Phosphinogold(I) Dithiocarbamate Complexes: Effect of the Nature of Phosphine Ligand on Anticancer Properties

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INTRODUCTION

Gold compounds in the oxidation states of +1 and +3 continue to be of interest in medicinal chemistry because their efficacies toward certain diseases can be fine-tuned.1,2 For instance, a number of gold(III) dithiocarbamates have been reported as anticancer agents by Fregona and co-workers.3 This includes the classics [Au(DMDT)X2] and [Au(ESDT)X2] (where X = Cl, Br; DMDT = 1,2-bis(diphenylphosphino)ethane, ESDT = 1,3-bis(diphenylphosphino)propane, and dpdh = 1,6-bis(diphenylphosphino)hexane). These gold compounds were characterized by a combination of NMR and infrared spectroscopy, microanalysis, and mass spectrometry; and in selected cases by single-crystal X-ray crystallography. Compounds 4–6, which have dpdh ligands, are unstable in solution for prolonged periods, with 4 readily transforming to the Au3 cluster [Au3S4(dppe)3]Cl2 (4a) in dichloromethane. Compounds 1–3 and 7–12 are all active against human cervical epithelioid carcinoma (HeLa) cells, but the most active compounds are 10 and 11, with IC50 values of 0.51 μM and 0.14 μM, respectively. Compounds 10 and 11 are more selective toward HeLa cells than they are toward normal cells, with selectivities of 25.0 and 70.5, respectively. Further tests, utilizing the 60-cell-line Developmental Therapeutics Program at the National Cancer Institute (U.S.A.), showed 10 and 11 to be active against nine other types of cancers.

Gold(III) thiolate compounds have also been of interest as anticancer agents since the first reports on the antiarthritic agent aurano-1,10,11 and other gold(I) thiolates12–14 were shown to possess anticancer properties. These include the report by Tiekink and co-workers that triorganophosphinogold(I) dithiocarbamates of general formula [(R3P)Au(S2CNR2)] were active against seven human cancer cell lines.13 Of these, [(Et3P)Au(S2CNMe2)] (Et = ethyl) was the most active against the IGROV ovarian cell line (ID50 = 12 ng/mL).13

An early report by Mirabelli et al.,14 supported by others,12,13 on various gold(I) compounds of structural formula R2P–Au–X (R = ethyl; X = thioglucose (SR)) has demonstrated that the presence of a P–Au–S motif in these compounds enhances their anticancer activity. Notably, phosphinogold(I) thiolate complexes of type [Au(PR3)(SR)] are more active than gold(I) thiolates of type [Au(SR)], suggesting that the presence of phosphine ligands increases the lipophilicity and membrane permeability of the phosphinogold(I) complexes that make them active.12

It has been established that gold compounds, such as aurano, act against cancer cells via the mitochondria by inhibiting thioredoxin reductase.11–18 In doing so, gold(I) binds the C-terminal of the redox-active selenocysteine, leading

Abstract: The reactions of potassium salts of the dithiocarbamates L (where L = pyrazolylthiocarbamate (L1), 3,5-dimethylpyrazolylthiocarbamate (L2), or indazolylthiocarbamate (L3)) with the gold precursors [AuCl{(Ph3P)2}], [Au2Cl2(dppe)], [Au2Cl2(dpdp)], or [Au2Cl2(dpph)] lead to the new gold(I) complexes [AuL{(Ph3P)2}] (1–3), [Au2L2(dppe)] (4–6), [Au2L2(dpdp)] (7–9), and [Au2L2(dpph)] (10–12) (where dppe = 1,2-bis(diphenylphosphino)ethane, dpdp = 1,3-bis(diphenylphosphino)propane, and dpph = 1,6-bis(diphenylphosphino)hexane). These gold compounds were characterized by a combination of NMR and infrared spectroscopy, microanalysis, and mass spectrometry; and in selected cases by single-crystal X-ray crystallography. Compounds 4–6, which have dpdp ligands, are unstable in solution for prolonged periods, with 4 readily transforming to the Au3 cluster [Au3S4(dppe)3]Cl2 (4a) in dichloromethane. Compounds 1–3 and 7–12 are all active against human cervical epithelioid carcinoma (HeLa) cells, but the most active compounds are 10 and 11, with IC50 values of 0.51 μM and 0.14 μM, respectively.
to cytotoxic effects. For these reasons, several phosphinogold(I) thiolate complexes have been prepared and investigated for their anticancer activities. Examples include the hydrophilic tetrakis(tris(hydroxymethyl)phosphino)gold(I) complex, which was reported to be cytotoxic toward HCT-15 tumor cells. Other examples are \([Au(PH_3)](sp pa)\) \((sp pa = sulfanylpropenolate)\), \([Au(PH_3)(K_TSC)]\) \((K_TSC = vitamin K_3 derivative)\), and the gold(I) 7-azacoumarin complex, which exhibit activity against HeLa and A2780 ovarian cells, \([Au(OmS)(Ph,P(CH_2)_2PPh_3)]\) \((Oms = lupinsulphide)\), exhibiting activity against ovarian carcinomas, and \([Et_3PAu-(S,CNEt_2)]\) and \([Ph_3PAu(S,CNC,C,H_3)]\), which exhibit activity against breast, ovarian, and colon cancer. This strongly suggests that one strategy to make gold(I) anticancer drugs is to prepare molecules that have a phosphine and a sulfur-containing ligand bound to gold(I) to have the P–Au–S motif.

