ANTITUMOR ACTIVITY OF GOLD(I), SILVER(I) AND COPPER(I) COMPLEXES CONTAINING CHIRAL TERTIARY PHOSPHINES

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Abstract.
The in vitro cytotoxicities of a number of gold(I), silver(I) and copper(I) complexes containing chiral tertiary phosphine ligands have been examined against the mouse tumour cell lines P815 mastocytoma, B16 melanoma [gold(I) and silver(I) compounds] and P388 leukaemia [gold(I) complexes only] with many of the complexes having IC50 values comparable to that of the reference compounds cis-diamminedichloroplatinum(II), cisplatin, and bis[1,2-bis(diphenylphosphino)ethane]gold(I) iodide. The chiral tertiary phosphine ligands used in this study include (R)-(2-aminophenyl)methylphenylphosphine; (R,R)-, (S,S)- and (R*,R*)-1,2-phenylenebis(methylphenylphosphine); and (R,R)-, (S,S)- and (R*,R')-bis{(2-diphenylphosphinoethyl)phenylphosphino}ethane. The in vitro cytotoxicities of gold(I) and silver(I) complexes containing the optically active forms of the tetra(tertiary phosphine) have also been examined against the human ovarian carcinoma cell lines 41M and CH1, and the cisplatin resistant 41McisR, CH1cisR and SKOV-3 tumour models. IC50 values in the range 0.01 - 0.04 μM were determined for the most active compounds, silver(I) complexes of the tetra(tertiary phosphine). Furthermore, the chirality of the ligand appeared to have little effect on the overall activity of the complexes: similar IC50 data were obtained for complexes of a particular metal ion with each of the stereoisomeric forms of a specific ligand.

Introduction.
Bis(ditertiary phosphine) complexes of gold(I), silver(I) and copper(I) have attracted much interest over the past decade as certain of these complexes have been shown to display antitumour activities comparable to cis-diamminedichloroplatinum(II), cisplatin, for example, bis[1,2-bis(diphenylphosphino)ethane]gold(I) chloride, [Au(dppe)2]Cl, and its silver(I) and copper(I) derivatives.1 The mode of action of these complexes is not known but it is believed to be quite different to that of cisplatin. The complex [Au(dppe)2]Cl has been shown to inhibit DNA, RNA and protein synthesis; to cause DNA single strand breaks; and to induce mitochondrial damage in hepatocytes. Indeed, despite the early promise of [Au(dppe)2]Cl and its derivatives, the compounds have subsequently been shown to be too toxic for clinical use due to side-effects related to the disruption of mitochondrial function in hepatocytes.2,3

We have recently synthesised a number of related gold(I), silver(I) and copper(I) complexes containing the substituted (2-aminophenyl)phosphines AMPP [(±)-(2-aminophenyl)methylphenylphosphine] and ADPP [(2-aminophenyl)diphenylphosphine] and investigated their in vitro cytotoxic properties against three mouse tumour cell lines, P815 mastocytoma, B16 melanoma and P388 leukaemia.4 Certain of these complexes exhibited antiproliferative properties comparable to those of the two reference compounds cisplatin and [Au(dppe)2]Cl. Furthermore, the complexes [Au(ADPP)2]PF6 and [Au((±)-AMPP)2]PF6 were shown to rapidly accumulate in mitochondria by virtue of their lipophilic, cationic nature and to inhibit the activity of ATP synthase leading to rapid cell death.5

In this paper we report on the in vitro cytotoxic properties of a range of gold(I), silver(I) and copper(I) complexes containing chiral tertiary arsine and phosphine ligands against the P815 tumour cell line. IC50 values have also been determined for certain of these complexes against the tumour cell lines B16, P388, 41M, and CH1, and the cisplatin resistant 41McisR, CH1cisR and
SKOV-3 tumour models. While the chirality of platinum-based antitumour agents is known to have a dramatic influence on their activity, this effect has not previously been investigated for gold(I), silver(I) or copper(I) complexes containing tertiary phosphine ligands.

