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Structural and solution chemistry of gold(I) and silver(I) complexes of bidentate pyridyl phosphines: selective antitumour agents☆

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Contents

Abstract

The 1:2 adducts of Ag(I) and Au(I) with 1,2-bis(di-*n*-pyridylphosphino)ethane (d*n*pype) for $n = 2$, 3 and 4 have been synthesised and solution properties characterised by multinu-

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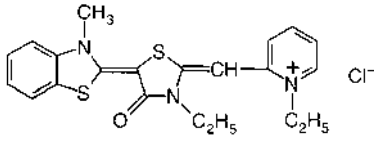
clear NMR spectroscopy. The complexes are hydrophilic analogs of the lipophilic Au(I) antitumour complex $[Au(dppe)_2]^+$ and the degree of hydrophilicity depends critically on the position of the N atom in the pyridyl ring. The complexes of d3pype and d4pype are simple monomeric $[M(d3pype)_2]^+$ and $[M(d4pype)_2]^+$ species which have a much higher water solubility than the 2-pyridyl complexes which crystallise in the solid state as dimeric $[\{M(d2pype)_2\}_2]^{2+}$. In solution these 1:2 M:d2pype species exist as equilibrium mixtures of monomeric, dimeric and trimeric (Ag) or tetrameric (Au) clusters. The Au(I) and Ag(I)pyridyl phosphine complexes have been evaluated for antitumour activity against a panel of cultured human ovarian carcinoma cell lines. The results show both potent and selective activity for the compounds with IC_{50} values ranging from 0.18 to 1500 μM. There is a correlation between the degree of antitumour selectivity and the octanol/water partition coefficients with the greatest selectivity (500-fold range) found for the most hydrophilic complex $[Au(d4pype)_2]Cl$. Clinical development of the parent compound $[Au(dppe)_2]^+$ was halted by liver toxicity and the hydrophilic pyridylphosphine analogs are significantly less toxic than $[Au(dppe)_2]^+$ when exposed to isolated rat hepatocytes. Convenient synthetic routes to the bidentate pyridyl phosphines d2pype, d3pype and d4pype are also described.

Keywords: Metallodrugs; Lipophilic cations; Pyridylphosphines; NMR spectroscopy

1. Introduction

New approaches are needed to overcome the two overriding problems in cancer chemotherapy-the common occurrence of drug-resistant tumour cells and the lack of selectivity of cancer drugs in differentiating between tumour cells and normal tissues. Most of the existing clinical drugs are non-selective, depending on the more rapid proliferation of cancer cells and act by targeting the DNA of tumour cells (by inhibiting biosynthesis or direct damage) and are susceptible to similar resistance mechanisms.

Lipophilic cations are provoking interest as a new class of antitumour drugs with the potential to selectively target mitochondria in tumour cells. These compounds concentrate in mitochondria due to their lipophilic-cationic character and exhibit preferential cytotoxicity to carcinoma cells with hyperpolarised membranes [1–4]. Over the past 20 years several structurally diverse lipophilic cations have demonstrated strong activity in tumour models, e.g. Rhodamine-123 [5], dequalinium [6], AA1 [7], bis-quaternary ammonium heterocycles [8] and triarylalkyl phosphonium salts [9]. Until recently their development was hindered by severe host toxicity but the first example of this class of agent (MKT-077 (**1**)) has entered phase 1 clinical trial [10].



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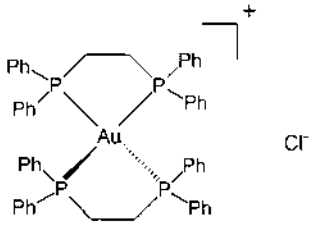
MKT-077 accumulates in tumour cell mitochondria inducing ultrastructural changes, loss of mitochondrial DNA, generalised perturbation of mitochondrial membrane and non-specific damage to membrane enzymes. In normal rat tissues it causes partial and reversible impairment of mitochondrial function [11].