For phosphinogold(I) complexes, the nature of the phosphine ligand appears to be important in regulating their anticancer behavior. For example a report on the anticancer phosphine ligand appears to be important in regulating their motif.

We report here new phosphinogold(I) dithiocarbamate complexes, using triphenylphosphate, 1,2-bis-(diphenylphosphino)ethane \((dppe)\), 1,3-bis-(diphenylphosphino)-propane \((dppp)\), 1,6-bis-(diphenylphosphino)hexane \((dphp)\), and the pyrazol-1-yl- and indazol-1-yl-based dithiocarbamate ligands \((dtc)\) to test the hypothesis that combining alkane chain length in the diphosphino ligand and using dithiocarbamate as the thiolate ligand can lead to new gold anticancer compounds. Our choice of pyrazoles and indazole as the backbone of the dithiocarbamate is based on their medicinal properties.

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respectively; the $^{31}$P{1H} NMR spectra showed broad singlets between 29.0 and 33.0 ppm. The spectroscopic data for 4−6 are similar to the data for the bis(diphenylphosphino)digold(I) cations with azotate neutral heterocycles [μ-(dppe)Au₂(pzH)₂]ClO₄ and [μ-dppmAu₂(pzH)₂]ClO₄ (pzH = pyrazole). 45

Attempts to grow crystals of compound 4 were unsuccessful. On prolonged standing, a solution of 4 in a mixture of dichloromethane and diethylether at room temperature led to the isolation of the chloride salt of the cationic gold(I) cluster [Au₁₈S₈(dppe)₆]²⁺ (4a), whose molecular structure was established by single crystal X-ray crystallography (Table 1, Figure 1).

Table 1. Crystallographic Data for Compounds 3, 4a, and 12

|        | 3     | 4a    | 12    |
|--------|-------|-------|-------|
| empirical formula fw | C₁₅₀H₁₄₈Au₁₈Cl₂P₁₂S₈ | C₄₆H₄₂Au₂N₄P₂S₄ | 1234.95 |
| temp (K) | 100(2) | 105(2) | 150(2) |
| wavelength (Å) | 0.71073 | 0.71073 | 0.77490* |
| cryst syst | monoclinic | monoclinic | monoclinic |
| space group | P2₁/n | P2₁/n | P2₁/n |
| a (Å) | 9.1382(12) | 17.6854(7) | 13.499(11) |
| b (Å) | 22.487(3) | 34.4361(14) | 11.560(19) |
| c (Å) | 11.3760(14) | 29.1894(12) | 15.396(15) |
| β (deg) | 91.541(2) | 92.4570(10) | 92.4570(10) |
| volume (Å³) | 2336.8(5) | 17760.5(12) | 2277(5) |
| Z | 4 | 4 | 2 |
| density (calc) (Mg/m³) | 1.855 | 2.342 | 1.801 |
| abs. coeff. (mm⁻¹) | 6.560 | 15.077 | 8.334 |
| F(000) | 1264 | 11376 | 1196 |
| reflections collected | 36929 | 188093 | 31033 |
| completeness to θ | 99.9% | 99.8% | 99.2% |
| goodness-of-fit on F² | 1.19 | 1.02 | 1.02 |
| final R indices [I > 2σ(I)] | R1 = 0.0216, wR2 = 0.0542 | R1 = 0.0473, wR2 = 0.1258 | R1 = 0.0404, wR2 = 0.0984 |
| R indices (all data) | R1 = 0.0216, wR2 = 0.0544 | R1 = 0.0677, wR2 = 0.1347 | R1 = 0.0356, wR2 = 0.1071 |
| larg. diff. peak hole (e.Å⁻³) | 1.15 and −0.88 | 2.14 and −1.44 | 1.45 and −0.86 |

“Using synchrotron radiation tuned to $\lambda = 0.7749$ Å.