Materials and Methods.
(a) Syntheses. The ligands (-)-(2-aminophenyl)methylphenylphosphine,7 (R*,R*)-1,2-phenylenebis(methylphenylphosphine)8 and (R*,R*)-1,2-bis[(2-diphenylphosphinophenoylethyl)phenylphosphino)ethane9 were synthesized and resolved following literature procedures. The complexes containing (R,R)-(S,S)- and (R*,R*)-diasor-diph [M = Ag(I) or Cu(I)]10 and (R,R)- and (S,S)-tetraphos [M = Au(I), Ag(I) or Cu(I)]9 were prepared as previously described in the literature. The synthesis of [Au2[(R*,R*)-tetraphos]2](PF6)2, [Au2[(R*,S*)-tetraphos]2](PF6)2, [Ag2[(R*,R*)-tetraphos]2](PF6)2, and [Cu [(R*,R*)-tetraphos]]PF6 will be reported elsewhere.11

Synthesis of [L-2]-Bis[(R)-(2-aminophenyl)methylphenylphosphine]gold(I) Iodide Ethanol Solvate, [Au{(R)-AMPP}2]I. A solution of the optically active ligand (R)-AMPP (0.17 g, 0.79 mmol) in ethanol (5 mL) was added to a solution of tetrabutylammonium diiodoaurate(I) (0.27 g, 0.39 mmol) in ethanol (15 mL) with stirring. The white crystalline product was filtered off, washed with ethanol and dried in vacuo (0.24 g, 83%). (Found: C, 42.3; H, 4.2; N, 3.3. Calc. for C28H34AuI2N2OP2: C, 42.0; H, 4.3; N, 3.5%). 1H NMR (CDCl3): δ 2.26 (6H, 2JmH 8.5 Hz, PMe), 4.31 (4H, NH2), 6.63-7.67 (18H, aromatics). 31P-{1H} NMR (CDCl3): δ 51.11 (s, 2P). M(MeNO2): 92 cm²mol⁻¹; AM(MeCN): 92 cm²mol⁻¹.

Synthesis of [T-4]-Bis[(R*,R*)-1,2-phenylenebis(methylphenylphosphino)benzene]gold(I) Iodide, [Au{(R*,R*)-diph}2]. Tetra-n-butylammonium diiodoaurate(I) (0.22 g, 0.32 mmol) was dissolved in ethanol (20 mL) and the ligand (R*,R*)-diph (0.20 g, 0.62 mmol) was slowly added with stirring. The solution was filtered for 30 min and the solvent was removed by evaporation. The residue was dissolved in acetone (5 mL) and diethyl ether was added dropwise to give a white crystalline product which was filtered off, washed with diethyl ether and dried in vacuo (0.24 g, 80%). m.p. 160 °C (Found: C, 50.0; H, 4.3. Calc. for C40H40AuI2P4: C, 49.6; H, 4.2%). 1H NMR (CDCl3): δ 2.27 (12H, PMe), 7.12-7.53 (28H, aromatics). 31P-{1H} NMR (CDCl3): δ 4.63 (4H, 6. P). ΛM (MeNO2): 93 cm² S mol⁻¹; ΛM (MeCN): 94 cm² S mol⁻¹.

