Hz, cytosine *H*-6). Conjugates 8a,c–e and **9b,c** in Table II were prepared in an analogous manner.

Biological Studies. Tumor Cells and Animals. L1210/0 and L1210/*ara*-C(I) lymphoid leukemia cells were purchased from Arthur D. Little, Inc. (Cambridge, MA) and L1210/*ara*-C(II) was obtained from Dr. Ralph J. Bernacki from Roswell Park Memorial Institute. The cells were routinely transplanted in DBA/2J mice, which were supplied by Roswell Park Memorial Institute.

Antitumor Activity in Vivo. DBA/2J male mice in groups of six (wt 20–30 g) were inoculated ip with 1×10^6 L1210/0 or 1×10^5 L1210/*ara*-C(I) or L1210/*ara*-C(II) lymphoid leukemia cells,[26] and a sonicated solution of the conjugates was given ip as reported earlier.[12] Each drug was tested over a wide range of doses. The results from the representative dose levels are shown in Tables III and IV.

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Registry No. 1a (X = H), 25666-00-6; 1a (X = TBDMS), 125592-12-3; 1a (X = Tr), 103321-06-8; 1a (X = R_1 = H), 53023-42-0; **1b** (X = H), 25666-01-7; **1b** (X = TBDMS), 95244-99-8; **1b** (X = Tr), 91274-06-5; **2a** (X = TBDMS), 125592-15-6; **2a** (X = Tr), 103612-81-3; **2b** (X = TBDMS), 125592-14-5; **2b** (X = Tr), 125592-13-4; **2c** (X = TBDMS), 125592-17-8; **2d** (X = TBDMS), 125592-18-9; **2e** (X = TBDMS), 125592-16-7; **3a**, 103612-83-5; **3b**, 125592-19-0; **3c**, 103304-70-7; **3d**, 103304-71-8; **3e**, 125592-22-5; **4a**, 103612-82-4; **4b**, 125592-20-3; **5a**, 103612-84-6; **5b**, 125592-21-4; **5c**, 125592-23-6; **5d**, 125592-24-7; **5e**, 125592-25-8; **6**, 125592-26-9; **7**, 87713-33-5; **8a**·2Na, 125592-29-2; **8b**·2Na, 125592-28-1; **8b**·2NH_3, 125592-27-0; **8c**·2Na, 125592-30-5; **8d**·2Na, 125592-31-6; **8e**·2Na, 125592-32-7; **9b**·2Na, 125592-33-8; **9c**·2Na, 125592-34-9; stearyl bromide, 112-89-0; cetyl bromide, 112-82-3; palmitoyl chloride, 112-67-4.

Cytotoxicity and Antitumor Activity of Some Tetrahedral Bis(diphosphino)gold(I) Chelates[1]

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Department of Chemistry, Birkbeck College, University of London, Gordon House and Christopher Ingold Laboratory, 29 Gordon Square, London WC1H 0PP, UK, and Departments of Molecular Pharmacology and Medicinal Chemistry, Smith Kline and French Laboratories, P.O. Box 1539, King of Prussia, Pennsylvania 19406-0939. *Received August 14, 1989*

We report the cytotoxicity toward B16 cells and antitumor activity in three transplantable tumor models of a series of ionic, tetrahedral, bischelated gold diphosphine complexes of the type $[Au^I(R_2PYPR_2')_2]X$, where Y = $(CH_2)_2$, $(CH_2)_3$, or *cis*-CH=CH. The anion (X = Cl, Br, I, CH_3SO_3, NO_3, PF_6) had little effect upon activity. The R = R′ = phenyl complexes 1, 7, and 8 [Y = $(CH_2)_2$, $(CH_2)_3$, *cis*-CH=CH, X = Cl] were the most active against P388 leukemia, with an increase in lifespan ranging from 83 to 92% and were also active against M5076 sarcoma and B16 melanoma. Complexes with pyridyl or fluorophenyl substituents had reduced activities. For the latter, ^{19}F and ^{31}P NMR were used to verify the formation of bischelated gold(I) complexes in solution. The reduced activity of the complex with R = Et and R′ = Ph and inactivity with R = R′ = Et are discussed in terms of their increased reactivity as reducing agents. ^{31}P NMR studies show that $[Au^I(Et_2P(CH_2)_2PPh_2)_2]Cl$ readily reacts with serum, albumin, and Cu^{2+} ions to give oxidized ligand.

The linear two-coordinate triethylphosphine gold(I) complex auranofin (1-thio-β-D-glucopyranose-2,3,4,6-tetraacetato-*S*)(triethylphosphine)gold(I), "Ridaura", Smith Kline and French Laboratories) is an orally active antiarthritic agent[2] with in vitro antiproliferative effects against B16 melanoma cells and P388 leukemia cells as well as cultured human cancer cells.[3,4] It is active against intraperitoneally (ip) implanted P388 leukemia in mice,[5] but only when administered ip, and it is inactive in other tumor models.[6] This restricted range of activity may be related to facile ligand-exchange reactions which auranofin can undergo. In plasma and in cells the tetraacetyl-β-D-thioglucose ligand is readily displaced by other thiolate ligands, and the phosphine ligand can be released and undergo oxidation to the oxide.[7–9]

We have recently prepared stable four coordinate gold(I) diphosphine complexes.[10,11] These ionic, chelated, tetrahedral complexes exhibit a spectrum of chemical activity different from that of auranofin. For example, they do not readily react with thiols.[12] Moreover extensive testing of bis[1,2-bis(diphenylphosphino)ethane]gold(I) chloride, ($[Au(dppe)_2]Cl$, 1) showed that it was active in several tumor models.[12] Further interest in the tetrahedral complexes has arisen from the observation that the bridged digold complexes $[\mu\text{-}Ph_2P(CH_2)_nPPh_2]Au^I_2X_2$ (A, X = Cl or β-D thioglucose) readily undergo rearrangement reactions in the presence of thiols or blood plasma to give

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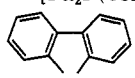
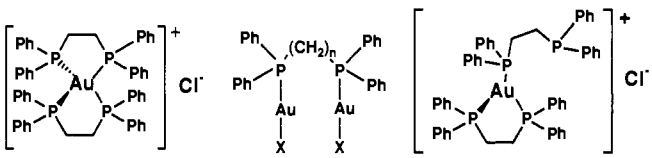
Table I. Tetrahedral Gold Complexes[a]