Figure 1. Molecular structure of 3 drawn with 30% probability ellipsoids. H atoms are omitted for clarity. Selected bond lengths [Å] and angles [deg]: Au1−P1, 2.2533(6); Au1−S1, 2.3272(6); P1−C1, 1.811(2); N1−C1, 1.399(3); S2−C1, 1.663(3); P1−Au1−S1, 17.536(2); C1−S1−Au1, 100.04(9); C9−P1−Au1, 118.20(8); S2−C1−S1, 124.85(15).

Scheme 2. Preparations of (Diphosphino)alkylgold(I) Dithiocarbamato Complexes 4−12

[pzH]₃[ClO₄] and [μ-dppmAu₂(pzH)₂]ClO₄ (pzH = pyrazole)

Attempts to grow crystals of compound 4 were unsuccessful. On prolonged standing, a solution of 4 in a mixture of dichloromethane and diethylether at room temperature led to the isolation of the chloride salt of the cationic gold(I) cluster [Au₁₈S₈(dppe)₆]²⁺ (4a), whose molecular structure was established by single crystal X-ray crystallography (Table 1, Figure 1).
Each S atom is implied a mixed-valent Au0 hydrated cluster [Au18S8(dppe)6]2+. At the core of the cluster is a Au6S2 geometry of six Au atoms from a capping Au2S triangle. Each of the Au atoms in other S atoms ligate one Au atom from the cubane and two Au atoms coordinate to three Au atoms of the cubane, whereas the bidentate dppe ligands. The Au−P distances vary between 2.9147(7) and 3.2838(8) Å; the Au−S bond length falls within a 2.343(3)−2.374(3) Å range, and the 12 Au−P distances average 2.260(7) Å, a value similar to that in compound 4. The diphosphine backbone and aurophilicity of the two Au atoms in 4 must have aided the cluster formation since increase in the alkyl chain length in the diphosphine gave stable diphosphinogold(I) dithiocarbamate complexes (vide infra).

(Diphosphino) Propyl and Hexyl Gold(I) Dithiocarbamate Complexes (7–12). To avoid the cluster formation observed during crystallization of 4, the −CH2− linker count within the dithiocarbines was increased to three in dppp and six in dphp. Their respective diphosphinogold(I) dithiocarbamates (7–12) were synthesized from the reaction between [Au2Cl2(dppe)] or [Au2Cl2(dpdp)] and 2 equiv of L1, L2, or L3 (Scheme 2). The 1H and 31P{1H} NMR spectra of 7–12 showed similar patterns as those of 4–6, with the CH2 protons in the backbone of the dithiocarbine ligands appearing upfield in the region of 1.49−2.93 ppm and singlets for phosphorus between 29.0 and 33.0 ppm. These complexes were stable in solution for several weeks, with no signs of decomposition. ESI-MS of 9 (m/z = 1190.88, Figure S1, Supporting Information) showed molecular ions that correspond to a binuclear complex with a molecular ion peak at m/z = 1190.88, and the crystal structure of 12 (Figure S2b) further indicates that the length of the alkyl linker in the dithiocarbine ligand is important in preventing compounds 7–12 from transforming into clusters.

