Correlation of the in Vitro Cytotoxic and in Vivo Antitumor Activities of Gold(I) Coordination Complexes

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A series of gold(I) coordination complexes including analogues of the antiarthritic agent auranofin 1 were evaluated for in vitro cytotoxic potency against both B16 melanoma cells and P388 leukemia cells and in vivo antitumor activity against P388 leukemia in mice. A number of the complexes showed potent cytotoxic activity in vitro and antitumor activity in vivo, with the phosphine-coordinated gold(I) thiosugar complexes demonstrating the greatest in vitro and in vivo activity. The data compiled for 63 complexes of the general structural formula LAuX provide the basis for the following observations: (1) potent in vitro cytotoxic activity is observed for substituted (phosphine)gold complexes, (2) lack of potency in vitro correlates well with lack of antitumor activity, (3) potent cytotoxicity in vitro is not necessarily predictive of activity in vivo, (4) in vivo antitumor activity is generally optimized by ligation of Au(I) with a substituted phosphine and a thiosugar.

The use of certain platinum-containing compounds against neoplastic diseases[1] and the reported evidence for the antitumor activity of complexes of rhodium,[2] germanium,[3] and palladium[4] underscore the potential of transition-metal compounds as therapeutic agents in the clinical treatment of neoplastic diseases.

Surprisingly, despite the intense interest in other metals, gold compounds remain relatively unexplored as antineoplastic agents. The antiarthritic orally absorbed phosphine-coordinated gold(I) compound auranofin[5] ('Ridaura', Smith Kline and French Laboratories; 1) has been shown to inhibit the responsiveness of human lymphocytes both in vivo and in vitro.[6,7] Simon et al. have reported on the in vitro proliferative effects of auranofin (1) against cultured human cancer cells.[8] Auranofin has also been shown to have a significant antitumor effect in mice inoculated with the lymphocytic leukemia P388.[9,10]

The mechanism(s) by which 1 produces these effects is unclear.[10] To explore further the antitumor properties of gold compounds and to gain insight into possible mechanism(s) of action, we have attempted to define the structure–activity relationships of a series of coordinated gold(I) complexes with respect to their cytotoxic potency against transformed cells in vitro and in vivo antitumor activity.

Experimental Section

Melting points were determined in open glass capillaries with a Thomas-Hoover melting point apparatus and are uncorrected. NMR and IR spectra of all new compounds were consistent with assigned structures.

Preparation of Complexes. Gold thioglucose (**37**; Sigma Chemical Co.), gold sodium thiomalate (**39**; Aldrich Chemical Co.), and triethylphosphine oxide (Pfaltz and Bauer, Inc.) were commercial items. The phosphine ligands were obtained from Strem Chemical Co.

Method A. [2,3,4,6-Tetrakis-*O*-[(methylamino)carbonyl]-1-thio-β-D-glucopyranosato-*S*](triethylphosphine)gold (3). A mixture of **2** (5.2 g, 0.01 mol) and methyl isocyanate (100 g, 1.75 mol) was stirred 4 h at ambient temperature, and the volatiles were removed at reduced pressure. Chromatography (dry column silica gel, gradient elution, 1–5% $CH_3OH/CHCl_3$) of the residue (8.1 g) gave 2.6 g (34%) of analytically pure product as an amorphous solid as the monohydrate. Anal. ($C_{20}H_{38}N_4O_9AuPS\cdot H_2O$) C, H, N.

(2,3,4,6-Tetramesyl-1-thio-β-D-glucopyranosato-*S*)(triethylphosphine)gold (4). A mixture of 41 (1.12 g, 3.2 mmol) and (2,3,4,6-tetrakis-*O*-(methylsulfonyl)-1-thio-β-D-glucopyranosato-*S*)silver (kindly provided by Dr. I. Lantos, Smith Kline and French Laboratories; 2.0 g, 3.2 mmol) in tetrahydrofuran (200 mL) was stirred overnight at ambient temperature. The AgCl was collected and the solvent removed in vacuo. The residue was chromatographed (silica gel $CHCl_3$) to give 2.5 g (93%) of 4 as an amorphous solid. Anal. ($C_{16}H_{34}O_{13}AuPS_5$) C, H.

Method B. (2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-galactopyranosato-*S*)(triethylphosphine)gold (6). A solution of K_2CO_3 (0.77 g, 5.6 mmol) in water (5 mL) was added to 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose (2.0 g, 5.5 mmol) in ethanol (40 mL)/water (10 mL) at 0 °C followed by addition of 41 (1.98 g, 5.6 mmol) in ethanol (30 mL)/CH_2Cl_2 (5 mL). The mixture was stirred at 0 °C for 45 min, diluted with water (200 mL), extracted with CH_2Cl_2 (2 × 50 mL), dried ($MgSO_4$), and filtered and the solvent removed in vacuo. Chromatography (silica gel for dry column, ether/acetone, 6:1) of the residue gave 2.0 g (53%) of product as an amorphous solid; $[\alpha]^{25}_D$ (1% CH_3OH) −128.0°. Anal. ($C_{20}H_{34}O_9AuPS$) C, H, P.