$[Au^I(R_2PYPR_2')_2]X$

complex	R, R'	Y	X	formula[b]	mp, °C	% yield	recryst sol.[c]	method
1	C_6H_5	$(CH_2)_2$	Cl	$C_{52}H_{48}AuClP_4 \cdot H_2O$	165–277	56, 70	A, B; E, C	A, E
2	C_6H_5	$(CH_2)_2$	Br	$C_{52}H_{48}AuBrP_4$[d]	182–188	73	C, D	B
3	C_6H_5	$(CH_2)_2$	I	$C_{52}H_{48}AuIP_4$	182–187	34	C, D	B
4	C_6H_5	$(CH_2)_2$	NO_3	$C_{52}H_{48}AuNO_3P_4 \cdot H_2O$	190–200	76	C, E	C
5	C_6H_5	$(CH_2)_2$	CH_3SO_3	$C_{53}H_{51}AuO_3P_4S$	209–210	50	C, F	D
6	C_6H_5	$(CH_2)_2$	$HO(CH_2)_2SO_3$	$C_{54}H_{53}AuO_4P_4S$[d]	199–201	41	C, F	D
7	C_6H_5	$(CH_2)_3$	Cl	$C_{54}H_{52}AuClP_4 \cdot H_2O$[d]	191–192	75	E	A, E
8	C_6H_5	*cis*-(CH=CH)	Cl	$C_{52}H_{44}AuClP_4$	226–250	88	E	E
9	3-F-C_6H_4	$(CH_2)_2$	Cl	$C_{52}H_{40}AuClF_8P_4 \cdot 0.5H_2O$	235–245	37	B, G	F
10	4-F-C_6H_4	$(CH_2)_2$	Cl	$C_{52}H_{40}AuClF_8P_4$	229–230	32	C, D	A
11	C_6H_5, CH_3CH_2	$(CH_2)_2$	Cl	$C_{36}H_{48}AuClP_4$[d]	170–200	90	H	A
12	2-C_5H_4N	$(CH_2)_2$	Cl	$C_{44}H_{40}AuClN_8P_4 \cdot H_2O$	257–258	48	D, H	A
13	4-C_5H_4N	$(CH_2)_2$	Cl	$C_{44}H_{40}AuClN_8P_4 \cdot 3HCl \cdot H_2O$	291–293	50	D, H	F
14	$[Ph_2P(CH_2)_2P(Ph)CH_2]_2$		Cl	$C_{42}H_{42}AuClP_4 \cdot 1.5H_2O$	241–242	33	D, H	A
15		$(CH_2)_2$	NO_3	$C_{52}H_{40}AuNO_3P_4$	290–292	26	C, D	G
16	CH_3CH_2	$(CH_2)_2$	PF_6	$C_{20}H_{48}AuF_6P_5$	240–245	83	I	C

[a] Compounds exhibited NMR spectra consistent with structures. [b] All compounds gave elemental analyses within ±0.4% of theoretical values except where noted. [c] Solvents: A = $CHCl_3$; B = toluene; C = H_2O; D = MeOH; E = acetone; F = *i*-PrOH; G = CH_2Cl_2; H = Et_2O; I = EtOH; J = hexane. [d] **2**, C: calcd, 58.17; found, 57.28; Br: calcd, 7.44; found 5.77; **6**, C: calcd, 58.81; found, 59.26; **7**, C: calcd, 60.83; found, 60.08; **11**, C: calcd, 51.65; found, 52.18.

monomeric tetrahedral bis(diphosphine) species.[13] Moreover, the activity of this series of complexes, and their diphosphines, against P388 leukemia is optimal for ligands which can form stable chelate rings (n = 2, 3, or *cis*-CH=CH).[14]



(1) (A) (B)

In this paper we report the effect of variations in chelate ring size, phosphine substituents, and counteranions of chelated bis(diphosphine)gold(I) complexes $[Au(R_2P(CH_2)_nPR'_2)_2]X$ (similar to **1**) on their cytotoxicity and activity against P388 leukemia, B16 melanoma, and M5076 reticulum cell sarcoma.

Firstly, variations in the counteranion were examined. If the active species were the chelated bis(diphosphine)gold(I) cation, then the anion would be expected to have little effect on activity. Secondly, variations were introduced which would affect the dynamics of chelate ring opening as depicted in B, thought to be a key factor in the mechanism of action:[15,16] fluorination of the phenyl rings to change P basicity, increasing the rigidity or length of the bridge between the two phosphorus centers in the bisphosphine ligand, and decreasing the lipophilicity of the diphosphine ligand (introducing high aqueous solubility).

Results

Gold Complexes. The tetrahedral Au(I) complexes studied in this paper were soluble in a wide range of solvents including $CHCl_3$, acetone, DMSO, and methanol. The phenyl-substituted complexes were only sparingly soluble in H_2O; the mixed phenyl–ethyl complex bis[1-(diethylphosphino)-2-(diphenylphosphino)ethane]gold(I) chloride $[Au^I(eppe)_2]Cl$, **11**) was an exception, being highly soluble in H_2O (>24 mM), as were the pyridyl complexes (**12**, **13**). These complexes are generally stable in solution for >24 h. In contrast, the bis[1,2-bis(diethylphosphino)ethane]gold(I) cation $[Au^I(depe)_2]^+$ required stabilization by a large counteranion. Decomposition into the annular digold complex $Au^I_2(depe)_2Cl_2$ and other products readily occurred.[11] PF_6^- complex **16** was found to be stable for at least 24 h in methanol/saline (1:1 v/v).

Several of these complexes exhibited ill-defined melting behavior (Table I). This was investigated in more detail for **1**, $[Au^I(dppe)_2]Cl$, a well-defined compound of known crystal structure.[10,17] Melting appeared to by a three-stage process, involving changes in crystal morphology and possibly loss of solvent of crystallization. The process was complicated further by oxidation of the ligand at slow rates of heating.