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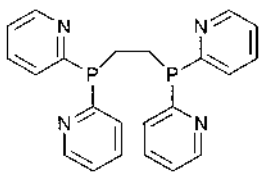
Metal diphosphine complexes such as the Au(I) complex $[Au(dppe)_2]^+$ (**2**) represent another class of lipophilic cationic antitumour agents. $[Au(dppe)_2]Cl$ was shown to exhibit a spectrum of antitumour activity in mouse tumour models [12]. There was some evidence for an antimitochondrial mode of action [13,14] as well as the occurrence of DNA strand breaks and DNA-protein cross-links in tumour cells [12]. For complexes of the type $[Au(R_2P(CH_2)_nPR'_2)_2]^+$ highest activity was found where $R = R' = Ph$ and $n = 2$, 3 or *cis*-CH=CH [15] and activity was retained when Au(I) was substituted by Ag(I) and Cu(I) [16–18]. Replacement of the phenyl substituents on the phosphine by other substituents, lead to a decrease, or loss of antitumour activity [18]. This may be related to the higher reactivity of alkyl- compared to aryl-phosphine towards disulphide bonds, and consequent oxidation (detoxification) in vivo [15,18].

Pre-clinical development of $[Au(dppe)_2]^+$ was abandoned after the identification of severe hepatotoxicity in dogs [19], attributed to alterations in mitochondrial function [20,21]. $[Au(dppe)_2]^+$ is extremely lipophilic (containing eight hydrophobic phenyl substituents) and consequently non-selectively targets mitochondria in *all* cells. Studies of isolated hepatocytes and hepatocyte mitochondria showed very rapid uptake of $[Au(dppe)_2]^+$, changes in O_2 consumption, mitochondrial membrane depolarisation, Ca^{2+} efflux, uncoupling of oxidative phosphorylation, ATP depletion and loss of hepatocyte viability within 1 h of drug exposure [20,21].

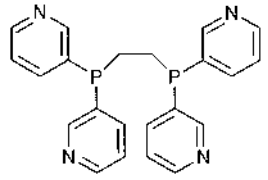
Several classes of lipophilic cations have demonstrated that antitumour selectivity is increased as the lipophilic-hydrophilic balance is varied (e.g. bisquaternary ammonium heterocycles [22] and triarylalkylphosphonium salts [9]). In recent work we have adopted the approach of modifying the diphosphine ligands of metal complexes related to $[Au(dppe)_2]^+$ in order to vary the hydrophilic character of the complexes and achieve greater selectivity for tumour cells versus normal cells. In order to retain aromatic substituents which appear to be important for antitumour activity [18] we have replaced some, or all by hydrophilic pyridyl groups. Our work was stimulated by the observation that the bis-chelated Au(I) complex $[Au(d2pype)_2]Cl$, (where d2pype is 1,2-bis(di-2-pyridylphosphino)ethane) was found to exhibit activity in mice bearing P388 leukaemia, whereas, the corresponding

4-pyridyl complex $[Au(d4pype)_2]Cl$ was inactive [15]. We have now shown that the position of the N atom in the pyridyl ring finely modulates the lipophilic-hydrophilic balance by influencing the structural types that exist for Au(I) and Ag(I) complexes. The different solubility profiles of these complexes influences their cellular uptake and hence differences in antitumour selectivity and potency.

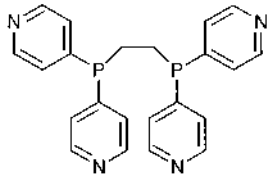
Convenient synthetic routes methods to the bidentate pyridyl phosphines $R_2P(CH_2)_2PR_2$ (R = 2-, 3- and 4-pyridyl) are also described.



d2pype



d3pype

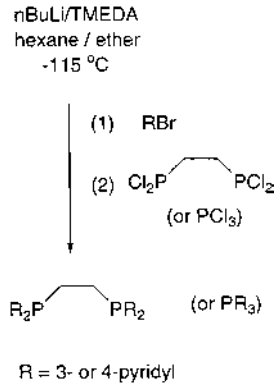


d4pype

2. Synthesis of bidentate pyridyl phosphine ligands

1,2-Bis(di-2-pyridylphosphino)ethane (d2pype) has been prepared previously by reaction of 2-pyridyllithium and 1,2-bis(dichlorophosphino)ethane in anhydrous ether [23,24]. However, the standard butyllithium metal halogen exchange method fails, or gives very low yields, when applied to the synthesis of 3- or 4-pyridyl substituted bidentate phosphines [24,25]. Similar problems have been encountered in the attempted synthesis of monodentate ligands such as tris-3- and tris-4-pyridylphosphines [26].