(Thiosemicarbazido-*S*)(triethylphosphine)gold(I) (18). An ethanol solution (60 mL) of thiosemicarbazide (1.37 g, 15 mmol) and 41 (5.25 g, 15 mmol) was refluxed for 45 min and the solvent evaporated to dryness. Benzene (50 mL) was added to the residue and the resulting white solid collected to give 5.39 g (81%); mp 117–119 °C. Anal. ($C_7H_{19}N_3AuPS\cdot HCl$) C, H, N.

Method C. (Nitrato)(triethylphosphine)(2,2′-thiodiethanol)gold(I) (26). $AgNO_3$ (1.7 g, 10 mmol) in acetonitrile (50 mL) was added to a solution of 41 (3.5 g, 10 mmol) and thiodiglycol (1.2 g, 10 mmol) in acetonitrile (25 mL) and stirred at ambient temperature overnight. AgCl was removed and the solvent evaporated in vacuo. The residue was dissolved in CH_2Cl_2, treated with activated carbon, and filtered and ether was added. The solvent was decanted and the residue washed with ether and

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0022-2623/86/1829-0218$01.50/0

decanted. The oily product was pumped in vacuo to give 3.95 g (79%) of **26** as a hydrate. Anal. ($C_{10}H_{25}NO_5AuPS \cdot {}^3/_4H_2O$) C, H, N.

Gold 2-Sulfidoethyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (40). A suspension of gold 2-sulfidoethyl β-D-glucopyranoside (5.1 g, 11.7 mmol) in a mixture of acetic anhydride (100 mL) and pyridine (10 mL) was refluxed for 10 min. The mixture was a cooled, filtered, and concentrated in vacuo. Water was added and the resulting solid collected and recrystallized from dioxane/water. Chromatography of this material on silica (dioxane) gave 1.6 g (23%) of **40**. Anal. ($C_{16}H_{23}AuO_{10}S$) C, H, Au.

Cyano(triethylphosphine)gold(I) (43). A mixture of **41** (3.5 g, 10 mmol) in CH_2Cl_2 (25 mL) and KCN (1.5 g, 23 mmol) in water (20 mL) was stirred at ambient temperature overnight, the organic layer was separated, washed with water, and dried (Na_2SO_4), and the solvent was removed in vacuo. Recrystallization of the residue from CH_2Cl_2/petroleum ether gave 2.4 g (70%) of **43**; mp 111–112 °C. Anal. ($C_7H_{15}AuNP$) C, H, N.

(Nitrato)bis(triethylphosphine)gold(I) (44). $AgNO_3$ (0.17 g, 1 mmol) in acetonitrile (20 mL) was added to a solution of **45** (0.47 g, 1 mmol) in acetonitrile (30 mL). After 2 h, the AgCl was removed and the solvent evaporated. The residue was repeatedly dissolved in methylene chloride and ether added and the solvent decanted. The resulting product was dried in vacuo to give 0.4 g (82%) of **44** as an amorphous solid; mp 77–79 °C. Anal. ($C_{12}H_{30}AuNO_3P$) C, H, N.

Method D. Chloro(triallylphosphine)gold(I) (48). Thiodiglycol (7.0 g, 56 mmol) in ethanol (35 mL) was added to chloroauric acid trihydrate (11.0 g, 28 mmol) in water (50 mL) kept at 0 °C. Triallylphosphine (5.0 g, 32 mmol) in ethanol (30 mL) was added to the colorless gold solution over 10 min to afford a viscous precipitate. After 20 min the mixture was extracted with $CHCl_3$, the extract was washed with water, dried ($MgSO_4$), and filtered, and the solvent was evaporated to give 11 g of crude, yellow, oily product. Dry column chromatography (silica gel/ether) gave 5.0 g (46%) of clear oil. Anal. ($C_9H_{15}AuClP$) C, H, P.

The complexes used in these studies are described in Table I.

Biology. Cell Culture Techniques. B16 melanoma (highly metastatic subline, F10)[11] were used and maintained as a monolayer culture in minimal essential media (MEM; Grand Island Biological Col., Grand Island, NY) supplemented with 10% calf serum and 100 units/mL penicillin and 100 μg/mL streptomycin in a 5% CO_2 humidified incubator at 37 °C. Asynchronous populations of cells were harvested and replated at 5000 cells/plate in sterile 60 mm × 15 mm petri plates. Plates were incubated overnight to allow attachment to the plate. Cells were treated with gold compounds (dissolved in 100% ethanol at 2 mM and then diluted in the appropriate concentration in MEM) under sterile conditions and allowed to react for 2 h followed by aspiration of medium. Plates were washed one time with 5 mL of phosphate-buffered saline (PBS), followed by the addition of 5 mL of fresh media. Plates were incubated for 5 days at 37 °C in a CO_2 incubator. Viability was measured by the ability of a cell to form a colony of greater than 50 cells. Colonies were fixed with 0.5% crystal violet in 95% ethanol. Plates were dried and counted with a Biotran III Automatic Count Totalizer (New Brunswick Scientific Co., Edison, NJ). Mean and standard deviation of triplicate samples were determined for each drug concentration. The data were expressed by plotting the log of the survivial fraction (number of colonies in drug treated plates/number of colonies in controls) vs. the drug concentration.