Synthesis of [T-4]-Bis[(R,R)-1,2-phenylenebis(methylphenylphosphino)benzene]gold(I) Iodide, [Au{(R,R)-diph}2]. Prepared as for [Au{(R*,R*)-diph}2] except using tetrabutylammonium diiodoaurate(I) (0.16 g, 0.23 mmol) and the ligand (R,R)-diph (0.15 g, 0.47 mmol). The product was obtained as white crystals after recrystallisation from acetone - diethyl ether (0.21 g, 94%). m.p. 168 °C (Found: C, 52.0; H, 4.4; N, 4.5. Calc. for C40H40AgF6P6: C, 52.1; H, 4.7; N, 4.7%). 1H NMR (CDCl3): δ 18.82 (br s, 9 H, PMe), 4.75 (6H, 6.80-7.54 (m, 27 H, aromatics). 31P-{1H} NMR (CDCl3): δ -22.5 (s, 3 P). ΛM (MeNO2): 83 cm² S mol⁻¹; ΛM (MeCN): 84 cm² S mol⁻¹.
(b) Biological procedures and materials. (i) Mouse tumour cell lines: P815 mastocytoma cells were cultured in Eagle’s Minimum Essential Medium F15 with 10% foetal bovine serum; B16 cells in McCoy’s medium RPMI 1640 with 10% foetal bovine serum; and P388 leukaemia cells in Dulbecco’s Modified Eagle Medium H16 with 10% horse serum (herein EC10). All cells were cultured and incubated in a Forma Scientific Infrared CO2 incubator at 37 °C in 5% CO2. Cell suspensions were centrifuged using a Jouan centrifuge model CR 4 22. Linbro 96 round bottom well tissue culture plates were used for the thymidine incorporation assay. Cells were harvested using a Pharmacia Version 1.02 Micro Cell Harvester and incorporated thymidine was counted using a Pharmacia 1205 Betaplate liquid scintillation counter. Thymidine incorporation assays were performed following a literature procedure. The compounds tested were dissolved in dimethylsulfoxide (0.2 mL) to give a concentration of 0.02 M and then diluted to 2 x 10^{-5} M using EC10.

(ii) Human ovarian carcinoma cell lines: The biological properties of the CH1, 41M and SKOV-3 cell lines have been described previously. The cell lines were grown as monolayers in Dulbecco’s modified Eagle plus 10% foetal calf serum, 100 μg mL^{-1} streptomycin, 100 μg mL^{-1} penicillin and 2 mM glutamine in a 5% CO2 atmosphere. The cell lines were checked regularly for mycoplasma infection. Cytotoxicity assessment was undertaken using the sulphorhodamine B assay as described previously. The complexes were dissolved in dimethylsulfoxide, diluted in distilled water and added to the cultures at concentrations ranging from 0.005 to 5 μM in quadruplicate. The final concentration of dimethylsulfoxide in the culture medium was < 0.5%. The cells were exposed to the drug containing medium for 96 h. The IC_{50} values were determined as the drug concentration reducing absorption (564 nm) of sulphorhodamine B-stained wells to 50% of that of the control wells.

Results.

Complexes. A number of univalent gold, silver and copper complexes have been utilised in the present study containing the ligands (R)-(2-aminophenyl)methylphenylphosphine (AMPP); (R,R)-, (S,S)- and (R*,R*)-1,2-phenylenebis(methylphenylphosphine) (diph) and their arsenic analogues (dias); and (R,R)-, (S,S)-, (R*,R*)- and (R*,S*)-bis[(2-diphenylphosphinoethyl)phenylphosphino]-ethane (tetraphos).†

![Complex structures](image)

(Only one enantiomer of each ligand is shown.)

The complexes that were used in this work are listed in Tables 1-4. We have previously described the preparation and characterisation of many of these complexes. Complexes containing (R)-AMPP were prepared in a similar fashion to that reported for their racemic analogues. Gold(I) compounds of diph were synthesised by reacting two equivalents of the appropriate form of the ligand with NBu₄[AuL₂]. We have previously prepared the corresponding hexafluorophosphate salts via an alternate route.†

† No gold(I) complexes of dias; silver(I) compounds of (R,R)-dias, (R,R)-diph and (R*,S*)-tetraphos; and copper(I) complexes of (R)-AMPP, (R*,S*)-tetraphos and (S,S)-diph were studied in this work.

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Biological Studies. The in vitro cytotoxic properties of the gold(I), silver(I) and copper(I) complexes have been assessed by measuring their effect on proliferation of the mouse tumour cell lines P815 mastocytoma, B16 melanoma [gold(I) and silver(I) compounds] and P388 leukaemia [gold(I) complexes only].