We investigated the possible reasons for the difficulties of synthesis of the 3- and 4-pyridyl phosphines by this method and found that while significant metal halogen exchange had occurred, compounds such as butyl substituted pyridines and dipyridines formed from addition and coupling reactions were major by-products. These reactions compete with nucleophilic attack by pyridyllithium on the chlorophosphine. In addition, the low yields of compounds indicated that

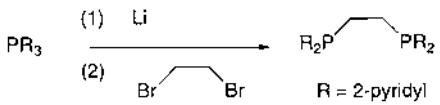


Scheme 1.

pyridyllithium was quite unreactive towards the chlorophosphine. We have overcome these problems by modification of the standard reaction conditions (Scheme 1) [27] in which the addition of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) increases the reactivity of the pyridyllithium towards the chlorophosphine while the combination of very low temperatures and careful timing in the addition of the various reagents suppress competitive addition/coupling reactions.

With this procedure the bidentate 3-and 4-pyridyl phosphines d3pype and d4pype were prepared in good to excellent yields [27]. The method should be generally applicable to the synthesis of a wide variety of mono- and bi-dentate 3- and 4-pyridyl tertiary phosphines, which until now have not been readily accessible [28].

The 2-pyridyl compound d2pype can be prepared also by an alternative method, involving treatment of 1,2-dibromoethane with two equivalents of lithium di-2-pyridyl-phosphide, generated from tris(2-pyridyl)phosphine and lithium metal (Scheme 2) [27]. This route is an attractive alternative to the use of $Cl_2P(CH_2)_2PCl_2$ which is hazardous to synthesise in the laboratory, and although commercially available, is expensive. Difficulties in obtaining the corresponding 3- and 4-pyridyl metal phosphides have so far precluded synthesis of compounds d3pype and d4pype by this route.



Scheme 2.

3. Structural and solution chemistry of 1:2 adducts with bidentate 2-, 3- and 4-pyridyl phosphines

3.1. Silver(I) complexes

In previous work the solution structures of 1:2 adducts of $AgNO_3$ with the bidentate aryl phosphines, dppe, dppp, *cis*-dppey, depe and eppe were investigated [29,30]. ^{31}P solution NMR studies of these complexes showed evidence for only monomeric, bis-chelated ionic complexes of type $[Ag(P–P)_2]NO_3$ with uncoordinated anion and bidentate phosphine ligands. These complexes have greatly enhanced kinetic and thermodynamic stabilities with respect to similar AgP_4 complexes containing monodentate phosphines. The ^{31}P spectra consist of two overlapping doublets (intensity ratio 51:49) in which the $^1J(^{31}P–^{107,109}Ag)$ spin–spin couplings are resolved at ambient temperature.

^{31}P-, ^1H- and ^{13}C-NMR spectra of the 1:2 adducts of $AgNO_3$ with d3pype and d4pype recorded in aqueous solution were consistent also with monomeric, bis-chelated structures. The $^1J(^{31}P–^{107,109}Ag)$ spin–spin couplings were resolved at ambient temperature and the values (ca. 230, 265 Hz) are typical of those expected for bis chelated complexes with tetrahedral AgP_4 coordination [31].