Antitumor Evaluation of Gold Complexes. P388 leukemia cells (10^6) were inoculated ip in $B6D2F_1$ mice. Twenty-four hours later, if the tumor inoculum proved to be free of bacterial contamination (as determined by 24-h incubation in thioglycolate broth), animals were randomized into groups of six and housed in shoe-box cages. Gold complexes were dissolved in a minimal volume of either *N,N*-dimethylacetamide (DMA) or 95% ethanol. An equal volume of saline was added; if the drug precipitated, an equal volume of Cremophor-El (polyethoxylated castor oil, Sigma) was added and then saline qs to a concentration such that the desired dose was delivered in 0.5 mL. The final concentration of DMA, ethanol, and Cremophor was 10%. Dilutions for lower doses were made with saline; thus, there is a decreasing proportion of organic solvents in the vehicle with decreasing dosage. Formulations were prpared immediately prior to injection. The gold complexes were administered ip on days 1–5 (i.e. treatment was initiated 24 h after tumor inoculation). Each experiment includes three groups of six animals as untreated controls and animals treated with a positive control, *cis*-diamminedichloroplatinum (CDDP; Sigma Chemical Co., St. Louis, MO), at two dose levels. Animals were monitored daily for mortality and experiments were terminated after 45 days. The end point is median survival time (MST) and increase in lifespan (ILS), which is the percentage of increase in MST relative to untreated controls. Untreated controls inoculated ip with 10^6 P388 leukemia cells generally survive for a median of 10 or 11 days. In 66 experiments, CDDP at 2 mg/kg per day produced 125 ± 38% ILS. In all these experiments CDDP was active (>50% ILS).

Results and Discussion

In Vitro Cytotoxicity. Table II shows that cytotoxicity (i.e. in vitro potency) to B16 mouse melanoma cells grown in vitro was evident for a broad spectrum of gold(I) coordination complexes. With the majority of compounds tested, a 2-h exposure at concentrations of ≤10 μM significantly reduced the viability of B16 melanoma cells. With few exceptions (**9**, **48**, **51**), the (phosphine)gold(I) coordination complexes were potent cytotoxic agents. The gold(I) thiolates **36–40** had low cytotoxicity, as did complexes of chlorogold(I) with sulfides **57**, **59**, **60**, pyridine **58**, hexamethylenetetramine **61**, and cyclooctene **62**. The only non-phosphine gold(I) complex with potent cytotoxic activity was chloro(triethylarsine)gold(I) (**56**) and this complex was 8-fold less potent than the analogous phosphine complex **41**.

In Vivo Antitumor Activity. As **1** has been shown to have antitumor activity in mice bearing ip P388 leukemia,[9,10] we systematically evaluated the influence of substituents on the antitumor activity of this class of gold complexes (Table II). Removal or replacement of the acetyl groups in the thiosugar did not markedly influence the in vivo potency (maximally tolerated dose) or efficacy (maximum increase in lifespan), though the mesylate **4** was somewhat less active. Replacement of 1-β-D-thioglucose with other thiosugars (series B) such as 1-α-D-thioglucose (**5**), thiogalactose (**6**), and thiomaltose (**7**) yielded compounds with similar potency and efficacy. A thioxylose analogue **8** was essentially inactive but was just as toxic to mice as the active analogues. A number of (triethylphosphine)gold(I) thiolates were evaluated (series C). Except for the *S*-methyl compound **9**, these compounds had in vivo potencies similar to that of **1**. The majority of these compounds had marginal activity against ip P388 (30–50% ILS) though a few (**13**, **20**, **23**, **26**) had activity approaching that of **1**.

In series D we explored the importance of the substitution of the phosphine on the activity of analogues of **1**. Activity was evident for all compounds in this series including complexes of various alkyl-, aryl-, alkoxy-, and amino-substituted phosphines (**27–35**). The least active compound was the triphenylphosphine (**30**) and the most active in vivo was the diethylisopropoxyphosphine (**34**). These substituents were also evaluated in series D/F in which the tetraacetylthioglucose ligand is replaced by a chlorine. These compounds **47–55** along with **41** appear to be less active in vivo than similarly substituted compounds with the tetraacetylthioglucose (i.e. **41** vs. **1**, **47** vs. **27**, **50** vs. **29**, **52** vs. **31**, **49** vs. **35**). The chlorogold(I) phosphine complexes with the greatest activity were the tribenzyl- and dibenzylphenyl-substituted analogues **54** and **55**.