Table II. ^{19}F and ^{31}P NMR Chemical Shifts for 3- and 4-Fluoro-Substituted Diphenyldiphosphinoethane Ligands (P–P), Their Oxides, and Bridged Digold Dichloro and Bischelates

	δ/ppm				
substitn	P–P	P(O)–P	P(O)–P(O)	[(μP–P)-$Au^I_2Cl_2$]	$[Au^I(P–P)_2]Cl$
			^{19}F		
3-F	−112.3	−110.3 −111.9	−110.0	−108.1	−109.8
4-F	−112.2	−106.2 −111.4	−105.7	−104.2	−108.0
			^{31}P		
3-F	−11.5	31.4[a] −10.6	31.4	32.7	22.7
4-F	−14.7	33.0[b] −14.0	32.4	31.1	20.8

[a] J = 49 Hz. [b] J = 47 Hz.

NMR Studies of Fluorinated Complexes. Both ^{19}F and $^{31}P\{^1H\}$ NMR studies were performed on the bis(diphosphine)gold(I) complexes with 3- and 4-fluorophenyl

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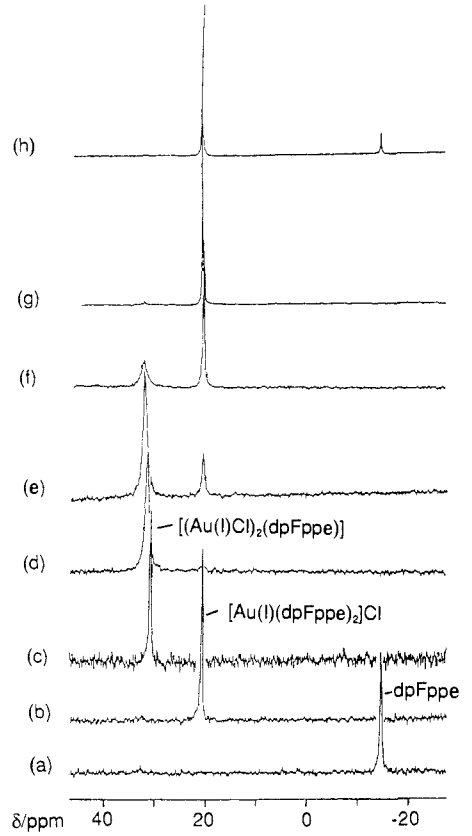

Figure 1. ^{19}F NMR spectra at 298 K of (a) 1,2-bis[bis(4-fluorophenyl)phosphino]ethane (dpFppe); (b) $[Au^{I}(dpFppe)_2]Cl$ (**10**); (c) $[(Au^{I}Cl)_2(dpFppe)]$; (d–h) as in c in the presence of 0.5, 1.0, 2.0, 3.0, or 4.0 molar equiv of dpFppe.

ligands (**9**, **10**) to confirm the presence of tetrahedral coordination geometry in solution. This was done by comparing spectra of free ligands with those of their bridged digold dichloro complexes and isolated bis(diphosphine)gold(I) complexes. Chemical shifts are listed in Table II. Formation of the latter was then monitored by titrating bridged complexes with excess diphosphine ligand. For both the 3- and 4-fluoro derivatives, both ^{19}F and $^{31}P\{^{1}H\}$ NMR spectra of these titrations show that the chelated bis(diphosphine) complex is present in solution even at low ratios of ligand to bridged complex (0.5:1). These $^{31}P\{^{1}H\}$ NMR titrations show the presence of a resonance for free ligand only when the Au/ligand ratio exceeds 1:2, i.e. when the formation of chelated complex is complete. At lower ratios these peaks were broadened by exchange.

As an example, $^{31}P\{^{1}H\}$ NMR spectra of the titration of the 4-fluorophenyl phosphine ligand are shown in Figure 1. This exactly parallels the behavior observed previously for unsubstituted diphenylphosphine ligands, such as dppe,[10,11] which have already been shown to form tetrahedral complexes.[10,17]

It was evident from NMR spectra that fluoro-substituted ligands were more susceptible to oxidation (in air) than unsubstituted analogues. Observed chemical shifts are included in Table II.

Effect on Tumor Cells in Vitro. These Au(I) diphosphine complexes were highly cytotoxic to B16 cells in vitro. Their IC_{50} concentrations are given in Table III. Ethyl-substituted complex **16** inhibited the cloning efficiency at a concentration almost 4-fold greater than complexes containing phenyl substituents. Cytotoxic potencies of **1** and **7** to B16 cells were not markedly influenced by the presence of fetal calf serum (fcs) in the incubation medium. For these complexes IC_{50} values were less than 2-fold lower in medium not containing serum as compared to drug exposure in medium containing 10% fcs.

Table III. Activity of Tetrahedral Bis(diphosphine)gold(I) Complexes in Mice Bearing Ip P388 Leukemia and in Vitro Cytotoxic Activity against B16 Melanoma Cells

complex	MTD,[a] μmol/kg per day	% ILS[b]	IC_{50},[c] μM
1	3	83 ± 25, n = 33	4.5
2	2	70, 83	*d*
3	2	60, 150	*d*
4	3	90 ± 17, n = 5	4
5	2	81 ± 10, n = 3	*d*
6	2	55, 78	*d*
7	3	89 ± 28, n = 3	0.6
8	2	92 ± 26, n = 7	2
9	10	45, 55	*d*
10	3	55, 50	*d*
11	4	54 ± 16, n = 4	5
12	8	75 ± 5, n = 5	*d*
13	6	inactive	*d*
14	3	40, 65	*d*
15[e]	4	61, 33	*d*
16	5	40, 30	17
cisplatin	7	125 ± 38, n = 66	*d*

[a] Maximally tolerated for $B6D2F_1$ mice on an ip every day for 5 days regimen. [b] Maximum increase in lifespan produced in mice bearing ip P388 leukemia; figures separated by commas represent data generated in separate experiments. A drug is considered to be active in this model if it produces >30% ILS. [c] Concentration which inhibits cloning efficiency of B16 melanoma cells by 50% on a 2-h exposure. [d] Not determined. [e] The bridged digold complex $ClAu^{I}(P\text{–}P)Au^{I}Cl$ of this ligand (**15a**) gave an ILS of 55% at an MTD of 5 μmol/kg per day.