Table 1. \(\text{IC}_{50}\) values for gold(I) complexes and cisplatin against P815 mastocytoma, B16 melanoma, and P388 leukaemia tumour models

| Complex | \text{IC}_{50} (\mu M) | P815 | B16 | P388 |
|---------|-----------------------|------|-----|------|
| \([\text{Au}(\pm\text{-AMPP})_2]^4\) | 9.25 | 2.60 | 5.80 |
| \([\text{Au}(\text{R}-\text{AMPP})_2]I\) | 9.00 | 3.00 | 5.50 |
| \([\text{Au}(\text{R},\text{R}^*)\text{-diph}]_2\] | 0.75 | 5.00 | 0.20 |
| \([\text{Au}(\text{R},\text{R})\text{-diph}]_2\] | 0.90 | 4.70 | 1.10 |
| \([\text{Au}(\text{S},\text{S})\text{-diph}]_2\] | 2.45 | 3.50 | 2.30 |
| \([\text{Au}_2(\text{R},\text{R}^*)\text{-tetraphos})_2](\text{PF}_6)_2\] | 0.87 | 0.55 | 0.60 |
| \([\text{Au}(\text{dppe})_2]^4\] | 0.22 | 5.20 | 0.10 |
| cis-\([\text{PtCl}_2(\text{NH}_3)_2]^4\] | 14.50 | 0.90 | 5.00 |

Table 2. \(\text{IC}_{50}\) values for silver(I) complexes against P815 mastocytoma and B16 melanoma mouse tumour cell models

| Complex | \text{IC}_{50} (\mu M) | P815 | B16 |
|---------|-----------------------|------|-----|
| \([\text{Ag}(\pm\text{-AMPP})_3]^4\) | 9.50 | 5.00 |
| \([\text{Ag}(\text{R}-\text{AMPP})_3]^4\) | 9.25 | 5.00 |
| \([\text{Ag}(\text{R},\text{R}^*)\text{-diph}]_2]^4\] | 0.14 | 4.00 |
| \([\text{Ag}(\text{S},\text{S})\text{-diph}]_2]^4\] | 1.62 | 4.30 |
| \([\text{Ag}(\text{R},\text{R})\text{-dias}]_2]^4\] | 0.75 | 1.30 |
| \([\text{Ag}(\text{S},\text{S})\text{-dias}]_2]^4\] | 0.32 | 1.40 |
| \([\text{Ag}_2(\text{R},\text{R}^*)\text{-tetraphos})_2](\text{PF}_6)_2\] | 0.04 | - |

Table 3. \(\text{IC}_{50}\) values for copper(I) complexes and the free ligands against P815 mastocytoma mouse tumour cell model

| Complex | \text{IC}_{50} (\mu M) |
|---------|-----------------------|
| \([\text{Cu}(\pm\text{-AMPP})_2]^4\) | 49.00 |
| \([\text{Cu}(\pm\text{-AMPP})_3]^4\) | 6.00 |
| \([\text{Cu}(\text{R},\text{R}^*)\text{-diph}]_2]^4\] | 0.14 |
| \([\text{Cu}(\text{R},\text{R})\text{-diph}]_2]^4\] | 1.45 |
| \([\text{Cu}(\text{R},\text{R}^*)\text{-dias}]_2]^4\] | 0.57 |
| \([\text{Cu}(\text{R},\text{R})\text{-dias}]_2]^4\] | 0.52 |
| \([\text{Cu}(\text{S},\text{S})\text{-dias}]_2]^4\] | 0.35 |
| \([\text{Cu}(\text{R},\text{R}^*)\text{-tetraphos}]_2]^4\] | 0.15 |
| \([\text{Cu}(\text{R},\text{R})\text{-tetraphos}]_2]^4\] | 0.16 |
| \([\text{Cu}(\text{S},\text{S})\text{-tetraphos}]_2]^4\] | 0.14 |

**Ligand**

\((\text{R},\text{R}^*)\text{-diph}\) 0.30
\((\text{R},\text{R})\text{-diph}\) 0.27
\((\text{S},\text{S})\text{-diph}\) 0.49
\((\text{S},\text{S})\text{-tetraphos}\) 0.85
The IC₅₀ values (concentrations resulting in 50% inhibition of labelled thymidine) for these complexes and the reference compounds cisplatin and [Au(dppe)₂] are given in Tables 1-3. IC₅₀ values for the ligands (R*,R*), (R,R) and (S,S)-diph, and (S,S)-tetraphos against the P815 mastocytoma cell line are also included in Table 3. The *in vitro* cytotoxicities of gold(I) and silver(I) complexes containing the optically active or *meso* [for Au(I) only] forms of the tetra(tertiary phosphine) tetraphos have also been examined against the human ovarian carcinoma cell lines 41M and CH1, the acquired cisplatin resistant 41McisR and CH1cisR tumour models, and the intrinsically cisplatin resistant SKOV-3 line. IC₅₀ values (determined via a sulforhodamine B assay) for these complexes are given in Table 4.