In contrast, ^{31}P-NMR solution spectra of the 1:2 adduct of Ag(I) with d2pype were considerably more complex. Variable temperature data recorded in $CH_3OH–CD_3OD$ solution are shown in Fig. 1. At 298 K the spectrum consisted of two overlapped doublets (δ 7.3, $^1J(^{107,109}Ag–^{31}P)$ 231, 266 Hz) and two pairs of broadened multiplets at δ 3.1 and 12.3. On cooling the solution the peaks sharpened and the pair of doublets at δ 7.3 gradually decreased in intensity while the other resonances increased in intensity. The fine structure of these multiplets was fully resolved at 263 K where a new set of peaks centred at δ 1.0, 6.1 and 15.9 became visible and increased in intensity with decreasing temperature. These spectra are interpretable as an equilibrium mixture of monomeric $[Ag(d2pype)_2]^+$, dimeric $[\{Ag(d2pype)_2\}_2]^{2+}$ and trimeric $[\{Ag(d2pype)_2\}_3]^{3+}$ species in which the d2pype ligands coordinate in both bridging and chelated modes via the phosphorus atoms (Scheme 3).

The assignment of the dimeric and trimeric structures was based on the results of ^{31}P-COSY and $[^{31}P–^{109}Ag]$ HMQC NMR spectra (Fig. 2). The COSY spectra showed cross-peaks between the sets of multiplets confirming that they correspond to two distinct spin-systems. In the $[^{31}P–^{109}Ag]$ HMQC spectrum $^{31}P–^{109}Ag$ two-dimensional cross-peaks were consistent with the expected two non equivalent P environments and one type of Ag environment for the dimer and three non-equivalent P environments and two non equivalent Ag environments for the trimer.

Crystals of the 1:2 adduct of $AgNO_3$ with d2pype obtained from CH_2Cl_2/Et_2O were shown by single crystal X-ray structure determination to be the dimer $[\{Ag(d2pype)(\mu\text{-}d2pype)\}_2][NO_3]_2 \cdot 2CH_2Cl_2$ [31]. Each silver ion is coordinated by one chelated and two bridging d2pype ligands forming a ten-membered ring in a double boat conformation. This structural type is rare for group 11 bidentate phosphine complexes, being recorded previously only for $[Ag(dppe)_2]_2(NO_3)_2$ [32],

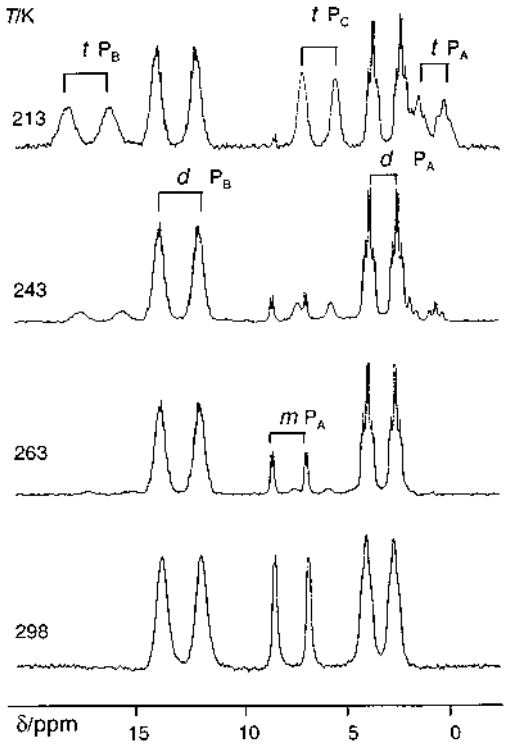
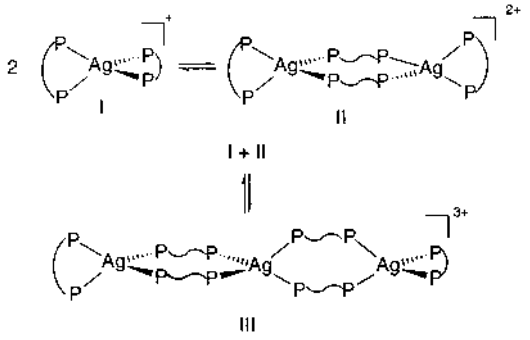


Fig. 1. 161.9 MHz ^{31}P-{^{1}H}-NMR spectra of $\{[Ag(d2pype)_2]NO_3\}_n$ in CH_3OH–30% CH_3OD at 298, 263, 243 and 213 K. The resonances are assigned to non-equivalent chelated (P_A) and bridging (P_B and P_C) phosphine environments in the monomeric (m), dimeric (d) and trimeric (t) species $\{[Ag(d2pype)_2]NO_3\}_n$ (Scheme 3) (Adapted from Ref. [31]).