In Vivo Antitumor Activity. In Table III, antitumor activities of the series of Au(I) diphosphine complexes against ip P388 leukemia in mice are compared. As described previously,[12] $[Au^{I}(dppe)_2]Cl$ (**1**) exhibits reproducibly high activity against this ip tumor when administered ip: an average of 83% increase in lifespan (ILS) at its maximally tolerated dose (MTD) (3 μmol/kg per day × 5) over 33 separate dose–response studies. The prolongation of lifespan at the MTD corresponded to a net cell kill of ca. 2 log units; i.e. at the end of the 5 day course of treatment tumor cell burden was reduced by 99% compared to the start of therapy. As shown in Table III, activity and potency were retained when the counteranion was varied from Cl^- to Br^-, I^-, NO_3^-, $CH_3SO_3^-$, or $HO(CH_2)_2SO_3^-$. Activity comparable to that of **1** was retained when the $(CH_2)_2$ bridge was changed to $(CH_2)_3$ (**7**) or *cis*-CH=CH (**8**), but with ethyl and phenyl substituents as in **11**, the fused phenyl ring as in **15**, or the "tetraphos" complex **14**, the activity was reduced with respect to that of **1** at a comparable MTD.

The 3- and 4-fluorophenyl-substituted complexes **9** and **10** exhibited marginal activity (45–55%) and the complex containing only ethyl substituents (**16**) showed little activity in this tumor model. Curiously, 2-pyridyl complex **12** exhibited good activity, whereas 4-pyridyl complex **13** was inactive.

Some of these Au(I) complexes were also evaluated against ip M5076 reticulum cell sarcoma and B16 melanoma tumors (Table IV). In general, the phenyl-substituted complexes that exhibited good activity against ip P388 leukemia were active in these models also at an MTD of 2 μmol/kg per day. 2-Pyridyl and 4-pyridyl complexes **12** and **13** were inactive against M5076 reticulum cell sarcoma, but **12** was active against B16 melanoma.

Reactivity in Model Systems

Serum and Albumin. These reactions were studied because it is known that serum can reduce the cytotoxicity

Table IV. Activity of Tetrahedral Bis(diphosphine)gold(I) Complexes in Mice Bearing M5076 Reticulum Cell Sarcoma or Ip B16 Melanoma

complex	MTD,[a] μmol/kg per day	% ILS[b] M5076	% ILS[b] B16
1	2	57 ± 15, n = 5	38 ± 9, n = 5
3	2	52	not tested
7	2	116	34 ± 6, n = 3
8	2	69	35
11	2	46	inactive
12	8	inactive	43
13	4	inactive	not tested
14	2	inactive	not tested
16	4	inactive	not tested
cisplatin	5	103 ± 11, n = 14	75 ± 15, n = 7

[a] Maximally tolerated dose for B6D2F$_1$ mice on an every day ip for 10 days regimen. [b] Maximum increase in lifespan produced in mice bearing ip implanted tumor; figures separated by a comma represent data generated in separate experiments. A drug is considered to be active against either tumor if it produces >25% ILS.

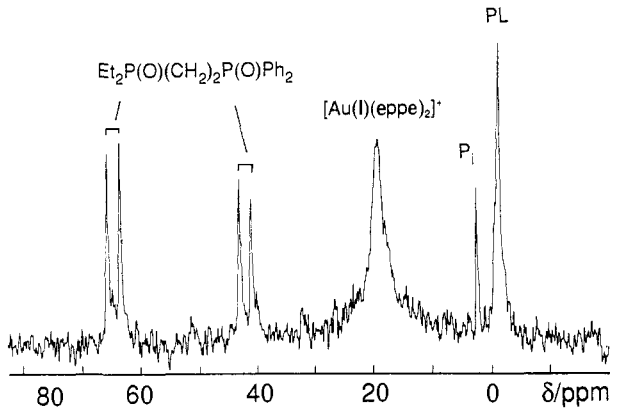

Figure 2. $^{31}P[^1H]$ NMR spectrum (24 MHz) of bovine serum containing 3.5 mM $[Au^I(eppe)_2]Cl$ (11) after an average reaction period of 18 h (45° pulses, 2.5-s pulse delay). Assignments: PL, phospholipids; P_i, inorganic phosphate.

of some gold phosphine complexes.[6,8,12]

Figure 2 shows the $^{31}P\{^1H\}$ spectrum of bovine serum after an average incubation period of 18 h with 11 (3.5 mM). In addition to the phospholipid and P_i resonances, the spectrum contains a very broad peak at 19.8 ppm ($\Delta\nu_{1/2}$ = 60 Hz) assignable to $[Au^I(eppe)_2]^+$. The AA'BB' spin system gives rise to a deceptively simple singlet.[11] The pair of doublet resonances at 63.9 ppm and 42.2 ppm are assignable to the phosphine oxide $Ph_2P(O)(CH_2)_2P(O)PEt_2$ by comparison with the spectrum of the authentic species. In a control experiment only a trace of the dioxide was present in a 2 day old solution of 11 in D_2O.

After an average of 8 h of incubation of a solution of bovine serum albumin (1 mM) and 0.1 M phosphate buffer (pH 7) with 11 (4 mM), the $^{31}P\{^1H\}$ spectrum contained a broad resonance at 20.2 ppm ($\Delta\nu_{1/2}$ = 60 Hz) attributable to $[Au^I(eppe)_2]^+$ and a pair of doublets at 63.9 and 42.3 ppm due to $Ph_2P(O)(CH_2)_2P(O)Et_2$. After 4 days the solution had thickened, peaks for the dioxide had increased in intensity, and the resonance for $[Au^I(eppe)_2]^+$ had broadened further so that it was barely distinguishable from the base line. After a further day the solution had solidified, suggesting that complete protein denaturation had occurred.

Reactions with Cu(II) Ions. Cu(II) ions potentiate the cytotoxicity of some diphosphine ligands[18] and react with these and with $[Au(dppe)_2]Cl$ to form Cu(I) diphosphine complexes.[12] We therefore examined the question as to whether or not similar reactions could occur for the water-soluble complex 11. When $CuSO_4$ (1 mol equiv) was added to a solution of 11 in D_2O (24 mM), a white precipitate formed immediately. The ^{31}P NMR spectrum of the solution suggested that it contained the annular complex $[Au^I_2(eppe)_2]^{2+}$ (δ 39.3, characteristic splitting pattern for AA'BB' spin system[11]), together with minor amounts of unidentified species (δ 78.1, 68.9, 63.0, and 49.1). The precipitate was probably a Cu(I) complex, as this is consistent with its color (white), the high Au/Cu ratio in the solution (5:1 as determined by atomic absorption spectrophotometry), and ^{31}P NMR spectrum (in $CDCl_3$, broad peak at −13.3 ppm, a shift similar to that of the major insoluble product from reaction of $[Au^I(dppe)_2]Cl$ (1) with $CuSO_4$[12]). There were also minor peaks at 50.5, 40.3, and 31.5 ppm.