**Table 4. IC₅₀ values for gold(I) and silver(I) complexes containing (R,R)-, (S,S)-, and (R*,S*)-tetraphos against P815, 41M, 41McisR, SKOV-3, CH1 and CH1cisR tumour cell lines**

| Complex | IC₅₀ (µM) | P815 | 41M | 41McisR | SKOV-3 | CH1 | CH1cisR |
|---------|----------|------|-----|---------|--------|-----|--------|
| [Au₂{(R,R)-tetraphos}₂](PF₆)₂ | 0.82 | 0.42 | 0.55 | 0.47 | 0.40 | 0.20 |
| [Au₂{(S,S)-tetraphos}₂](PF₆)₂ | 0.95 | 0.39 | 0.43 | 0.45 | 0.34 | 0.16 |
| [Au₂{(R*,S*)-tetraphos}₂](PF₆)₂ | - | 0.35 | 0.47 | 0.30 | 0.31 | 0.15 |
| [Ag₂{(R,R)-tetraphos}₂](PF₆)₂ | 0.03 | 0.029 | 0.021 | 0.052 | 0.016 | 0.015 |
| [Ag₂{(S,S)-tetraphos}₂](PF₆)₂ | 0.02 | 0.029 | 0.022 | 0.077 | 0.016 | 0.012 |

**Discussion.**

*Nature of Complexes.* The structures of certain of the complexes used in this work have been confirmed by X-ray analysis. Apart from the gold(I) complex of (R)-AMPP, viz. [Au{(R)-AMPP}₂]PF₆, which is believed to have a linear geometry about the metal centre with the ligands bound in a monodentate fashion via the phosphorus donor atom, in all other cases a tetrahedral stereochemistry prevails. The complex [Ag{(R)-AMPP}₃]PF₆ is believed to contain one bidentate ligand and two ligands bound in a monodentate fashion via the phosphorus donor atom. A similar structure has been proposed for related complexes of the type [ML₃]PF₆ [where M = Cu(I) or Ag(I); and L = (2-aminophenyl)diphenylphosphine, ADPP, or (±)-AMPP]. Gold(I) and silver(I) complexes of tetraphos are dimeric in the solid state, they form bimetallic helicates having double-helical or side-by-side helical structures.

All of the complexes containing (R)-AMPP, dias or diph undergo rapid ligand exchange reactions in solution with the gold(I) compounds of the latter being the least kinetically labile. Ligand exchange reactions for complexes containing the tetra(tertiary phosphine) appear to be much slower, for example, no exchange between the gold(I) complexes and added free ligand in d₆ DMSO was observed in the ³¹P-{¹H} NMR spectra. Molar conductance measurements on gold(I), silver(I) and copper(I) complexes of tetraphos in acetonitrile, however, indicate that an equilibrium between monomeric and dimeric forms probably exists in solution.

*Biological Studies.* As previously noted, the nature of the metal ion and the counterion made very little difference to the cytotoxicity of the complexes. The silver(I) complexes of tetraphos were the exception. They exhibited significantly higher activities towards the P815 melanoma cell line than their gold(I) and copper(I) counterparts. This trend is not confined to one cell line but is clearly apparent in all of the cell lines tested including the cisplatin resistant models 41McisR, CH1cisR and SKOV-3. IC₅₀ values in the range 0.01 - 0.04 µM were determined for the silver(I) complexes of tetraphos. They are the most active of the compounds utilised in the present study.

