Anti-tumor Activity of Two Binuclear Gold(I) Complexes with Bridging Dithiolate Ligands

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Abstract

Two new binuclear complexes of gold with bridging dithiolate ligands were synthesized and characterized: $(\phi_3PAu)_2(\mu\text{-DTE})$ and $(Et_3PAu)_2(\mu\text{-DMTA})$; H_2DTE = dithioerylthritol and H_2DMTA = 2,5-dimercapto-1-thia-3,4-diazole. These compounds and three gold compounds with antiarthritic activity (gold sodium thiomalate, gold thioglucose and Et_3PAuCl) were tested for antitumor activity using the Ehrlich–Ascites tumor cell in mice. Only $(\phi_3PAu)_2(\mu\text{-DTE})$ showed significant activity.

Introduction**

Gold, lithium and platinum, are the bases for clinically used chemotherapeutic regimens in the treatment of rheumatoid arthritis [1], manic depressive psychosis [2] and cancer [3], respectively. The success of *cis*-platin in the treatment of testicular, ovarian and other cancers has prompted considerable interest in the possibility that complexes of other metals may have significant anti-tumor activity. Ruthenium [4] and copper [5] have been investigated extensively. Gold, because it has immunoregulatory properties [6] should be investigated more extensively.

The novel anti-arthritic agent auranofin, (2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-S-)triethylphosphine gold(I), is active against He–La cells and P-388 leukemia in mice [7, 8], but not against other tumors [9]. Other trialkylphosphine gold(I) complexes are active and the potency is dependent on the phosphine moiety and maximized by use of a thiosugar as the second ligand [10].

Therefore we have undertaken an effort to synthesize a number of gold derivatives related to known anti-tumor and anti-arthritic complexes and to test them against the Ehrlich Ascites tumor in mice. The syntheses and anti-tumor activities of two binuclear gold(I) compounds are reported here.

Experimental

Analyses

CHN analyses were performed by Mr. Keith Krumnow of the UWM Chemistry Department staff. Gold analysis was performed on an IL 357 flame atomic absorption spectrometer using dilutions of Spex $KAu(CN)_2$ in 2% KCN solution as the standard.

Spectra

IR spectra were obtained on a Nicolet MX-10 Spectrometer. ^1H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker WP250 instrument using TMS and external H_3PO_4 as the standards.

Reagents

Et_3PAuCl and AuSTm (Aldrich Chemical Co.) were generously provided by Smith Kline and French Laboratories; K_2DTMA by Vanchem, Ltd. H_2DTE and AuSTg were purchased from Sigma Biochemical (St. Louis, Mo, USA).

$(Ph_3PAu)_2(\mu\text{-DTE})$

Ph_3PAuCl (520 mg, 1.05 mmol) was dissolved in 5 ml of $CHCl_3$ and EtOH. 81 mg (0.52 ml) of dithioerythritol (Sigma) was added with stirring. A very small amount of white precipitate appeared and was filtered. The filtrate was strongly acidic, indicating that reaction had occurred, and was taken up to pH = 5 with NaOH (0.1 M). The solution was evaporated to dryness (amber oil), 50 ml of ice-cold water were added twice and the aqueous portion was discarded. The oil was kept over P_4O_{10} for 2

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**AuSTg = aurothioglucose; AuSTm = gold sodium thiomalate; C = control; DMSO = dimethylsulfoxide; H_2DTE = dithioerythritol; K_2DMTA = 2,5-dimercapto-1,3,4-thiadiazole, dipotassium salt; T = treated.

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days, then attached to the vacuum line to yield an efflorescent solid which was kept under nitrogen. *Anal.* Calc. for $C_{40}H_{38}Au_2P_2O_2S_2$: C, 44.87; H, 3.55. Found: C, 45.32; H, 3.36%.