The reaction of 11 with Cu(II) was detectable at much lower concentrations by electronic absorption spectroscopy. The broad absorption band of $[Au^I(eppe)_2]^+$ (31 μM) with initial λ_{max} of 281 nm shifted to 277 nm on addition of 0.2 molar equiv of Cu(II) sharpened, and reached maximum intensity at a 1:1 [Au]/[Cu] molar ratio.

Discussion

Within the series of tetrahedral, chelated Au(I) diphosphines $[Au^I(R_2P(CH_2)_nPR_2')_2]X$, the high antitumor activity (high ILS) and potency (low MTD and IC_{50}) shown by 1 (n = 2, R = R' = Ph, X = Cl) are also observed for all complexes containing 5- or 6-membered chelated rings (n = 2, 3, or *cis*-CH=CH) which have phenyl-substituted phosphines (R, R'). Changing the anion X had little effect on the antitumor activity, as might be expected since these complexes would dissociate in highly polar media[10] to give $[Au^I(P-P)_2]^+$ and X^- ions. However, ion pairing, especially with endogenous Cl^-, could be important in determining the transport of these cations across membranes. It is noteworthy that in the crystal structure of $[Au^I(dppe)_2]Cl$ (1) there appears to be a close association of chloride with the hydrophobic surface of the $[Au^I(dppe)_2]^+$ cation.[15-17]

Replacement of phenyl substituents by ethyls reduced both the cytotoxic potency and activity against P388 leukemia. $[Au^I(depe)_2]PF_6$ (16) is inactive against ip P388 leukemia and is 4 times less toxic to B16 melanoma cells in vitro than 1. A similar trend has been observed for their ligands.[14] In contrast to dppe, $Et_2P(CH_2)_2PEt_2$ (depe) exhibits no antitumor activity and is relatively nontoxic to mice.[14] This can be rationalized by the ease with which depe undergoes oxidation in aqueous media in comparison with phenyl-substituted diphosphines, which in turn is related to the difference in autoxidation pathways for alkyl and aryl phosphines.[19] Although coordination to Au(I) can protect the phosphine ligand from oxidation, $[Au^I(depe)_2]^+$ has a lower thermodynamic stability than 1 and readily decomposes in the presence of Cl^- ions to give the annular complex $Au^I_2(depe)_2Cl_2$ together with oxidized depe.[10,11] Studies reported here show that the phenyl and ethyl substituted complex 11 reacted slowly with components of bovine serum to give oxidized ligand. It reacted similarly with serum albumin, probably by cleavage of the disulfide bonds. We have previously observed similar reactions between albumin and $[Au^I(PEt_3)_2]^+$.[20] Alkyl phosphines themselves cleave disulfide bonds.[16] The re-

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activity of these water-soluble ethyl-substituted complexes may be contrasted with that of 1, which has a low reactivity toward disulfide bonds in model systems and in plasma,[12] although it appears to undergo a minor side reaction with lipoproteins in plasma.[21] It is likely that ethyl-substituted complexes will take part in oxidative side reactions in vivo more readily than the phenyl-substituted complexes, and this may account for their reduced antitumor activities. Direct comparisons between 11 and 1 in model reactions are made difficult by differing solubilities of the two complexes: only 11 is highly soluble in aqueous solvents. This marked difference in lipophilicity may play a major role in partitioning of the complex in plasma, its transfer into cells, and consequently its cytotoxicity.

The introduction of fluoro substituents into phenyl rings caused some decrease in activity against P388 leukemia and a significant loss of potency (MTD) for the 3-substituted (9) compared to the 4-substituted derivative (10). The ^{19}F and ^{31}P NMR titrations of bridged digold complexes containing fluorinated diphenyldiphosphine ligands with excess ligand confirmed that the tetrahedral Au(I) chelates (9 and 10) were kinetically and thermodynamically stable in solution. Resonances for these complexes were present at low Au/ligand ratios, and exchange with free ligand was relatively slow on the NMR time scale. A similar pattern was observed during the ^{31}P NMR titration of $[Au^I_2(dppe)Cl_2]$ with dppe,[10,14] and isolated complexes containing $[Au^I(dppe)_2]^+$ have been shown by X-ray crystallography to have flattened tetrahedral geometry.[10,17]

Complexes with fluoro-substituted ligands were noticeably less stable to autoxidation. The significant loss of potency (MTD) of the 3-substituted compared to the 4-substituted derivative could be related to differences in rates of autoxidation. However, other factors such as differences in the basicity of these ligands and steric interactions on the surface of the ion could be important.

The complex with 2-pyridyl substituents (12) had comparable antitumor activity to 1 in P388 leukemia, but was less potent (i.e. a higher dose was required to effect the observed ILS). Curiously, 4-pyridyl complex 13 was inactive against P388 leukemia although slightly more toxic to mice. A similar trend was observed for bridged digold diphosphine complexes of these ligands.[14] Further reactivity and partitioning data for these compounds are required in order to rationalize these trends in activity.

The four coordinate Au(I) complexes with phenyl-substituted diphosphines were also active against the ip tumors M5076 reticulum cell sarcoma and B16 melanoma (Table IV). This broader range of antitumor activity compared to that of the two-coordinate Au(I) complex auranofin[6] may be related to their differing chemical reactivities.