$[Cu(dmpe)_2]_2(BF_4)_2$ [33] and $[Ag(dmpe)_2]_2(BPh_4)_2$ [34]; the majority of reported structures existing as the tetrahedral monomer [30,35].

^{31}P-NMR spectra recorded at 295 K for solutions of $\{[Ag(d2pype)_2]NO_3\}_n$ of equal concentration in a range of solvents showed the relative percentages of the



Scheme 3.

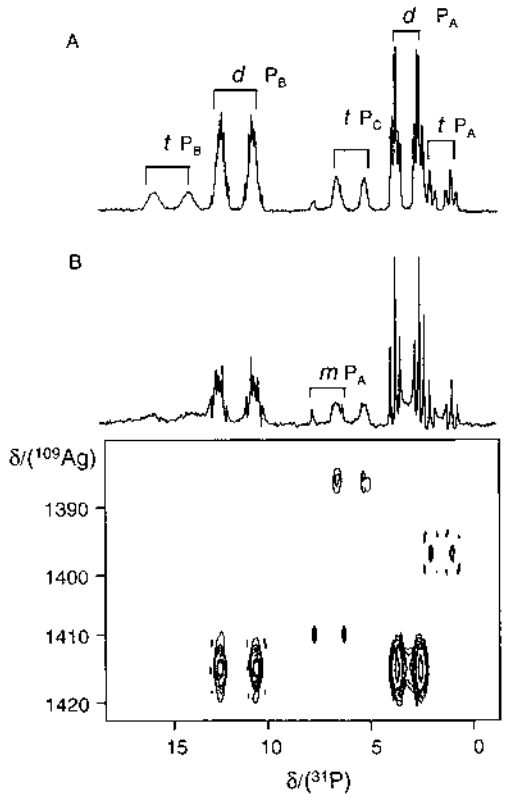


Fig. 2. The normal ^{31}P-{^{1}H}-NMR spectrum of a solution of {[Ag(d2pype)$_2$]NO_3}$_n$ at 243 K (A), and the [^{31}P, ^{109}Ag] spectrum in CD_3OD at the same temperature (B) (see Fig. 1 for assignments). (Adapted from Ref. [31]).

monomer and dimer in solution to be strongly solvent dependent with the concentration of the monomer decreasing from ca. 67% in $CHCl_3$ and CH_2Cl_2 to 19% in methanol and 12% in ethanol, while in acetonitrile, the dimer was found to be the only species present in the solution. ^{31}P-NMR signals assignable to both the monomeric and dimeric species were observed for {[Ag(d2pype)$_2$]$^+$}$_n$ in blood plasma at 37°C showing that association equilibria must be taken into account when considering the likely speciation of the complex in vivo [36].

3.2. Gold (I) complexes

As for the Ag(I) system evidence from ^{31}P-, ^{1}H- and ^{13}C-NMR spectra were consistent with simple monomeric, bis-chelated structures for the 1:2 adducts of Au(I) with d3pype and d4pype. Both [Au(d3pype)$_2$]Cl and [Au(d4pype)$_2$]Cl form hydroscopic, semi-crystalline solids and are soluble in water, dmso and methanol, partially soluble in ethanol and insoluble in CH_2Cl_2 [37]. The single crystal X-ray structure of [Au(d4pype)$_2$]Cl · HCl · 6H_2O shows a monomeric complex in which