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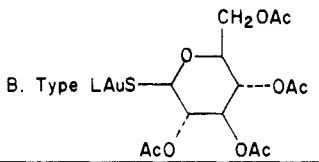
Table I. Chemical Data for Gold(I) Complexes

A. Type $(CH_3CH_2)_3PAuSR$

no.	R	formula	mp, °C	crystn solvent	a^{25}_D, deg	anal. or ref
1	2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl	$C_{20}H_{34}AuO_9PS$				5
2	β-D-glucopyranosyl	$C_{12}H_{26}AuO_5PS$				5
4	2,3,4,6-tetramesyl-β-D-glucopyranosyl	$C_{16}H_{34}AuO_{13}PS_5$				C, H
5	2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl	$C_{20}H_{34}AuO_9PS$				19
6	2,3,4.6-tetra-*O*-acetyl-β-D-galactopyranosyl	$C_{20}H_{34}AuO_9PS$				C, H, P
7	hepto-*O*-acetyl-β-maltosyl	$C_{32}H_{50}AuO_{17}PS$		methanol	+23.1	C, H, P
8	1,2-*O*-isopropylidene-5-α-D-xylofuranosyl	$C_{14}H_{26}AuO_4PS$				C, H, P
9	CH_3	$C_7H_{18}AuPS$				20
10	$CH_2(CH_2)_6CH_3$	$C_{14}H_{32}AuPS$		oil		C, H, P
11	$CH(CO_2H)CH_2CO_2H$	$C_{10}H_{20}AuO_4PS$				21
12	2-morpholinoethyl	$C_{12}H_{27}AuNOPS$	146–147.5	$CHCl_3$/ether		C, H, N
13	2′-ethyl-1-β-D-glucopyranosyl	$C_{14}H_{30}AuO_6PS$				5
14	2′-ethyl-1-thio-β-D-glucopyranosyl	$C_{14}H_{30}AuO_5PS_2$				5
15	glutathionyl hydrochloride	$C_{16}H_{31}AuN_3O_6PS$	115–120	lypholized		C, H, N, Au, 12
16	CN	$C_7H_{15}AuNPS$				20
17	$C(NH_2)_2{\cdot}HCl$	$C_7H_{18}AuN_2PS{\cdot}HCl$				21
18	$C(NH_2)NHNH_2$	$C_7H_{19}AuN_3PS$	117–119	benzene		C, H, N
19	phenyl	$C_6H_{20}AuPS$				20
20	2-aminophenyl	$C_{12}H_{21}AuNPS$	87–88.5	CH_3OH		C, H, N
21	2-pyridyl	$C_{11}H_{19}AuNPS$	81–83	CH_3OH/H_2O		C, H, N
22	4-pyridyl	$C_{11}H_{19}AuNPS$	46–48			C, H, N
23	2-thiazolinyl	$C_9H_{19}AuNPS$	70–71	acetone/H_2O		C, H, N
24	2-benzimidazolyl	$C_{13}H_{20}AuN_2PS$	222–223	ethanol		C, H, N
25	2-benzoxazolyl	$C_{13}H_{18}AuNOPS$	100–102	CH_3OH/H_2O		C, H, N
26	$(CH_2CH_2OH)_2NO_3^-$	$C_{10}H_{25}AuNO_5PS$		oil		C, H, N

B. Type LAuS



no.	L	formula	mp, °C	crystn solvent	a^{25}_D, deg	anal. or ref.	method
27	$(CH_3)_3P$	$C_{17}H_{28}CuO_9PS$	177–178	ethanol		C, H	B
28	$[(CH_3)_3CH_2]_3P$	$C_{23}H_{40}AuO_9PS$	137–138	CH_3OH/H_2O		C, H	B
29	$[(CH_3)_2N]_3P$	$C_{20}H_{47}AuN_3O_9PS$				19	
30	$(C_6H_5)_3P$	$C_{32}H_{34}AuO_9PS$				20	
31	$(CH_3CH_2)_2C_6H_5P$	$C_{24}H_{34}AuO_9PS$	amorphous	methanol	−61.6	C, H, P	B
32	$CH_3CH_2(C_6H_5)_2P$	$C_{28}H_{34}AuO_9PS$	amorphous	methanol	−56.6	C, H, P	B
33	$(CH_3CH_2)_2CH_3CH_2OP$	$C_2H_{34}AuO_{10}PS$				22	
34	$(CH_3CH_2)_2[(CH_3)_2CH_2O]P$	$C_{21}H_{36}AuO_{16}PS$				22	
35	$(CH_3CH_2)_2[HO(CH_2)_3CH_2]P$	$C_{22}H_{38}AuO_{10}PS$		methanol	47.2	C, H, P	B

C. Type $[AuSR']_n$

no.	R′	formula	mp, °C	crystn solvent	anal. or ref	method
36	2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl	$(C_{14}H_{19}AuO_9S)_n$			23	
37	β-D-glucopyranosyl	$(C_6H_{11}AuO_5S)_n$			Sigma	
38	CH_2CH_2OH	$(C_2H_5AuOS)_n$			23	
39	$CH(CO_2Na)CH_2CO_2Na$	$(C_4H_5AuO_4S{\cdot}2Na)_n$			Aldrich	
40	2′-ethyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl	$(C_{16}H_{23}AuO_{10}S)_n$	205–208	dioxane/water	C, H, Au	C