Auranofin undergoes facile thiol-exchange reactions, which may be important in its antiarthritic activity, allowing transfer of gold onto proteins and into cells, and perhaps the displacement and mobilization of Cu(I).[7-9] The chelated bis(diphosphino) complexes are stable to thiol exchange, and complex 1 is inactive in the carageenan assay for antiinflammatory activity.[24] Similarly, when the incubation medium contains fetal calf serum (fcs), the cytotoxicity of auranofin to B16 melanoma cells is reduced by 1 order of magnitude, due to gold binding to serum proteins,[6] whereas the in vitro cytotoxicities of complexes 1 and 7 reported here were not markedly affected by fcs. We have observed the same pattern of behavior with bischelated Ag(I) complexes, which undergo ligand exchange more readily than Au(I) analogues.[22] This was reflected in a reduced cytotoxicity of these Ag(I) complexes when the incubation medium contained fcs.[22]

Cu(II) ions may play a role in the cytotoxicity of diphosphine ligands and their metal complexes.[16,23] $[Au^I(dppe)_2]Cl$ (1) reacts with Cu(II) to give the annular complex $[Au^I_2(dppe)_2]^{2+}$ and a Cu(I) dppe complex.[12] The present studies have shown that the mixed phenyl–ethyl analogue 11 reacts in a similar manner with Cu(II) ions to give the annular complex $[Au^I_2(eppe)_2]^{2+}$ and a Cu(I) eppe complex. It is possible that the formation of a Cu(I) diphosphine complex in vivo may be involved in the cytotoxic mechanism. Cu(II) ions can potentiate the cytotoxicity of phosphine ligands,[18] which are themselves active but less potent.[14]

We have reported previously[22,23] that Ag(I) and Cu(I) analogues of the tetrahedral chelated Au(I) diphosphine complexes exhibit antitumor activities comparable to those of 1 against ip P388 leukemia in mice and are equally potent. Similar decreases in activity were observed on changing phenyl substituents for ethyls. This led us to propose[16] that the cytotoxic mechanism of these complexes depends on a subtle combination of thermodynamic and kinetic stabilities, so that the metal protects the ligand from unfavorable oxidation reactions and delivers it to cells, yet there is sufficient lability in the metal–phosphorus bond that the phosphine is able to react at the critical target sites.

Cardiac, hepatic, and vascular toxicities of $[Au^I(dppe)_2]^+$ complexes have so far proved to be limiting factors in preclinical trials.[25] Studies on hepatocytes[26] have suggested that cell injury may be caused by disruption of mitochondrial function. $[Au^I(dppe)_2]^+$ induces a rapid, dose-related collapse of the inner mitochondrial membrane electrochemical potential, resulting in an efflux of calcium via reversal of the ruthenium red-sensitive calcium uniporter and an uncoupling of mitochondrial oxidative phosphorylation. On the other hand, the primary cytotoxic lesion may involve the formation of DNA–protein cross-links.[27] It may therefore be possible to separate the mechanisms of antitumor action and those of side-effects to aid the further evaluation of bisphosphine complexes.

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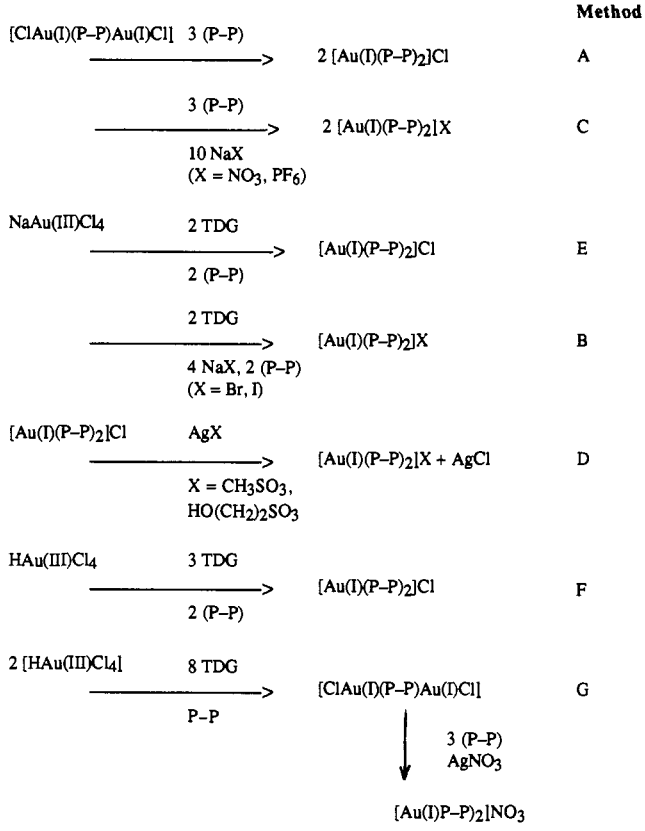
Scheme I.[a] Outline of Syntheses of the Tetrahedral Au(I) Complexes by Methods A–G



[a] TDG is thiodiglycol.

Although they are less active than cisplatin in the tumor models studied here, the difference in mechanism of action from cisplatin (for which the primary lesions are intrastrand DNA crosslinks[28]) and the improvement in ILS on concurrent administration with cisplatin[12] make these diphosphine complexes potentially valuable for combination therapy.

Experimental Section

Melting points were determined in open glass capillaries with a Thomas-Hoover melting point apparatus, with a Kofler melting point hot stage made by Reichart, or with a Mettler FP 82 hot stage and an Olympus CH microscope. Elemental analyses were carried out by the Microanalysis Department, University College, London, UK or by Edith Reich, Analytical Chemistry Department, SK&F Laboratories.

NMR Measurements. $^{31}P\{^1H\}$ NMR spectra were recorded on a JEOL FX-60 spectrometer (24.2 MHz) in 10-mm tubes or on a Bruker WM200 spectrometer (81.0 MHz) in 15-mm tubes. The external reference was 85% H_3PO_4. These shifts can be converted to the reference we have used previously [85% H_3PO_4 in D_2O (85:15 v/v)] by adding 1.9 ppm. ^{19}F NMR spectra (188.3 MHz, not proton decoupled) were recorded on the WM200 spectrometer in 10-mm tubes and were referenced to external $CFCl_3$. For the titrations, aliquots of dissolved ligands were added to solutions of bridged complexes. Solvents were $CDCl_3$ (^{19}F) or $CDCl_3/CHCl_3$ (1:1 v/v ^{31}P). Initial concentrations were between 1.5 and 2.2 mM.