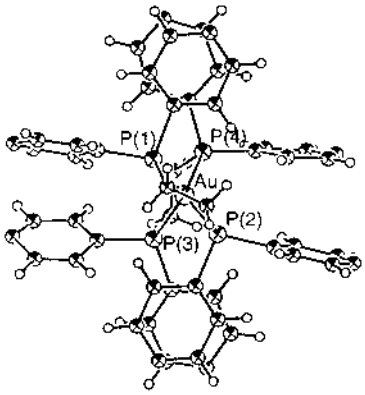


Fig. 3. Representative view of the $[Au(d4pype)_2]^+$ cation of $[Au(d4pype)_2]Cl \cdot HCl \cdot 6H_2O$. (Adapted from Ref. [37]).

one of the 4-pyridyl rings is protonated. A projection of the cation is shown in Fig. 3. The two ligands are coordinated in a bidentate fashion leading to a distorted tetrahedral geometry about the Au atom.

For the 1:2 adduct of Au(I) with d2pype the complex isolated from methanol solution has a dimeric structure as shown by the single crystal X-ray structure determination (Fig. 4). $[Au(d2pype)_2]_2Cl_2 \cdot 14H_2O$ consists of a dimeric cation, $[(d2pype)Au(\mu_2\text{-}d2pype)_2Au(d2pype)]^{2+}$ in which the two halves of the cation dimer are related by a centre of crystallographic symmetry with each gold atom coordinated to one bidentate and two bridging d2pype ligands with the bridging ligands and gold atoms forming a ten-membered ring in a double boat conformation [37]. The structure is similar to that determined for the analogous Ag(I) complex, and suggests that this dimeric structure is stabilized in the solid state

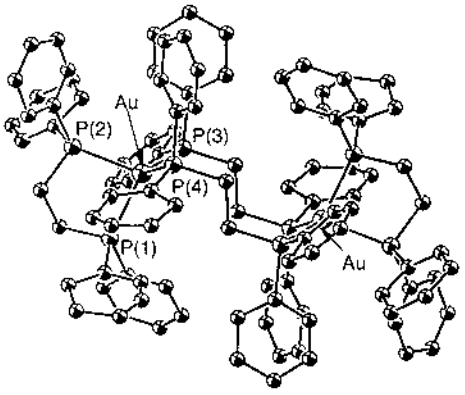


Fig. 4. Representative view of the dimeric $[Au_2(d2pype)_4]^{2+}$ cation of $[Au_2(d2pype)_4]Cl_2 \cdot 14H_2O$. (Adapted from Ref. [37]).

with respect to the monomer by the d2pype ligand. In contrast to $[Au(d3pype)_2]Cl$ and $[Au(d4pype)_2]Cl$, the 1:2 Au:d2pype complex is not significantly soluble in water but is soluble in dmso, dmf, methanol, ethanol and CH_2Cl_2.

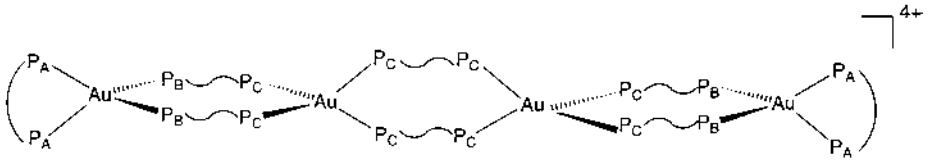
^{31}P-NMR studies of the 1:2 Au:d2pype adduct show a similar, but not identical, trend to those of the Ag(I) system. In dmf and CD_2Cl_2 solutions we observed single ^{31}P-NMR resonances attributable to monomeric $[Au(d2pype)_2]^+$, but in methanol dynamic behaviour was evident [37]. At 293 K, in addition to the $[Au(d2pype)_2]^+$ resonance at δ 26.8 a pair of multiplets were visible at δ 25.3 and 18.8. These were assignable to the dimeric complex $[\{Au(d2pype)_2\}_2]^{2+}$ based on a ^{31}P-COSY spectrum which showed cross-peaks between the two multiplets confirming that they are part of the same spin-system. On cooling the solution in the temperature range 293–253 K the $[Au(d2pype)_2]^+$ resonance gradually decreased in intensity while the two multiplet resonances attributable to the dimer increased in intensity. These data are consistent with a similar equilibrium between the monomer and dimer to that observed for the Ag(I) complex.