Crystal data, together with the data collection and refinement parameters, are presented in Table 1. The molecular drawing of 12 is shown in Figure 3. The dinuclear complex resides on a crystallographic inversion center, and only one-half of it is symmetry independent; the most important distances are discussed. The symmetry-independent half of complex 12 can...
be considered a congener of complex 3. In complex 12 the coordination environment about the Au atoms is nearly linear, with the P1–Au1–S1 angle spanning 173.41(6)°; the Au1–S1 (2.312(3) Å) and Au1–P1 (2.255(2) Å) distances are typical and in good agreement with the ones observed in complex 3. The single C1–S1 distance to the ligating atom S1 (1.720(7) Å) is longer than the formally double C1=–S2 bond of 1.656(6) Å, but both values closely match the corresponding values in complex 3. In the lattice, pairs of clusters are packed with an inversion center at the center of the Au–S···Au–S parallelepiped. Within each parallelepiped, the nonbonding Au···Au distance measures 3.986(3) Å, and the Au···S distance is 3.598(4) Å. Both distances exceed the sum of the van der Waals radii of the involved elements; thus, there are no aurophilic interactions in the lattice. The structural findings for complex 12 are similar to those reported by Uson et al.12 and Cookson et al.31 for the gold(I) complexes [Au(PPh3)(S2C-aza-15-crown-5)] and [Au(C3H7NS)2]3, respectively. The other bonding distances and angles are in the same range and correspond with the expected values. Crystal data, together with the data collection and refinement parameters, are presented in Table 1.

**Biological Activity.** Fifteen compounds (L1–L3, 1–12) were initially screened for their ability to inhibit cell growth on human cervical epitheloid carcinoma (HeLa) cells in vitro. All data were acquired in triplicate, and the final values were recorded as averages. The dose values that caused 50% inhibition of cell growth (IC50) are listed in Table 2. To establish the activities of phosphine gold(I) complexes it was important to first establish the activities of the ligands (L1–L3) to determine whether activities of the metal complexes could be due to the presence of the dithiocarbamate ligands. All the free ligands were inactive against HeLa cells.

The gold(I) complexes were grouped into three sets according to ligand type, PPh3 (1–3), dppp (7–9), and dpdh (10–12), for testing. Complexes 4–6 (the dppe set) were not tested because of the instability of 4 in solution discussed earlier. Although complexes 1–3, 7–9, and 12 had quite good IC50 values (2.2–7.0 μM), their tumor specificity (TS) values were very low (Table 2), and they were less active than cis-platin (0.476 μM). The low TS factors suggest that these compounds were toxic to both tumor and normal cells and were thus not further investigated. Compounds 10 and 11 had IC50 values of 0.51 μM and 0.14 μM, respectively, compared to 0.45 μM for cis-platin. These two gold compounds (10 and 11) displayed very good TS values of 25.0 and 70.5, respectively (Table 2). Our findings are comparable to and in some cases much better than activities reported for phosphine gold(I) thiolate complexes12,13,23 which further buttresses the conception that a P–Au–S motif enhances the therapeutic effect of phosphine gold(I) thiolate compounds as anticancer agents. Complexes 10 and 11 were further investigated against a panel of 60 cancer cell lines of the Developmental Therapeutics Program (DTP) at the National Cancer Institute (U.S.A.), where the concentration that inhibits growth of cells by 50% (GI50), the concentration that causes total tumor growth inhibition (TGI), and the least concentration required to kill 50% of tumor cells (LC50) were determined. The 60 cell lines were organized into subpanels, representing various histologies, for example, nonsmall cell lung-, colon-, breast-, ovarian-, renal-, prostate-, and CNS cancers, leukemia, and melanoma. Because of the extensive data, we have highlighted only the most important findings in Tables 3 and 4, but more data are provided as Supporting Information (Figures S2 and S3; Tables S1 and S2). Complexes 10 and 11 were, in general,

![Figure 3. Molecular structure of 12 drawn with 50% probability ellipsoids. H atoms are omitted for clarity. Selected bond length [Å] and angles [deg]: Au1–P1, 2.255(2); Au1–S1, 2.312(3); P1–C9, 1.818(6); N1–C8, 1.390(6); S1–C1, 1.720(7); S2–C1, 1.656(6); P1–Au1–S1, 173.41(6); C1–S1–Au1, 100.9(2); C15–P1–Au1, 112.6(8); S2–C1–S1, 124.1(4). Selected symmetry-related atoms are labeled with a superscript.](image-url)
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Table 3. Selected DTP Antitumor Inhibition Results of Complex 10

| type of cancer | panel/cell line | GI50 (μM) | TGI (μM) | LC50 (μM) |
|---------------|----------------|----------|----------|-----------|
| leukemia      | RPMI-8226      | 0.19     | 0.53     | >100      |
|               | SR             | 0.19     | 2.41     | >100      |
| NNSM cell line| A549/ATCC      | 0.38     | >100     | >100      |
| colon         | COLO205        | 0.31     | >100     | >100      |
|               | KM12           | 0.42     | 1.73     | >100      |
| melanoma      | UACC-62        | 0.38     | >100     | >100      |
| ovarian       | OVCAR-3        | 0.70     | 14.20    | >100      |
| renal         | RXF393         | 0.45     | >100     | >100      |

