12(4)	−0.14(4)	−0.09(4)	−0.03(3)	−0.01(3)
N(10)	N(11)	N(18)	C(19)	O(20)	C(21)
0.02(2)	0.02(2)	−0.01(2)	−0.03(2)	−0.01(2)	0.07(2)
O(22)					
−0.01(2)					
The angle between planes I and II is 3.3(5)°					

[a]Atoms included in the calculation of the planes.

additional proton on the N(10) atom participates in a short intramolecular hydrogen bond between N(10) and the carbonyl oxygen atom O(22) [N(10) ...O(22) = 2.59(1) Å]. Thus, in addition to the essentially coplanar phenyl and substituted uracil groups, there is a third ring in DZH_2^+, formed by this short hydrogen bond. Owing at least partly to this bond, the phenyl and uracil groups are so oriented that the whole DZH_2^+ cation is almost planar (Table 4). The same planarity is found in the complex $[DZH_2]_2$-$[AuBr_2]Br \cdot H_2O$.

Bond lengths and angles compare reasonably well with those reported for other complexes of DZH [24, 29]. In the pyrimidine ring all C–C and C–N

TABLE 5. All Possible Hydrogen Bonds (Å) and some Short Intermolecular Distances (Å) Between $AuCl_2^-$ and DZH_2^+ Ions

N(10)–O(22)	2.59(1)	Au–C(13)	3.41(1)
Cl(1)–O(24)	3.22(1)	Au–C(4)	3.74(1)
Cl(2)–N(18)	3.28(1)	Cl(1)–N(14)	3.33(1)
N(18)–O(23)	2.79(1)	Cl(1)–C(7)	3.61(2)
O(23)–O(20)	2.90(1)	Cl(2)–N(16)	3.41(1)
O(23)–O(24)	2.83(2)	Cl(2)–C(9)	3.76(2)

distances are intermediate between single and double bond lengths, as expected from electronic delocalization. The N=N bond distance of the azo group [1.28(1) Å] and the nearest C–N distances [C(9)–N(10) = 1.44(1) and C(12)–N(11) = 1.33(1) Å] indicate electron delocalization also in this part of the DZH_2^+ cation.

Although all hydrogen atoms could not be located and none of the positions were refined, the short intermolecular distances between non-hydrogen atoms clearly indicate bonds between $AuCl_2^-$, DZH_2^+ and the crystal water molecules (*cf.* Table 5 and Fig. 3). The possible hydrogen bond distances suggest that both chlorine atoms act as proton acceptors, and both hydrogen atoms of N(18) and probably all hydrogen atoms of the crystal water molecules are involved in the hydrogen bonds. The other short interatomic distances between $AuCl_2^-$ and DZH_2^+ ions, besides these possible hydrogen bond contacts, indicate only van der Waals interactions (Table 5).

$AuLCl_2$ Complexes

Air stable complexes of the general formula $AuLCl_2$ (where LH = DZH, DZCH and $DZBH_2$) were obtained in good yield from $NaAuCl_4 \cdot 2H_2O$ and the corresponding 6-amino-1,3-dimethyl-5-arylazouracil derivative. The conductivity measurements indicate that the complexes are non-electrolytes in methanolic solution.

The similarity of the IR spectra of the complexes suggests an analogous coordination mode for all three ligands. Table 6 lists the most significant IR bands for the complexes together with those for the corresponding ligands. All the complexes show a sharp band due to the N–H stretching vibration of the deprotonated N-coordinated 6-amino group at about 3350 cm^{-1} [20]. The N=N stretching bands are shifted (by *ca.* 100 cm^{-1}) to lower wavenumber relative to the uncoordinated ligand, indicating that a nitrogen atom of the azo group is involved in the coordination to gold(III). In addition, the IR spectrum of the complex $Au(DZBH)Cl_2$ shows a sharp band assignable to the O–H stretching vibration of the free acid group at 3606 cm^{-1}, by which five-coordination of gold(III) can be ruled out.

TABLE 6. Infrared Data (cm^{-1}) for the Gold(III) Complexes

	ν(N–H)	ν(N=N)	ν(Au–Cl)
DZH	3269	1522	
$Au(DZ)Cl_2$	3342	1381	359
DZCH	3257	1529	
$Au(DZC)Cl_2$	3342	1383	361, 337
$DZBH_2$	3400–2400[a]	1524	
$Au(DZBH)Cl_2$	3369	1385	362, 342

[a]Wide band.

In the far-IR spectra there are two strong bands at about 360 and 340 cm^{-1} (a wide band at 359 cm^{-1} for $Au(DZ)Cl_2$), which can be assigned to the Au–Cl stretching vibrations where the Cl atoms are *trans* to the nitrogen atoms of the azo and deprotonated amino groups, respectively. Such a small difference in the position of the bands is indicative of the similar *trans*-influence of the two nitrogen atoms. Our values for the Au–Cl stretching vibration are in agreement with the value of 370 cm^{-1} found [30] for the Au–Cl stretching vibration where the Cl atom is *trans* to the nitrogen atom of the azo group in $Au(Az)Cl_2$ (Az = anionic azobenzene).

In good accord with the IR data, the results of an X-ray crystal structure determination of the complex $Au(DZC)Cl_2$ [29] indicate that the ligand is *cis*-coordinated through the nitrogen atom of the deprotonated amino group and the nitrogen of the azo group bonded to the phenyl ring. Two *cis* chlorine atoms occupy the remaining positions in the coordination sphere, defining a nearly square-planar coordination geometry, which is typical for Au(III).