D. Type $[(CH_3CH_2)_3P]_nAuY$

no.	Y	*n*	formula	mp, °C	crystn solvent	anal. or ref	method of prep
41	Cl	1	$C_6H_{14}AuClP$			5	
42	CH_3	1	$C_7H_{18}AuP$			25	
43	CN	1	$C_7H_{15}AuNP$	111–112	CH_2Cl_2/pet. ether	C, H, N	C
44	NO_3^-	2	$C_6H_{15}AuNO_3P$	77–79	CH_2Cl_2/ether	C, H, N	C
45	Cl^-	2	$C_{12}H_{30}AuClP_2$			26	
46	$(C_6H_5)_3P$, Cl^-	1	$C_{24}H_{30}AuClP_2$	98 dec	benzene/hexane	C, H, Cl	C

E. Type LAuCl

no.	L	formula	mp, °C	crystn solvent	anal. or ref	method of prep
47	$(CH_3)_3P$	C_3H_9AuClP			5	
48	$(CH_2{=}CHCH_2)_3P$	$C_4H_{15}AuClP$		oil	C, H, P	D
49	$(CH_3CH_2)_2[HO(CH_2)_4]P$	$C_8H_{18}AuClOP$		oil	C, H, P	D
50	$[(CH_3)_2N]_3P$	$C_6H_{18}AuClN_3P$			19	
51	$(C_6H_5)_3P$	$C_{18}H_{15}AuClP$			27	
52	$(CH_3CH_2)_2(C_6H_5)P$	$C_{10}H_{15}AuClP$	61–63	ether	C, H	D
53	$[(CH_3)_2CH]_2(C_6H_5)P$	$C_{12}H_{19}AuClP$	128–130	ethanol	C, H	D
54	$(C_6H_5CH_2)_3P$	$C_{21}H_{21}AuClP$	260–262	ethanol	C, H	D
55	$(C_6H_5CH_2)_2(C_6H_5)P$	$C_{20}H_{19}AuClP$	175–6	ethanol	C, H, P	D
56	$(CH_3CH_2)_3As$	$C_6H_{15}AsAuCl$			28	
57	$(CH_3CH_2)_2S$	$C_4H_{10}AuClS$			28	

Table I (Continued)

E. Type LAuCl						
no.	L	formula	mp, °C	crystn solvent	anal. or ref	method of prep
58	pyridine	C_5H_5AuClN			28	
59	pentamethylene sulfide	$C_5H_{10}AuClS$			30	
60	tetramethylene sulfide	C_4H_8AuClS	145–148	ethanol	C, H	D
61	hexamethylenetetramine	$H_{12}N_4AuCl$			31	
62	cyclooctene	$C_8H_{14}AuCl$			32	

As with cytotoxicity in vitro, antitumor activity was dependent on the existence of two coordinating ligands being coordinated with gold(I). The gold(I) thiolates (series E) were tolerated by mice at very high doses and were inactive against P388 leukemia in vivo.

Series F explores replacement of the tetraacetylthioglucose ligand of 1 with a number of simple ligands such as chloro (41), methyl (42), cyano (43), nitrato (44), and another phosphine (45, 46), the latter giving charged complexes. Each of these analogues retained potency in vivo and antitumor activity.

The last group of compounds, 56–62, includes chlorogold(I) complexes coordinated to ligands other than phosphines. The triethylarsine 56 was less potent than the analogous phosphine 41 and was inactive with respect to antitumor activity. The sulfido (57, 59, 60), pyridino (58), and cyclooctenyl (62) complexes, although much lower in potency than the phosphine complexes, demonstrated antitumor activity in vivo (>30% ILS).

The structure–activity relationship derived from these studies indicate that structural changes within ligand types coordinated to the gold atom can profoundly alter the in vitro cytotoxic and in vivo toxic potencies and the antitumor activity of various groups of compounds. For example, removal of the triethylphosphine moiety from 1 reduced the cytotoxic potency of the resulting complex (36) 150-fold. In general, phosphine-coordinated gold complexes were more potent in vitro than their non-phosphine substituted counterparts. This relationship may in part be the result of lipophilicity introduced into a gold complex by an alkyl- or phenylphosphine. For example, distribution and partition coefficient data show that Et_3PAuCl (41), Me_3PAuCl (47), Ph_3PAuCl (51), and 1 are significantly more lipophilic than gold thioglucose (37) and gold thiomalate (39).[12] This increase in lipophilicity may enhance the ability of the former compounds to associate with and/or transverse cellular membranes. The correlation of increasing lipophilicity and cytotoxic potency is in agreement with data reported by Otiko et al. in which ^1H NMR studies indicated that gold thiomalate (39) coats the surface of erythrocytes whereas 1 and chloro(triethylphosphine)gold(I) (41) enter red cells and undergo ligand exchange with intracellular sulfhydryl-containing molecules such as glutathione.[13] Alternatively, while 1, 41, 47 and 51 are lipophilic, they are also monomeric. This is in contrast to the polymeric nature of 37 and 39.[14] Thus, these gross structural differences might also account for the observed biological variations.