Table 4. Selected DTP Antitumor Inhibition Results of Complex 11

| type of cancer | panel/cell line | GI50 (μM) | TGI (μM) | LC50 (μM) |
|---------------|----------------|----------|----------|-----------|
| leukemia      | RPMI-8226      | 0.14     | 0.41     | >100      |
|               | SR             | 0.03     | 0.52     | 90        |
| NNSM cell line| A549/ATCC      | 0.35     | >100     | >100      |
| colon         | COLO205        | 0.11     | >100     | >100      |
| melanoma      | UACC-62        | 0.25     | >100     | >100      |
| ovarian       | OVCAR-8        | 0.60     | >100     | >100      |
| renal         | A498           | 0.38     | >100     | >100      |

We observed a similar activity pattern for complex 11 against all the cell lines, but 11 was even more potent than 10. For instance, the GI50 values of 11 against the leukemia cell lines RPMI-8226 and SR were 0.14 μM and 0.03 μM, respectively. In fact, the best activity registered for all the compounds tested was that of 11 against the SR cell line (0.03 μM) (Table 4). The TGI concentrations recorded were similarly quite low, RPMI-8226 (0.41 μM) and SR (0.52 μM), which corroborates the cytostatic properties of 11. Of interest is that compound 11, unlike 10, showed very good cytotoxic activity against the HCC-2998 cell line (LC50 = 2.99 μM). Compound 11 also showed activity against the colon cancer COLO205 and HCC-2998 cell lines, with GI50 of 0.11 μM and 0.25 μM, respectively. In general, complexes 10 and 11 recorded high cytostatic median values (GI50 = −6.25; TGI = −4.51) and cytotoxic median value (LC50 = −4.05) against the 60 cell lines (Table 5).

Table 5. Cytostatic and Cytotoxic Median Data of 10 and 11

| drug | MG-MID | Δ | range | MG-MID | Δ | range |
|------|--------|---|-------|--------|---|-------|
| 10   | −5.93  | 0.8| 2.73  | −4.36  | 1.92| 2.28  |
| 11   | −6.25  | 1.22| 1.96  | −4.51  | 1.87| 2.38  |

CONCLUSIONS

We have prepared the first examples of phosphinogold(I) dithiocarbamates derived from heterocycles. The stability of these phosphinogold(I) dithiocarbamate complexes depends on the nature of the phosphine ligand used. Triphenylphosphino and diphenylphosphinoalkyl ligands with alkyl chains longer than ethyl produce stable gold dithiocarbamates in solution, but the diphenylphosphinoethane(diphenylgold(I)) dithiocarbamates are unstable and were found to transform to a Au18 cluster. All the phosphinogold(I) dithiocarbamates that are stable in solution are active against HeLa cancer cells, suggesting the importance of the P=As–S moiety in conferring activity to the compounds. Compounds with hexyl chain were found to be the most active and extremely selective; in particular compounds 10 and 11 were 2.5 and 70.5 times more selective for HeLa cells than normal cells. Bis-(diphosphines) alkanes with longer CH2 linkers appear to hold better promise as anticancer agents than their shorter CH2-linker counterparts, similar to the observation by Horvath et al.27 We also found compounds 10 and 11 to have excellent activities for nine other cancer cell lines in vitro. Of the nine cancer cell lines tested, the best activity against RPMI-8226 was found for 10 (GI50 = 0.19 μM), while the best activity against SR cells (GI50 = 0.03 μM) was for 11. Although activities for 10 and 11 in vivo were not so great, we believe finding drug delivery vehicles to transport these two compounds would improve their activities in vivo. We are therefore investigating the use of various delivery vehicles for these compounds.