$$Au(d2pype)_2 + Au(d2pype)_2 \rightleftharpoons \{Au(d2pype)_2\}_2 \quad (1)$$

However, different behaviour was observed as the solution was cooled below ca. 253 K [37]. The intensity of the monomer peak remained constant whereas the multiplets of the dimer broadened and shifted apart. At temperatures below 243 K the low frequency multiplet split into two new broad peaks at δ 18.1 and 15.2 indicating the occurrence of further equilibria at low temperatures. Since there is little change in the concentration of the monomer it is possible that the equilibrium at low temperatures corresponds to the formation of the tetrameric cluster from the dimer, according to Eq. (2):

$$2\{Au(d2pype)_2\}_2 \rightleftharpoons \{Au(d2pype)_2\}_4 \quad (2)$$

A ^{31}P-COSY spectrum at 213 K, which showed connectivities between the three multiplets, and the intensity ratio P_A:P_B:P_C of 1:1:2 are consistent with the tetrameric cluster **3**.



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However, the connectivities in the ^{31}P-COSY spectrum are ambiguous as the peaks are very broad and there may be more than one species contributing to the central multiplet at δ 18.1. The possibility of aggregation to give other tetrameric or high order clusters can not be discounted.

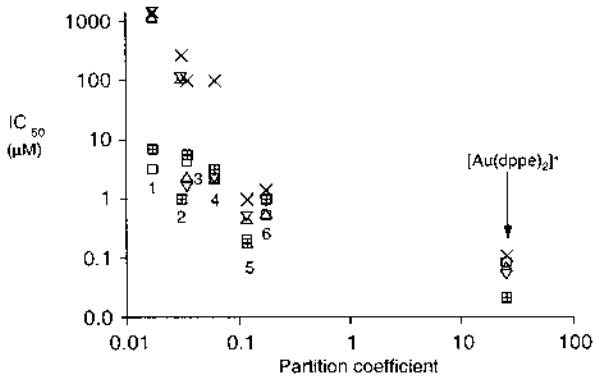

Fig. 5. Relationship between octanol-water partition coefficient and cytotoxic potency against cultured human ovarian tumour cells. The complexes are 1:2 M:P-P adducts of Au(I) (**1**,**3**,**5**) and Ag(I) (**2**,**4**,**6**) with the bidentate pyridyl phosphines d2pype (**5**,**6**), d3pype (**3**,**4**) and d4pype (**1**,**2**). The ovarian carcinoma cell-lines are CH1 (square), 41 M (triangle), cisplatin-resistant CH1cisR (quartered square) and 41McisR (upside down triangle) and the intrinsically cisplatin resistant SKOV3 (**X**). From Refs. [38,39].

4. Biological activity

4.1. In vitro activity against human tumour cell-lines

The 1:2 adducts of Au(I) and Ag(I) with the bidentate pyridylphosphines d2pype, d3pype and d4pype have octanol/water partition coefficients between 0.02 and 0.18 and are thus considerably more hydrophilic than the parent compound $[Au(dppe)_2]Cl$ (partition coefficient = 25.4). The compounds have been evaluated for antitumour activity against a panel of human ovarian carcinoma cell lines in vitro [38,39]. These include examples of cisplatin-sensitive (CH1, 41M) and -resistant cell lines with both acquired (CH1cisR, 41McisR) and intrinsic (SKOV3) resistance to cisplatin. These results show potent and selective activity for the hydrophilic metal phosphine complexes with IC_{50} values ranging from 0.18 to 1500 μM. There is a correlation between the degree of selectivity and octanol/water partition coefficients. (Fig. 5). For example, the Au(I) 4-pyridyl complex (most hydrophilic) exhibits the greatest selectivity (500-fold range) showing activity against the CH1 and CH1cisR cell lines (IC_{50} 3.09, 6.82 μM), but no activity in the 41M pair and SKOV3 ($IC_{50} > 1000$ μM). Increasing the lipophilicity (as in the Ag(I) 3-pyridyl complex) improves activity in the 41M pair, while retaining activity against the CH1 pair, but SKOV3 is resistant. The Au(I) 2-pyridyl complex is cytotoxic to the CH1 and 41M pairs (IC_{50} 0.2–0.5 μM) as well as the intrinsically cisplatin-resistant SKOV3 (1.0 μM). There is a general increase in potency with increase in lipophilicity (Fig. 5).