The ultimate intracellular target(s) through which gold–phosphine complexes produce their cytotoxic effects is unclear at present. Data from our laboratory demonstrate that the cytotoxic effect of auranofin is rapid and that there appears to be a direct relationship between cellular association of auranofin-derived gold and the cytotoxic effect of the drug.[10] In addition, this cytotoxic effect is not mediated through a cell cycle-specific or -dependent mechanism nor is there evidence for a preferential inhibition of DNA, RNA, or protein synthesis.[10,15] These findings in combination with the structure–activity relationships reported here (i.e., increased lipophilicity: increased cytotoxic potency) provide the basis for proposing a cytotoxic mechanism for this class of compounds in which the substituted phosphine moiety provides the lipophilicity required for penetration of the gold into the cellular membrane. During the process of integration into the membrane, gold-ligand-substitution reactions occur in which gold becomes ligated to membrane proteins which may lead to a variety of specific and/or nonspecific membrane perturbations and cell lysis. Recent data from our laboratory indicates that the initial ligand substitution reaction involves the exchange of the tetraacetylthioglucose moiety of auranofin for sulfhydryl-containing groups of the cellular membrane.[16] The subsequent ligand substitution reactions and the interactions of these metabolites of auranofin with cellular membranes and their structural and enzymatic components are currently under investigation.

The structure–activity relationships with respect to the in vivo antitumor activity of this group of gold compounds would not appear to be as well defined as that defined for the cytotoxic potency in vitro. While the largest percentage of highly active compounds are of the general formula $X_3PAuSGlu(Y)_4$ (series A, B, and D), there are also individual compounds within other series which have significant in vivo antitumor activity (20, 23, 26, 29, 34, 43, 54, and 55).

Analysis of the in vitro and in vivo data in Table II indicates that the in vitro assay would have provided an appropriate prescreen for the selection of compounds for evaluation in vivo. Although a high degree of cytotoxic potency in vitro does not indicate that the gold(I) complex will be active in vivo, a low degree of cytotoxicity (i.e. IC50 > 20 μM) is indicative that a compound will be inactive or, at best, marginally active in vivo. One notable exception is the chloro(diethylsulfido)gold(I) complex (57) which was inactive in vitro but was active in vivo albeit at very high dose levels. Analysis of the data in Table II also demonstrates that there is a correlation ($r = 0.80$) between in vitro cytotoxic potency (IC50) with in vivo potency (MTD) for this entire group of compounds (Figure 1). Compounds with low cytotoxicity are tolerated at higher dose levels in mice.

Although a possible explanation for the discrepancy between in vitro cytotoxic and in vivo antitumor activity demonstrated by many of the compounds is the difference in tumor cells used in the in vitro cytotoxicity experiments (B16 melanoma) and the in vivo antitumor studies (P388 leukemia), additional data shows that many of these com-

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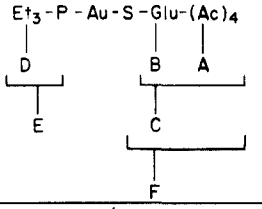
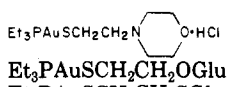
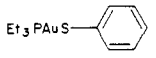
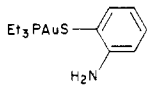
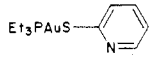
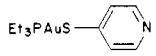
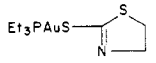
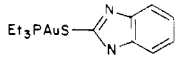
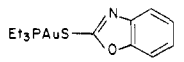
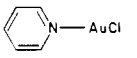
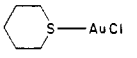
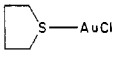
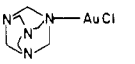
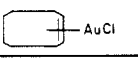
Table II. Biological Evaluation of Auranofin Analogues



no.	structure[a]	B16[b] IC50, μM	MTD,[c] μmol/kg	P388[d] ILS_{max}, %
	Series A			
1	$Et_3PAuSGlu(Ac)_4$	1.5	18	70
2	$Et_3PAuSGlu$	2	24	68
3	$Et_3PAuSGlu(CONHMe)_4$	7	21	58
4	$Et_3PAuSGlu(SO_2Me)_4$	3	19	40
	Series B			
5	Et_3PAuS-α-$Glu(Ac)_4$	4	18	65
6	$Et_3PAuSGal(Ac)_4$	4	18	88
7	$Et_3PAuSGlu(Ac)_3Glu(Ac)_4$	6	19	88
8	$Et_3PAuSXyl$(1-β-2-propylidene)	6	15	27
	Series C			
9	$Et_3PAuSCH_3$	60	110	36
10	$Et_3PAuSCH_2(CH_2)_6CH_3$	nt	17	32
11	$Et_3PAuSCH(COOH)$-CH_2COOH	1	22	46
12		nt	24	32
13	$Et_3PAuSCH_2CH_2OGlu$	2	14	55
14	$Et_3PAuSCH_2CH_2SGlu$	7	21	45
15	Et_3PAuS-glutathione·HCl	2	12	32
16	$Et_3PAuSCN$	1	21	36
17	$Et_3PAuSC(NH)NH_2$·HCl	1	14	41
18	$Et_3PAuSC(NH)NHNH_2$·HCl	1	11	36
19		6	19	36
20		1	18	63
21		2	14	46
22		1	9	36
23		4	19	60
24		5	17	45
25		3	11	27
26	$Et_3PAuS(EtOH)_2^+·NO_3^-$	10	19	64