EXPERIMENTAL SECTION

Materials and Instrumentation. All manipulations were performed under a dry, deoxygenated nitrogen atmosphere using Schlenk techniques. All commercially available chemicals were used as received. Pyrazol-1-ylthiocarbamate (L1), 3,5-dimethylpyrazol-1-yldithiocarbamate (L2), and indazol-1-ylthiocarbamate (L3) were synthesized according to literature methods.53–55 Gold starting materials [Au2Cl2(dppe)], [Au2Cl2(dppp)], and [Au2Cl2(dpph)] (dppe = 1,2-bis(diphenylphosphino)ethane; dppp = 1,3-bis(diphenylphosphino)propane; and dpph = 1,6-bis(diphenylphosphino)hexane) were synthesized according to the literature procedures.53–55 Infrared (IR) spectra were recorded as KBr pellets on a Bruker Tensor27 spectrophotometer. 1H, 13C{1H}, and 31P{1H} NMR spectra were recorded on a Varian 2000 spectrometer (1H, 300 MHz; 13C, 75.4 MHz; and 31P, 121.5 MHz) in CDCl3 or D2O at room temperature. Elemental analysis was performed on a Fisons elemental analyzer at the University of Cape Town, South Africa. ESI-MS spectra S5 and S7, Supporting Information, which in the DTP program indicates high activity across most of the panel of cell lines.

Following on the in vitro results, compound 11 was further tested in vivo in hollow fiber studies, but the activity data suggested that the compound could not be delivered to the targets. We are therefore investigating the use of various drug delivery vehicles, including the use of β– and γ–cyclodextrin, to deliver this drug in vivo.
were recorded on a Waters API Quattro Micro spectrometer at the University of Stellenbosch, South Africa. The mass spectra were collected using 3.0-s cyclic scans and applying the sample cone voltage of 15 V at the source block temperature of 100 °C. Desolvation temperature was 350 °C at desolvation cone gas flow rate of 350 L/h.

**Synthesis of Triphenylphosphinegold(II) Complexes. Pyrazolyl-1-dithiocarbamato-triphenylphosphinegold(II)** (1). Complex 1 was prepared by dissolving L1 (0.05 g, 0.03 mmol) in deionized water (10 mL), after which a solution of [AuCl(PPh3)] (0.15 g, 0.3 mmol in dichloromethane (10 mL) was added. The resultant biphasic mixture was vigorously stirred at room temperature for 30 min, during which time the color of the organic layer changed from colorless to red. The aqueous and organic layers were separated, and the organic layer was dried over anhydrous MgSO4. The solvent was removed from the organic extract in vacuo to afford an orange-yellow solid.

Yield = 0.17 g (93%). 1H NMR (CDCl3): δ 7.65 (s, 1H, 5-pz); 7.57 (s, 1H, 3-pz); 7.49 (m, 9H, Ph2P); 7.35 (m, 6H, Ph2P); 6.27 (s, 1H, 4-pz). 13C{1H} NMR (CDCl3): δ 213.4 (C(3-C=C)); 140.0 (C(5-C=C)); 134.2-129.1 (phenyl region); 140.8 (C(3-pz)); 106.1 (C(4-pz)). IR (KBr, cm⁻¹): vC=O = 1620, vC=C = 1121, vC=C = 844. 31P{1H} NMR (CDCl3): δ 35.2 (PPh2). Anal. Calc. for C26H20AuCl2N2PS2: C 29.71, H 2.32, N 5.09. Found: C 29.80, H 2.00, N 3.80%.

Compounds 5–12 were prepared using the procedure described for 4 above, using the appropriate starting materials.

**Bis-(3,5-dimethylpyrazolyl-1-dithiocarbamato-bis(diphenyolphosphino)ethane Dinuclear Gold(I))** (6). L3 (0.04 g, 0.19 mmol), [AuCl(dppe)] (0.08 g, 0.09 mmol). Yield = 0.07 g (63%). H NMR (CDCl3): δ 9.16 (s, 1H, Ha); 8.06 (s, 1H, Hb); 7.78 (m, 8H, Ph2P(Ph)(CH2)2Ph); 7.49 (m, 12H, Ph2P(Ph)(CH2)2Ph); 7.67 (d, 1H, 4-H); 7.37 (1H, 3-H); 7.43 (1H, 5-H); 2.87 (4H, CH2, Ph2P(Ph)(CH2)2Ph); 1.99 (m, 4H, Ph2PC2H2). IR (KBr, cm⁻¹): vC=O = 1612, vC=C = 1137. 31P{1H} NMR (CDCl3): δ 43.9 (PPh2). Anal. Calc. for C32H24Au2N2P2S4: C 41.79, H 2.45, N 6.19. Found: C 41.74, H 2.48, N 6.16.