$(Et_3PAu)_2(\mu$-DMTA)

Et_3PAuCl (670 mg, 1.9 mmol) dissolved in MeOH was added under stirring to an ice-cold solution of 2,5-dimercapto-1,3,4-thiadiazole dipotassium salt (216 mg, 0.96 mmol) in methanol. The solution was left stirring until room temperature was reached. A small amount of oil separated at the bottom of the flask, the supernatant solution was separated and after addition of anhydrous ether (1 volume) an opalescence appeared. The solution was kept overnight at −20 °C and a white ppt, identified as KCl, was collected. After 2 days at −20 °C, a larger amount of solid was collected and dried *in vacuo* over P_4O_{10}. *Anal.* Calc. for $C_{10}H_{30}Au_2N_2P_2S_3$: C, 21.60; H, 3.85; N, 3.60. Found: C, 21.47; H, 3.17; N, 3.67%.

Anti-tumor Testing

White Swiss ACR mice obtained from King Animal Laboratories, Madison, Wis., were housed in a temperature/humidity controlled animal room with 12 h light-dark cycle and fed Purina rodent chow and distilled water *ad libitum*. Six (or seven) mice were used for each experimental or control group. Ehrlich Ascites tumor cells (10^6) were injected into the mice on day 0. The drugs, dissolved in 0.1 ml of saline solution or DMSO/H_2O (1/1), were injected on days 1 through 7. The life spans and weight gain of the mice were followed for 36 days. Doses are reported as μmol Au/kg body weight and ranged from 30 to 6 μmol Au/kg. The negative control was saline solution; the positive control, 5-fluorouracil (6.0 mg/kg).

Results and Discussion

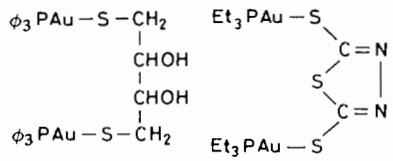
$(Et_3PAu)_2(\mu$-DMTA) and $(Ph_3PAu)_2(\mu$-DTE) were synthesized by double metathesis reactions of the dithiolate ligands with the appropriate chlorogold(I) phosphine complex:

$$K_2DMTA + 2Et_3PAuCl \longrightarrow (Et_3PAu)_2DMTA + 2KCl$$

$$H_2DTE + 2\phi_3PAuCl \longrightarrow (\phi_3PAu)_2DTE + 2HCl$$

A sharp decrease in the pH of the solution during the synthesis confirmed the displacement of protons from the ligand. The compounds were characterized by their infra-red spectra, elemental analyses and by their ^{13}C, ^{31}P and ^1H NMR spectra. Infra-red spectra confirmed the presence of the appropriate ligands in each complex. The ^{31}P spectrum of $(Et_3PAu)_2$-DMTA showed a single broad peak at 36.8 ppm from external H_3PO_4. Auranofin and triethylphosphine-(glutathionato-S)gold(I) also have similar chemical shifts, [10, 11] establishing that the gold is bound to the thiolate ligand and not the nitrogen of the heterocyclic ring. The ^{31}P NMR spectrum of $(\phi_3$-$PAu)_2(\mu$-DTE) consisted of a single peak at 34.96 ppm, consistent with the formulation and suggesting coordination via the thiolate groups of the ligand.

The structures of the compounds can be represented as shown below:



^{13}C spectra of the complexes (Table I) were consistent with the expected structures, but not otherwise noteworthy. The DTE complex, upon standing in DMSO reacted slowly (*ca.* 35–40% over 6 weeks) to form Ph_3PO, detected by its ^{31}P NMR, and presumably the gold thiolate, Au_2DTE:

$$(Ph_3PAu)_2(\mu\text{-DTE}) + 2DMSO \longrightarrow 2\phi_3PO + (CH_3)_2S + Au_2(\mu\text{-DTE})$$

A sample of ϕ_3PAuCl stored under the same conditions contained only a trace of ϕ_3PO, reflecting the instability of the product AuCl which would be formed by the analogous reaction.