4.2. In vitro hepatotoxicity studies.

Because the development of $[Au(dppe)_2]^+$ was halted by liver toxicity we are examining the toxicology of the analogs using isolated rat hepatocytes. Preliminary studies [38,39] demonstrate that the hydrophilic analogues are significantly less toxic than the parent compound. The octanol-water partition coefficient predicted their hepatotoxicity. Significantly, whereas cultures of isolated hepatocytes exposed to $[Au(dppe)_2]^+$ leaked lactate dehydrogenase in a concentration and time dependent manner, the Au(I) 4-pyridyl complex showed no evidence of damage to isolated rat hepatocytes exposed to concentrations of up to 100 μM for up to 24 h.

5. Concluding remarks

The 1:2 complexes of both Ag(I) and Au(I) with bidentate pyridylphosphine ligands are more hydrophilic than the phenyl-substituted analogs and the degree of hydrophilicity depends critically on the position of the pyridyl N atom. The complexes of d3pype and d4pype are simple monomeric $[M(d3pype)_2]^+$ and $[M(d4pype)_2]^+$ species. These have a much higher water solubility than the 2-pyridyl complexes which crystallise in the solid state as dimeric $[\{M(d2pype)_2\}_2]^{2+}$. The increased hydrophilic character of the monomeric 3-pyridyl and 4-pyridyl complexes is likely to be a consequence of the more exposed N atoms. The d2pype complexes exist in solution as equilibrium mixtures of monomeric, dimeric and trimeric (Ag) or tetrameric (Au) clusters. The underlying reasons why this behaviour is observed for d2pype complexes and not for dppe, d3pype or d4pype complexes is not apparent, but it is notable that the dimeric structure is known for the complex $\{[Ag(dppe)_2]^+\}_2$. Thus it is likely that $[M(P–P)_2]^+$ cations in these systems are not isolated species in solution but interact strongly with solvent according equilibrium (Eq. (3)) and for the d2pype systems the equilibrium is shifted to the right.

$$2[M(P–P)_2]^+ X^- \text{solv} \rightleftharpoons [\{M(P–P)_2\}_2]^{2+} 2X^- \text{solv} \quad (3)$$

Although the position of the pyridine nitrogen in the ring influences this chemistry we found no evidence for the coordination of the pyridyl N atoms to the metal in either the solid state or in solution. 2-Pyridyl phosphines have been shown to coordinate to Ag(I) in a P, N bidentate fashion [40], but only in situations when chloro/phosphorus coordination is not sufficient for coordinative saturation. In the present case there is an apparent strong preference for the MP_4 coordination sites through either chelation or bridging coordination of the diphosphine ligands.

The structural and solution chemistry of these complexes are relevant to the interpretation of their differing antitumour activities. The 2-pyridyl and 4-pyridyl analogs of $[Au(dppe)_2]Cl$ were evaluated previously for antitumour activity in mice bearing i.p P388 leukaemia and whereas the 2-pyridyl complex had comparable activity to $[Au(dppe)_2]Cl$, the 4-pyridyl analog was inactive. These differences may be related, at least in part, to differences in their uptake into cells as a consequence

of their different hydrophilic character. We have shown by ^{31}P-NMR experiments that whereas the 2-pyridyl complex readily partitions between plasma and red blood cells, the water soluble 4-pyridyl complex is retained in the blood plasma fraction. We are currently investigating the relationships between lipophilicity, cellular uptake and antitumour selectivity (in vitro and in vivo) of complex of this type using inductively coupled plasma mass spectrometry.

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