Preparation of Complexes. Diphosphine ligands for complexes 1–8, 11, 14, and 16 were purchased from Strem Chemicals Inc.; those for complexes **9**, **10**, **12**, **13**, and **15** were synthesized by published methods.[14] $NaAuCl_4$ and $HAuCl_4$ were obtained from Johnson Matthey plc, and thiodiglycol was from Sigma. Bridged digold(I) complexes ClAu(P–P)AuCl were prepared as described previously.[11,14]

An outline of these syntheses is shown in Scheme I. In general, synthetic routes to the tetrahedral complexes involved either reaction of the bridged digold(I) complex ClAu(P–P)AuCl with 3 mol equiv of ligand (P–P), (method A) or the addition of 2 molar equiv of ligand (P–P) to a solution of Au(I) generated in situ by reduction of Au(III) with thiodiglycol (methods E and F).[10,11] Exchange of Cl^- for other anions was accomplished either by addition of excess NaX (methods B and C) or AgX (methods D and G), with AgCl precipitation prior to workup.

Method A. Bis[1,2-bis(diphenylphosphino)ethane]gold(I) Chloride Hydrate (1). [1,2-Bis(diphenylphosphino)ethane]-bis[chlorogold(I)] (**15a**, 2.16 g, 2.5 mmol) was added in portions as a powder to a suspension of dppe (3.15 g, 7.9 mmol) in acetone (200 mL) kept at ambient temperature. A clear, colorless soltuion resulted. After stirring for 18 h the solvent was removed at reduced pressure until crystals appeared. Toluene was added and the mixture was cooled. Resulting crystals (several crops) were collected and dissolved in $CHCl_3$, the solution was treated with activated carbon and filtered, toluene was added (100 mL), and the solution was cooled to 0 °C. These crystals were collected and dried in vacuo to give 3.0 g (56%) of **1**; mp 264–277 °C (shrinks at ca. 170 °C). Anal. ($C_{52}H_{48}AuClP_4 \cdot H_2O$) C, H, P, Cl.

Method B. Bis[1,2-bis(diphenylphosphino)ethane]gold(I) Bromide (2). Thiodiglycol (0.33 g, 2.70 mmol) in acetone (2 mL) was added to a solution of $NaAu^{III}Cl_4 \cdot H_2O$ (0.5 g, 1.35 mmol) and NaBr (0.56 g, 5.4 mmol) in H_2O (5 mL). The solution was cooled to 0 °C and a solution of dppe (1.07 g, 2.70 mmol) in acetone (30 mL) was added. After 30 min the solvent was concentrated to 10 mL at ambient temperature, H_2O was added, and the precipitate was collected and recrystallized from H_2O/acetone to give 1.06 g (73%) of **2**; mp 182–188 °C. Anal. ($C_{52}H_{48}AuBrP_4$) C, H, P. Iodide complex **3** was prepared similarly, except 4 molar equiv of NaI dissolved in H_2O were added after the addition of dppe.

Method C. Bis[1,2-bis(diphenylphosphino)ethane]gold(I) Nitrate (4). $NaNO_3$ (0.4 g, 4.6 mmol) in H_2O (10 mL) was added to a clear solution of [1,2-bis(diphenylphosphino)ethane]bis-[chlorogold(I)] (**15a**, 0.4 g, 0.46 mmol) and dppe (0.55 g 1.39 mmol) in acetone (25 mL) kept at ambient temperature. After 30 min, H_2O (10 mL) was added and the solvent volume was reduced via slow evaporation at ambient temperature. The resulting product **4** was collected and dried in vacuo to give 0.6 g (61%) of **4**; mp 190–200 °C. Anal. ($C_{52}H_{48}AuNO_3P_4$) C, H, N, P.

Method D. Bis[1,2-bis(diphenylphosphino)ethane]gold(I) Methanesulfonate (5). A solution of $AgSO_3CH_3$ (0.19 g, 0.94 mmol) in 3:1 EtOH/H_2O (30 mL) was added to a solution of **1** (1.0 g, 0.97 mmol) in 3:1 EtOH/H_2O (50 mL) kept in the dark at ambient temperature. After 24 h the mixture was filtered through diatomaceous earth and the solvent volume was reduced in vacuo. The resulting precipitate was collected, washed with H_2O (50 mL) and dried under vacuum to give 0.67 g (63%) of **5**; mp 195–200 °C. Recrystallization from H_2O/*i*-PrOH gave 0.55 g (50%) of **5**; mp 209–210 °C. Anal. ($C_{53}H_{51}AuO_3P_4S$) C, H.

Method E. Bis[*cis*-1,2-bis(diphenylphosphino)ethene]-gold(I) Chloride (8). Thiodiglycol (0.33 g, 2.70 mmol) in 2.5:1 H_2O/acetone (7 mL) was added to $NaAu^{III}Cl_4 \cdot H_2O$ (0.5 g, 1.35 mmol) in H_2O (5 mL) at 0 °C. A solution of *cis*-dppey (1.07 g, 2.7 mmol) in acetone (25 mL) was added and the solvent volume was reduced to about 10 mL and cooled. The resulting crystals were collected, washed with H_2O, and dried in vacuo to give 1.2 g (88%) of **8**; mp 226–250 °C. Anal. ($C_{52}H_{44}AuClP_4$) C, H, P, Cl.

Method F. Bis[1,2-bis[bis(3-fluorophenyl)phosphino]-ethane]gold(I) Chloride (9). Thiodiglycol (1.03 g, 8.7 mmol) was added to a solution of $HAu^{III}Cl_4 \cdot H_2O$ (1.196 g, 2.9 mmol) in CH_3OH (5 mL)/H_2O (20 mL). Upon turning colorless, dmfppe (2.73 g, 5.8 mmol) in acetone (25 mL) was added and the mixture was kept at 0 °C for 18 h. The liquid portion of the reaction mixture was decanted to obtain the semisolid material which was triturated with ether. The resulting solid was collected and recrystallized from CH_2Cl_2/toluene to give 1.27 g (37%) of **9**; mp 235–245 °C. Anal. ($C_{52}H_{40}AuClF_8P_4 \cdot 0.5H_2O$) C, H.

Method G. Bis[1,2-bis(5*H*-dibenzophospholyl)ethane]-gold(I) Nitrate (15). First, the bridged complex μ-[1,2-bis(5*H*-dibenzophospholyl)ethane]bis[chlorogold(I)] was prepared as follows: thiodiglycol (3.22 g, 26.4 mmol) was added to $HAu^{III}Cl_4 \cdot 4H_2O$ (2.72 g 6.6 mmol) in H_2O (4 mL) kept at 0 °C. After

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becoming colorless, acetone (16 mL) was added and the mixture was warmed to ambient temperature. A solution of 1,2-bis(dibenzophospholyl-1)ethane[29] (1.18 g, 3 mmol) in warm acetone (100 mL) was added (immediate precipitate) and the mixture was stirred for 1 h at ambient temperature. The solid was collected, washed with 1:1 acetone/H_2O, and dried in vacuo to give 2.05 g (79%) of **15a**; mp > 300 °C. An analytical sample was obtained as needles from DMF; mp 325–350 °C dec. Anal. Calcd for $C_{26}H_{20}Au_2Cl_2P_2$: C, 36.34; H, 2.35. Found: C, 37.03; H, 2.50.