The two new compounds and several well-known gold compounds, gold thioglucose (AuSTg), gold thiomalate (AuSTm) and Et_3PAuCl, were tested for anti-tumor activity using Ehrlich Ascites tumor cells in Swiss ACR white mice (Table II). Tumor cells were injected into the mice on day 0. On days 1 through 7, the mice were injected with drug. The weight gain and life span of the mice were measured for 36 days. Two measures of the anti-tumor activity are reported. The average weight of the tumor-injected saline-treated control mice increases from 30 to *ca.* 63 g over the period of 20 days. Substantial reduction in tumor growth is evidence of anti-tumor or carcinostatic activity. A T/C survival ratio significantly greater than 100% indicates an increased life span as a result of treatment (T) compared to the saline-injected controls (C).

The $(\phi_3PAu)_2(\mu$-DTE) complex showed significant anti-tumor activity against the Ehrlich Ascites tumor cell. Using survival times for all mice, the T/C ratio was 128. However several mice died prematurely due to toxicity of the complex – *i.e.*, before the tumor had grown sufficiently to cause fatalities. If these two mice were excluded from the calculation, the T/C ratio was 167. $(Et_3PAu)_2(\mu$-DMTA) was extremely toxic at 15 and 30 μmol Au/kg, and the low T/C ratios reflect deaths due to the complex.

TABLE I. ^{31}P and ^{13}C NMR Data

Compound	Solvent	$\delta(^{31}P)$ (ppm)	
^{31}P NMR (*vs.* external H_3PO_4)			
$(Et_3PAu)_2(\mu$-DMTA)	DMSO-d_6	39.9	
	$CHCl_3$-d	36.8	
$(\phi_3PAu)_2(\mu$-DTE)	DMSO-d_6	35.0	
Compound	Atom	$\delta(^{13}C)$ (ppm)	J_{CP} (Hz)
^{13}C NMR (*vs.* TMS) measured in DMSO			
$(Et_3PAu)_2(\mu$-DMTA)	CH_3	8.9	–
	CH_2	16.9	33.9
	CS_2=N	165.7	–
$(\phi_3PAu)_2(\mu$-DTE)	$C_{o,m}$	133.5	14.0
	C_{para}	131.7	(1.6?)
	$C_{o,m}$	129.2	11.4
	C_{phos}	128.5	61 cps
	CHOH	76.2	–
	CH_2S	32.0	–

TABLE II. Activity of Gold Complexes against Ehrlich Ascites Tumor Cells[a]

Treatment	Dose (μmol Au/kg)	Weight[b]		Survival T/C (%)
		Day 10	Day 20	
Controls				
(–) saline	–	42	63	100
(+) 5-fluoruracil		35	53	124
$(\phi_3PAu)_2(\mu$-DTE)	15	31	33	128(167)
$(Et_3PAu)_2(\mu$-DMTA)	15	52	–	30
	30	38	39	55
AuSTg	15	44	65	97
	30	33	49	110
AuSTm	15	39	56	142
Et_3PAuCl	5	40	56	109

[a]Tumor was implanted on day 0 and the compounds were injected daily on days 1 through 7. [b]Average weight of mice, which reflects the extent of tumor growth.

AuSTg and Et_3PAuCl did not show significant activity against Ehrlich Ascites cells. AuSTm, evaluated by the tumor weight-gain, was not active, but did prolong survival times, as shown by the T/C ratio of 142. These findings are consistent with generally negative results of gold(I) thiolates when tested against other cell lines.

The Et_3PAuCl proved to be too toxic at 30 μmol/kg and the mice died rapidly, but at 5 μg/ml it was tolerated. High toxicity and severe gastrointestinal effects have previously been reported for Et_3PAuCl [11]. At the lower dose level it was not active against the tumor cells.

These results suggest that related phosphine complexes of H_2DTE and other similar dithiols should be synthesized and tested for anti-tumor activity. Mirabelli *et al.* have previously suggested that phosphinegold(I) thiosugar-derivatives should have potential anti-tumor activity [10], as found here.

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