**Bis(indazolyl-1-dithiocarbamato-bis(diphenyolphosphino)ethane Dinuclear Gold(I))** (7). L1 (0.08 g, 0.46 mmol), [AuCl(dppe)] (0.2 g, 0.23 mmol). Yield = 0.15 g (59%). H NMR (CDCl3): δ 8.73 (s, 2H, 5-H); 2.77 (m, 8H, Ph2P(Ph)(CH2)2Ph); 7.51 (s, 4H, 4-H); 1.78 (4H, CH2, Ph2P(Ph)(CH2)2Ph); 1.99 (m, 4H, Ph2PC2H2). IR (KBr, cm⁻¹): vC=O = 1618, vC=C = 1088. 31P{1H} NMR (CDCl3): δ 32.8. Anal. Calc. for C32H24Au2N2P2S4: C 41.79, H 2.45, N 6.19. Found: C 41.74, H 2.48, N 6.16.

**Bis-(pyrazolyl-1-ylthiocarbamate-bis(diphenyolphosphino)propane Dinuclear Gold(I))** (8). L3 (0.1 g, 0.46 mmol), [AuCl(dppe)] (0.2 g, 0.23 mmol). Yield = 0.13 g (50%). H NMR (CDCl3): δ 7.66 (m, 12H, Ph2P(Ph)(CH2)2Ph); 7.34 (m, 12H, Ph2P(Ph)(CH2)2Ph); 1.95 (m, 4H, Ph2PC2H2). IR (KBr, cm⁻¹): vC=O = 1613, vC=C = 1099. 31P{1H} NMR (CDCl3): δ 292. Anal. Calc. for C32H24Au2N2P2S4: C 41.79, H 2.45, N 6.19. Found: C 41.79, H 2.48, N 6.16.
Inorganic Chemistry

Synthesis of Dinuclear Bis(pyrazol-1-yldithiocarbamato)-bis(diphenylphosphino)hexane Gold(I) (10). L1 (0.02 g, 0.11 mmol), [Au2Cl2(dppp)] (0.05 g, 0.05 mmol). Yield = 0.05 g (70%). 1H NMR (CDCl3): δ 8.78 (d, 2H, 2JHH = 2.7 Hz, S-Ph); 7.80 (s, 3H, 2-PPh2). 13C{1H} NMR (CDCl3): 25.9 (2C, Ph2P(CH2)2-); 121.1 (C(8C-Ph, 9C-Ph)); 118.3 (C(6C-Ph)); 30.1 (4C, Ph2P(CH2)2(CH2)2-PPh2). IR (KBr, cm−1): ν = 1606, ν = 1150, ν = 993. 31P{1H} NMR (CDCl3): δ 29.2. ESI-MS: m/z = 11909 [Au3(1,3)(dppp)]+(5%). Anal. Calc. for C38H38Au2N4P2S4: C 40.22, H 3.37, N 4.94, S 11.30. Found: C 40.17, H 3.45, N 4.36, S 10.28%.

Synthesis of Dinuclear Bis(3,5-dimethylpyrazol-1-yldithiocarbamato)-bis(diphenylphosphino)hexane Gold(I) (11). L2 (0.09 g, 0.42 mmol), [Au2Cl2(dppp)] (0.2 g, 0.2 mmol). Yield = 0.12 g (50%). 1H NMR (CDCl3): δ 8.76 (d, 2H, 2JHH = 2.7 Hz, S-Ph); 7.78 (m, 8H, Ph2P(CH2)2-PPh2); 7.49 (m, 12H, Ph2P(CH2)2); 6.43 (s, 2H, 4JHH = 3.4 Hz, (Ph)2P(CH2)6-P(Ph)2-PPh2). 13C{1H} NMR (CDCl3): 25.6 (2C, Ph2P(CH2)2-); 215.9 (C(C6C-pz)); 153.3 (C(5-pz); 144.2 (C(3-pz)); 133.4 124.9 (C(4-pz)); 123.3 (C(5-pz)); 121.1 (C(8C-Ph, 9C-Ph)); 118.3 (C(6C-pz)); 30.1 (4C, Ph2P(CH2)6-P(Ph)2-PPh2); 121.1 (C(8C-Ph, 9C-Ph)); 118.3 (C(6C-pz)); 30.1 (4C, Ph2P(CH2)6-P(Ph)2-PPh2); 25.9 (2C, Ph2P(CH2)6-P(Ph)2-PPh2). IR (KBr, cm−1): ν = 1620, ν = 1108, ν = 960. 31P{1H} NMR (CDCl3): δ 32.3. Anal. Calc. for C46H42Au2N4P2S4: C 44.74, H 3.43, N 4.54, S 10.39. Found: C 44.37, H 3.05, N 4.62, S 10.56%.