Furthermore, the cytotoxicities of many of the complexes are comparable to that of cis-diammine-dichloroplatinum(II), cis-[PtCl₂(NH₃)₂], and bis[1,2-bis(diphenylphosphino)ethane]gold(I) iodide, [Au(dppe)₂]. Those containing the quadridentate tetra(tertiary phosphine) tetraphos were generally found to be more active than those incorporating the bidentate d(tertiary phosphines) diph and dppe, which in turn exhibited greater cytotoxicities than compounds with AMPP ligands. A similar trend is generally observed with respect to the kinetic stabilities of these complexes. The
data, however, do not reflect differences in kinetic stability based on the nature of the metal ion. The free tertiary phosphines themselves are also cytotoxic but generally to a significantly lesser extent than their metal complexes.

Complexes containing tertiary arsines are also active. The copper(I) and silver(I) compounds containing dias had a similar effect on the proliferation of P815 melanoma cells as their diph counterparts. The complexes of dias are significantly more labile than their diph analogues.\(^{10}\)

The chirality of the ligand had no apparent effect on the cytotoxicity of the complex. In most cases similar IC\(_{50}\) data were recorded irrespective of the isomeric form of the ligand coordinated to the metal ion. A ten-fold difference in activity was observed between [Ag\(((R\,*R\,^*)\,diph)\,PF_6\] and [Ag\(((S\,S\,-diph)\,PF_6\] against the P815 but not the B16 cell lines. This data supports the notion that the biological target of these complexes is different to that of cisplatin. In the case of chiral platinum-based complexes a significant difference in activity has been observed between the two enantiomers; those containing the \((R\,R)\) antipode of a range of optically active diamines were more cytotoxic than either the \((S\,S)\) or racemic forms of the corresponding ligands.\(^{6}\)

The IC\(_{50}\) values reported in this work were typically determined over a period of 22 h: treated samples were incubated for 18 h prior to the addition of \(^3\)H-thymidine and then further incubated for 4 h. The data reported in Table 4 (except against P815) was determined over a period of 96 h using a sulphorhodamine B assay. As we reported previously for gold(I) complexes containing \((\pm)\)-AMPP, (\(R\,^*R\,^*)\,-tetraphos, dppe or ADPP;\(^{5}\) a different reaction profile was observed when the \(^3\)H-thymidine incorporation assay was performed using the minimum time requirement for the experiment, i.e. \(^3\)H-thymidine was added after 0.25 h to the treated samples and then incubated for a further 0.5 h. No significant inhibition of DNA synthesis in the murine P388 leukaemia cell line was observed for cisplatin or [Au\(_2\,)\,(tetraphos)\,PF_6]^{2+} under these conditions. Complete inhibition of DNA synthesis takes ~20 h at a concentration of 10 \(\mu\)M and 100 \(\mu\)M for [Au\(_2\,)\,(tetraphos)\,PF_6]^{2+} and cisplatin, respectively. On the other hand, rapid inhibition of DNA synthesis was observed for gold(I) complexes containing AMPP, dppe or ADPP and furthermore their dose response did not substantially change over 24 h indicating that their effect was rapid and complete within 1 h. These complexes were also shown to cause a rapid increase in the mitochondrial membrane potential in intact P388 leukaemia cells which correlates with their early effect on DNA synthesis. The gold(I) complex of \((R\,^*R\,^*)\,-tetraphos, but not cisplatin, was also shown to cause an increase in the mitochondrial membrane potential when the experiment was performed over a period of 4 h. This mitochondrial activity has been shown to arise from inhibition of ATP synthase by the gold(I) complexes.\(^{5}\)

The IC\(_{50}\) data reported here further corroborate these earlier findings i.e. the primary target of gold(I), silver(I) and copper(I) complexes containing tertiary phosphine ligands is not DNA. A much more dramatic difference in IC\(_{50}\) values would have been expected for complexes containing different stereoisomeric forms of the same ligand, if this was the case. Rather these gold(I) complexes [and presumably their silver(I) and copper(I) analogues] act by rapid accumulation in mitochondria by virtue of their lipophilic cationic nature followed by inhibition of the ATP synthase which ultimately results in the observed inhibition of cellular proliferation.

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