no.	structure[a]	B16[b] IC50, μM	MTD,[c] μmol/kg	P388[d] ILS_{max}, %
	Series D			
27	$Me_3PAuSGlu(Ac)_4$	2	9	45
28	i-$Pr_3PAuSGlu(Ac)_4$	4	14	46
29	$(Me_2N)_3PAuSGlu(Ac)_4$	2	8	60
30	$Ph_3PAuSGlu(Ac)_4$	4	7	36
31	$Et_2PhPAuSGlu(Ac)_4$	2	13	55
32	$EtPh_2PAuSGlu(Ac)_4$	4	6	32
33	$Et_2EtOPAuSGlu(Ac)_4$	1	14	70
34	Et_2-i-$ProPAuSGlu(Ac)_4$	2	17	90
35	$Et_2HOBuPAuSGlu(Ac)_4$	8	17	58
	Series E			
36	$AuSGlu(Ac)_4$	150	110	14
37	AuSGlu	166	>300	15
38	$AuSCH_2CH_2OH$	140	500	9
39	$AuSCH(COOH)CH_2COOH$	60	350	24
40	$AuSCH_2CH_2OGlu(Ac)_4$	>100	106	0
	Series F			
41	Et_3PAuCl	1	14	36
42	Et_3PAuCH_3	1	36	55
43	Et_3PAuCN	0.4	17	68
44	Et_3PAuNO_3	2	13	41
45	$Et_3PAuPEt_3^+·Cl^-$	1	8	36
46	$Et_3PAuP(C_6H_5)_3^+·Cl^-$	6	13	52
	Series D/F			
47	Me_3PAuCl	6	16	34
48	$(allyl)_3PAuCl$	15	5	25
49	$Et_2HOBuPAuCl$	8	15	27
50	$(Me_2N)_3PAuCl$	0.7	13	31
51	Ph_3PAuCl	12	20	36
52	$Et_2PhPAuCl$	1	8	41
53	i-$Pr_2PhPAuCl$	3	9	36
54	$(PhCH_2)_3PAuCl$	4	19	91
55	$Ph(PhCH_2)_2PAuCl$	2	10	66
	Series E/F			
56	$Et_3AsAuCl$	8	40	18
57	Et_2SAuCl	>200	500	50
58		125	128	35
59		67	60	32
60		80	100	32
61		155	64	14
62		125	190	41

[a] Abbreviations used: Et, ethyl; Me, methyl; Glu, glucose; Ac, acetate; Gal, galactose; Xyl, xylofuranose; i-Pr, isopropyl; Bu, butyl; Ph, phenyl; nt, not tested. [b] In vitro cytotoxic potency was determined in a clonogenic assay with B16 mouse melanoma cells. Cells were treated with a compound for 2 h and clonogenic capacity of the cells was measured as a function of colonies formed by day 5. The IC50 value is the concentration of drug required to reduce the number of colonies formed by 50% relative to controls. [c] Maximally tolerated dose in tumor bearing mice, on a daily times 5 ip regimen. [d] Compounds were compared for antitumor efficacy given ip on days 1–5 to mice inoculated ip with 10^6 P388 leukemia cells. Activity was expressed as an increase in median life span (ILS) relative to control tumor-bearing mice. The value shown is the maximum ILS obtained in dose–response studies which bracket the maximally tolerated dose. In most instances the value shown is the average of two or more dose–response studies. An ILS value of ≥40% represents cell kill sufficient to result in a net reduction in tumor cell burden at the end of therapy; with a level of significance of $p \leq 0.05$.

pounds are cytotoxic to P388 cells in vitro and that the structure–activity relationships are equivalent to those observed with B16 cells in vitro. This comparative analysis of the in vitro chemosensitivity of B16 melanoma and P388 leukemia was conducted for 16 gold complexes (two examples of each group in Table II), and the data are shown in Table III. A linear regression analysis of the IC50 values for B16 cells vs. the IC50 values for P388 cells

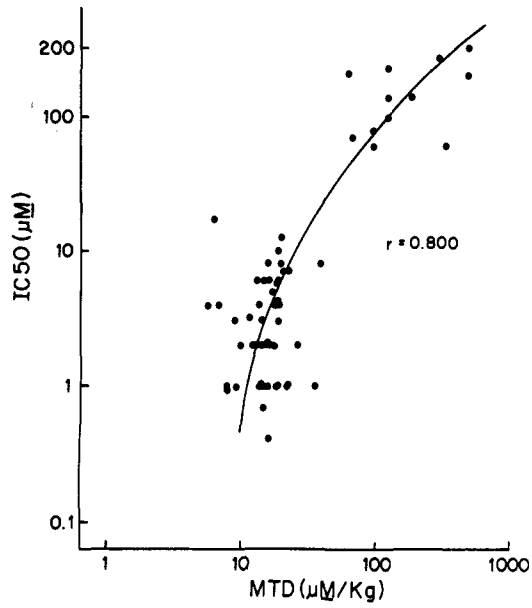


Figure 1. Relationship of in vivo cytotoxic potency and maximally tolerated dose in vivo of gold(I) complexes. IC50 and MTD values correspond to those tolerated in Table I.