SUMMARY

The absorption correction for the all three complexes was based on the neighboring atoms with relative isotropic displacement coefficients. All hydrogen atoms were included in the structure factor calculation at idealized positions and were allowed to ride on the neighboring atoms with relative isotropic displacement coefficients.57

TESTING OF COMPOUNDS FOR ANTICANCER ACTIVITY

Biological Reagents and Instrumentation. All commercial reagents were used as received. Phosphate-buffered saline (PBS), Eagle’s RPMI-1640 medium, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit, phothemagglutinin-protein form (PHA-P) and the 96-well flat-bottomed culture plates were all purchased from BD Biosciences Ltd. Cisplatin was purchased from Sigma Aldrich. Eagle’s medium with 0.1 mM nonessential amino acids was prepared by adding 2 mM l-glutamine, 1.0 mM sodium pyruvate, and 5% bovine fetal calf serum to the pure Eagle’s RPMI-1640 medium. A total of fifteen compounds (L1–L3, 1–3, and 7–12) were screened for their anticancer activities. Human cervix epithelial carcinoma (HeLa) cells and human lymphocytes (PBMCs), from preservative-free heparinised peripheral blood, were obtained from the Department of Pharmacology and Pretoria Medical Hospital, University of Pretoria, South Africa. The absorbance values were recorded on a Whittaker Microplate Reader 2001 spectrophotometer at 570 nm and the reference wavelength of 630 nm.

Two complexes (10 and 11) were further tested against the 60-cell-line panel at the DTP using their internal procedures.58,59 From this study, three important (cytostatic and cytotoxic) properties are evident, namely, cytostatic values that include molar concentrations of drug required for 50% growth inhibition (IC50), cytotoxic values that include molar concentrations of drug required for total growth inhibition (TG1), and cytotoxic values that include molar concentrations of drug required to kill 50% of the cell population (LC50).

Cell Culture and Drug Treatment. HeLa cells were cultured in Eagle’s medium with 0.1 mM nonessential amino acids, 2 mM l-glutamine, 1.0 mM sodium pyruvate, and 5% bovine fetal calf serum at 37 °C in an atmosphere of 5% CO2. Cells were placed in 96-well sterile plates, at a density of 1×104 cells/well in 100 μL of medium, and incubated for 1 h. Subsequently, the ligands or gold compounds were added, with concentrations ranging from 0 to 100 μM. Cytotoxicity was determined by using MTT to stain treated HeLa cells after 7 d, according to literature methods.60 MTT dye is reduced by living cells to yield a soluble formazan product that can be assayed colorimetrically. A20 0 μL volume of freshly prepared MTT (5 mg/mL) was added to each well, and the cells were incubated for another 4 h. MTT (5 mg/mL) was added to each well, and the cells were incubated for another 4 h. Cell survival was evaluated by measuring absorbance at 570 nm, using a Whittaker Microplate Reader 2001. All experiments were performed in triplicate.

The inhibition of the growth of normal cells by the complexes tested was also measured by employing human lymphocytes (PBMC) cells. The same procedure described above was used, except that the treated PBMC cells were incubated for 3 d as opposed to 7 d for HeLa cells. The aim of testing these compounds on normal cells was to determine whether the compounds could target the cancerous (HeLa) cells specifically and not the normal cells. Lymphocytes were divided into two groups, namely, (i) normal cells that were stimulated using PHA-P so as to increase their proliferation rate (stimulated lymphocytes) and (ii) unstimulated normal cells (resting lymphocytes).

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ASSOCIATED CONTENT

Supporting Information
Crystallographic information data (CIF); ESI-MS of 9; biological data for compounds 11 and 12. This material is available free of charge via the Internet at http://pubs.acs.org. The deposition numbers CCDC 680273, 949434, and 920397 contain the supplementary crystallographic data for compounds 3, 4a, and 12, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Notes
The authors declare no competing financial interest.

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