A suspension of 1,2-bis(dibenzophospholyl-1)ethane (0.47 g 1.2 mmol) in CH_3OH (80 mL) was added to a suspension of **15a** (0.34 g 0.4 mmol) in CH_3OH (40 mL). After 2 h (solution still slightly cloudy) $AgNO_3$ (0.14 g, 0.85 mmol) in CH_3OH (20 mL) was added and the mixture was stirred for 30 min. The precipitate was collected, the filtrate volume was reduced to 40 mL and H_2O (40 mL) was added. After cooling, the pale yellow, microcrystalline solid was removed, washed with H_2O, and dried in vacuo at 80 °C to give 0.25 g (60%) of **15**; mp 283–295 °C. Recrystallization from CH_3OH/CH_2Cl_2/toluene (1:1:5) gave an analytical sample; mp 292–294 °C (darkens 280 °C). Anal. ($C_{52}H_{40}AuNO_3P_4$) C, H, N.

Biological Evaluation. One million P388 leukemia cells were implanted intraperitoneally (ip) in $B6D2F_1$ mice. Twenty-four hours later animals were randomized into groups of six and housed in shoebox cages. All complexes tested were purified as described for the analytical samples. Gold complexes were dissolved in a minimal volume of either *N,N*-dimethylacetamide (DMA) or ethanol. An equal volume of saline was added; if the drug precipitated, an equal volume of Cremophor-EL (polyethoxylated castor oil, Sigma Chemical Co.) was added and then saline was added such that the desired dose was delivered in 0.5 mL. Formulations were prepared immediately prior to injection. These compounds were administered ip on days 1–5 (i.e. treatment was initiated 24 h after tumor innoculation) at five logarithmically spaced dosage levels to identify the maximally tolerated dose (MTD) and the level of antitumor activity produced at this dose. Each experiment included three groups of six animals as nontreated controls, and animals treated with a positive control, cisplatin (Sigma Chemical Co.), at two dose levels. Animals were monitored daily for mortality and experiments were terminated after 45 days. The end point was median survival time (MST) and increase in lifespan (ILS), which is the percentage of increase in MST relative to nontreated controls. Nontreated controls innoculated ip with 10^6 P388 leukemia cells generally survived for a median of 9–11 days. In 66 experiments, cisplatin 7 μmol/kg per day produced 125 ± 36% ILS. In all experiments cisplatin was active, giving ILS values of >50%.

Reticulum cell sarcoma M5076 and B16 melanoma cells were maintained by serial transplantation in syngenic mice and were implanted ip for drug evaluation. All tumors were obtained from the National Cancer Institute tumor bank at Frederick Cancer Research Center, Frederick, MD.

For evaluation in the slower growing solid tumor models, compounds formulated as described above were administered ip daily for 10 days beginning 1 day after tumor implantation. These compounds were administered in each tumor model at five logarithmically spaced dosage levels which encompassed the maximally tolerated dose (MTD). Activity was assessed by prolongation of median lifespan.

Cytotoxicity was determined in a clonogenic assay using B16 melanoma cells grown in monolayer culture as previously described.[12]

Reactions of $[Au^1(eppe)_2]Cl$ (11) with Bovine Serum and Albumin. Complex **11** (5.1 mg, 6 μmol) in H_2O (250 μL) was added to bovine serum (1.5 mL, Wellcome Laboratories, reconstituted in D_2O/H_2O 1:1). The final concentration of the complex was 3.5 mM. The resulting $^{31}P\{^1H\}$ NMR spectrum was acquired for a period of 36 h (average reaction time 18 h).

Complex **11** (5.2 mg, 6.3 μmol) in D_2O (0.5 mL) was added to a solution of bovine serum albumin (Sigma Chemical Co.) (84.4 mg, ca. 1.3 μmol) in 0.1 M phosphate buffer, pH 7 (1 mL), giving a final complex concentration of 4.2 mM. A white precipitate began to form, which cleared with gentle stirring. The $^{31}P[^1H]$ NMR spectrum was recorded over a period of 16 h and again after standing for 4 days at room temperature.

Reactions with Cu(II). Copper(II) sulfate pentahydrate (10.1 mg, 0.04 mmol) dissolved in H_2O (0.2 mL) was added to a solution of **11** (33.7 mg, 0.04 mmol) in D_2O (1.5 mL). A fine, white precipitate formed, which was removed by centrifugation and decantation and redissolved in $CDCl_3$. The $^{31}P\{^1H\}$ NMR spectra of both supernatant and redissolved precipitate were recorded. The reaction was also followed by electronic absorption spectroscopy (Perkin Elmer 554, 1-cm pathlength cells, 298 K). Stock solutions of **11** (62 μM) and $CuSO_4 \cdot 5H_2O$ (196 μM) were made up in 50 mM phosphate buffer pH 7 and aliquots of these were mixed together so that the concentration of the gold complex was constant at 31 μM and the [Au]/[Cu] ratios were 1:0, 1:0.2, 1:0.4, 1:0.6, 1:0.8, 1:1, or 1:2. Spectra were recorded over the range 200–450 nm after 5 min of reaction time.

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(30) Abbreviations: dppe, 1,2-bis(diphenylphosphino)ethane; depe, 1,2-bis(diethylphosphino)ethane; eppe, 1-(diethylphosphino)-2-(diphenylphosphino)ethane; *cis*-dppey, *cis*-1,2-bis(diphenylphosphino)ethene; dmFppe, 1,2-bis[bis(3-fluorophenyl)phosphino]ethane; dpFppe, 1,2-bis[bis(4-fluorophenyl)phosphino]ethane; TDG, thiodiglycol 2,2′-thiobis(ethanol); MTD, maximally tolerated dose; MST, median survival time; ILS, increase in lifespan.