In view of the above results, it is reasonable to conclude therefore that the complexes $Au(DZ)Cl_2$ and $Au(DZBH)Cl_2$ also exhibit a *cis*-square-planar structure (Fig. 4).

Growth Inhibition of HeLa Cells

Table 7 lists the percentage of growth inhibition of HeLa cells (human tumour cervix) due to $[DZH_2][AuCl_2] \cdot 2H_2O$ and the gold(III) complexes $AuLCl_2$.

With the gold(III) complexes $Au(DZ)Cl_2$ and $Au(DZC)Cl_2$, a 24 h exposure at a concentration of 10 μM considerably inhibited the growth of HeLa cells, the reduction in the number of colonies formed being greater than 50% relative to controls. However, with the complex $Au(DZBH)Cl_2$ a ten-fold higher concentration (100 μM) was required to produce similar growth inhibition. There is no obvious reason for this result, since the IR spectra indicate analogus structures for the three complexes, which differ only in the *ortho* substituent to the azo group on the phenyl ring of each ligand.

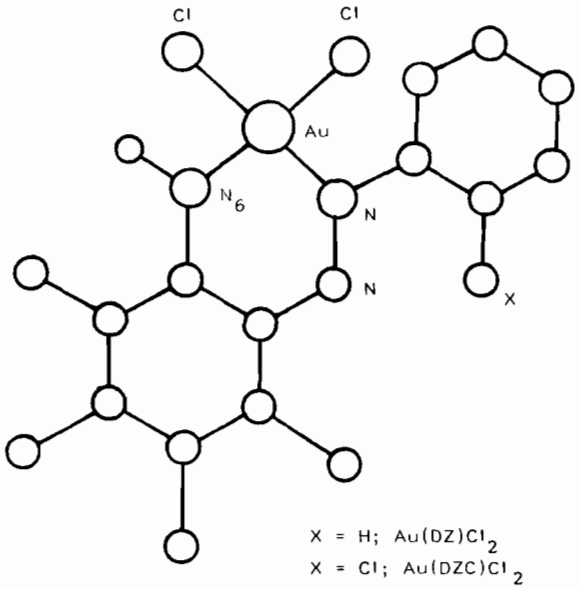

Fig. 4. Proposed structure for the complexes $AuLCl_2$.

The growth inhibition data at a concentration of 10 μM of $[DZH_2][AuCl_2]\cdot 1.5H_2O$ seem to indicate that this complex is a stronger growth inhibitor than the analogous bromogold(I) complexes [24], and similar to the gold(III) complex $Au(DZ)Cl_2$.

Recently, a correlation between earlier *in vitro* cytotoxic activity (given as the concentration of gold compound required to reduce the number of colonies formed by 50% relative to control, IC_{50}) and *in vivo* antitumour activity has been proposed [8, 12]: namely that, although a high degree of growth inhibition of tumour cells *in vitro* does not prove that the gold(III) complex will be active *in vivo*, generally a low degree of growth inhibition ($IC_{50} > 20$ μM) is indicative that the compound will be inactive or only marginally active *in vivo*. Thus, the *in vitro* assay may provide an appropriate prescreen for the selection of compounds for evaluation *in vivo*. In view of this, the complexes $[DZH_2][AuCl_2]\cdot 1.5H_2O$, $Au(DZ)Cl_2$ and $Au(DZC)Cl_2$ might have antitumour activity *in vivo*, whereas $Au(DZBH)Cl_2$ would more likely be inactive. Nevertheless, the *in*

TABLE 7. Growth Inhibition of HeLa Cells by the Gold(I) and Gold(III) Compounds

Compound	Concentration (μM)	Viability cells ($\times 10^4$)	Growth inhibition size (%)	
$[DZH_2][AuCl_2]\cdot 1.5H_2O$	10	10.20 ± 0.60	big	11.2
			small	66.6
			isolated cells	22.2
	50	9.00 ± 0.30	big	10.0
			small	47.5
			isolated cells	42.5
$Au(DZ)Cl_2$	10	20.88 ± 1.02	big	14.0
			small	50.0
			isolated cells	36.0
	20	17.60 ± 0.20	big	12.0
			small	42.5
			isolated cells	45.5
$Au(DZC)Cl_2$	5	19.75 ± 0.80	big	43.5
			small	36.0
			isolated cells	20.5
	10	11.54 ± 0.46	big	37.0
			small	42.0
			isolated cells	21.0
$Au(DZBH)Cl_2$	100	14.90 ± 0.60	big	11.0
			small	74.5
			isolated cells	14.5
	250	13.70 ± 0.50	big	13.5
			small	40.5
			isolated cells	46.0
Control		19.00 ± 1.25	big	79.7
			small	13.5
			isolated cells	6.8
Control + DMSO (10 μl)		16.92 ± 0.58	big	79.7
			small	11.9
			isolated cells	8.4

vivo antitumour activity of this last complex cannot be ruled out since some gold complexes inactive *in vitro*, exceptionally to the general rule, have proved to be active *in vivo* [12]. Work is in progress to further characterize the anticancer properties of our complexes *in vivo*.

Supplementary Material

Additional crystallographic information including lists of observed and calculated structure factors, anisotropic temperature factors and non-refined hydrogen atom coordinates is available from the authors on request.

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