Table III. Comparison of the in Vitro Cytotoxic Potency of Gold Complexes against P388 Leukemia and B16 Melanoma Cells

no.	P388 in vitro:[a] IC50, μM	(P388 IC50/B16 IC50)[b]	no.	P388 in vitro:[a] IC50, μM	(P388 IC50/B16 IC50)[b]
1	0.8	0.53	47	3	0.50
6	1	0.25	51	3	0.25
31	1	0.50	24	8	1.6
45	1	1.0	9	40	0.67
2	2	1.0	59	50	0.75
7	2	0.33	39	100	1.67
30	2	0.5	37	200	0.75
41	3	0.33	57	>200	1

[a] The in vitro chemosensitivity of P388 cells (maintained as suspension cultures) was determined in colony-forming assays in 0.17% agarose as described by Metcalfe et al.[33] Colony formation and IC50 values were measured as described for the B16 melanoma colony formation assay. [b] The ratio of the IC50 values determined for P388 and B16 cells in vitro.

provided a correlation coefficient of 0.89 and a slope of 1.12. From the data it would appear that, while in most cases the P388 cells were slightly more sensitive to the in vitro cytotoxic activity of these gold complexes, in general the rank order potency was eqivalent for both mouse tumor cell types. These data, as well as previously reported data, showing that compound 1 is inactive against B16 melanoma in vivo, demonstrates that for this group of compounds in vitro cytotoxic activity against a particular tumor cell type does not predict that a compound will have selective in vivo activity against that tumor.

These data indicate that the replacement of the chloride group with a thiosugar moiety in some manner provides a structural basis of selectivity for antitumor activity relative to toxicity by gold complexes within series A, B, and D. Obviously, the equation with respect to the parameters required for in vivo antitumor activity is considerably more complex than that of in vitro cytotoxic potency. Differences in pharmacokinetics, drug metabolism, and induction of metal binding proteins (e.g. metallothionein) in various organ sites, for example, could all be involved in the explanation as to why some of the extremely potent cytotoxic agents are not active against the in vivo tumor.

In conclusion, the data reported here indicate that a number of gold(I) coordination complexes, particularly (phosphine)gold compounds, possess potent in vitro cytotoxic activity and in vivo antitumor activity. We have recently reported that the in vivo antitumor activity of 1 as evaluated in a spectrum of mouse tumor models is limited.[1] Therefore, in an attempt to identify a compound with a greater degree and spectrum of antitumor activity these and other gold(I) complexes[17,18] are currently being evaluated in other animal models and in the human tumor stem cell clonogenic assay to better define their potential as antineoplastic agents.

Acknowledgment. We thank the Analytical, Physical, and Structural Chemistry staff including Edie Reich for elemental analyses and mass spectral and NMR data and Drs. B. Sutton, J. Hemple, and L. Kruse for discussion and interest and Dr. I. Lantos and K. Razgaitis for technical assistance and Dr. L. Webb for a sample of **15**. We thank Judy Seaman for her excellent typographical assistance.

Registry No. 1, 34031-32-8; 2, 34031-29-3; 3, 68611-89-2; 4, 68587-60-0; 5, 99395-45-6; 6, 99395-46-7; 7, 99327-18-1; 8, 99327-19-2; 9, 15685-02-6; 10, 99327-20-5; 11, 41581-85-5; 12, 99327-21-6; 13, 34031-34-0; 14, 34031-35-1; 15, 99327-22-7; 16, 14243-46-0; 17, 99327-23-8; 18, 99327-24-9; 19, 14243-43-7; 20, 54720-70-6; 21, 99327-25-0; 22, 99327-26-1; 23, 55927-96-3; 24, 55927-97-4; 25, 55927-98-5; 26, 99327-28-3; 27, 85533-74-0; 28, 85528-71-8; 29, 99327-29-4; 30, 85528-72-9; 31, 68611-95-0; 32, 99327-30-7; 33, 83582-27-8; 34, 83582-28-9; 35, 99327-31-8; 36, 69849-37-2; 37, 74610-70-1; 38, 99327-32-9; 39, 74916-57-7; 40, 83678-72-2; 41, 15529-90-5; 42, 34275-23-5; 43, 90981-41-2; 44, 99327-33-0; 45, 65583-79-1; 46, 65583-76-8; 47, 15278-97-4; 48, 99327-34-1; 49, 99327-35-2; 50, 99021-83-7; 51, 14243-64-2; 52, 55940-22-2; 53, 90479-64-4; 54, 72477-06-6; 55, 99327-36-3; 56, 99327-37-4; 57, 41867-99-6; 58, 22355-16-4; 59, 39929-23-2; 60, 39929-21-0; 61, 99327-38-5; 62, 12145-57-2; $HAuCl_4$, 16903-35-8; $(CH_2{=}CHCH_2)_3P$, 16523-89-0; 2,3,4,6-tetrakis-*O*-(methylsulfonyl)-2-thio-β-D-glucopyranosato)-*S*-silver, 68703-53-7; gold 2-sulfidoethyl-β-D-glucopyranoside, 99327-39-6.

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