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Synthesis, characterisation and in vitro evaluation of platinum(II) and gold(I) iminophosphine complexes for anticancer activity

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ABSTRACT

The reactions of iminophosphine ligands with $[PtCl_2(COD)]$, $[PtCl_2(DMSO)_2]$, and [Au(tht)Cl] has been investigated. The new platinum(II) and gold(I) complexes were characterised using elemental analysis, electrospray ionisation-mass spectrometry (ESI-MS), NMR (^1H and ^{31}P) and IR spectroscopy and X-ray diffraction studies. In vitro cytotoxic study results show that platinum and gold complexes block the proliferation of WHCO1 and KYSE450 cell lines with an IC_{50} range of 2.16–9.47 μM.

1. Introduction

The rapidly growing field of organometallic chemistry has expanded into the scope of biology and medical applications [1–3]. Metal complexes are also of great interest in bioinorganic chemistry. According to the International Agency for Research on Cancer (IARC), in the year 2005, oesophageal cancer was reported as the ninth most common cancer in the world, and the sixth leading cause of cancer-related deaths worldwide [4]. A unique epidemiological feature of oesophageal cancer is its very uneven geographic distribution, with high incidence found within sharply demarcated geographic confines [5]. These geographic 'hot spots' include areas in Northern Iran, Kazakhstan, South Africa, and Northern China [6]. On the African continent, a number of reports have documented a very high incidence of oesophageal cancer in South Africa, particularly in the Transkei districts [7].

Cancer is fundamentally a disease, in which the cells proliferate indefinitely, due to dysregulated control pathways. Consequently, cancer cells continue to grow and divide yielding an ever increasing mass referred to as a tumour [8]. The tumour grows invasively, destroying surrounding body tissues. Cancer cells from this primary tumour may then spread, or metastasize, to other parts of the body, where new tumours may begin to grow. Eventually the tumour load will cause death, often by physically blocking or compressing blood vessels or organs such as the brain, or disrupting critical processes.

Cancer makes a contribution to morbidity and mortality worldwide, with the International Agency for Research on Cancer (IARC) estimating that for 2008, there were 12.4 million new cases of cancer, 7.6 million deaths from cancer and 28 million people alive with cancer within 5 years from initial diagnosis. They do report that about half of the cases and about 60% of the mortalities were from medium and low income countries [9].

Ever since its approval in 1978 for the treatment of testicular and ovarian cancer, cisplatin has been used extensively in cancer therapy [10]. Transition metal-based anticancer drugs such as cisplatin have found widespread use, since they form highly reactive, charged, platinum complexes that bind to nucleophilic groups such as GC-rich sites in DNA, inducing DNA cross-links that result in apoptosis and cell growth inhibition. In SA, like in other countries worldwide, cisplatin is the drug of choice when treating oesophageal cancer with chemotherapeutic agents. However, cisplatin has numerous shortcomings, including induced cellular resistance, severe toxicity, and a spectrum of activity limited to a narrow range of tumor types. This has prompted considerable efforts to develop novel metal-based anticancer agents that could circumvent the limitations of cisplatin. As a result, thousands of platinum-based compounds have been synthesized and screened for toxicity and anticancer activity, although very few have been approved for clinical use [11–15].

Auranofin, an orally absorbed gold(I) phosphine complex, had been used for the treatment of rheumatoid arthritis [16], and early reports also suggested that this compound and other gold phosphine complexes were also active against the growth of cultured

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tumour cells [17–20]. Auranofin itself has proved cytotoxic against HeLa cells in vitro and P388 leukaemia cells in vivo [21,22]. Recent advances in medicinal inorganic chemistry demonstrate significant prospects for the utilization of Au(I) and Au(III) and their coordination complexes as drugs, presenting an interesting arena for inorganic chemistry. Significant progress in gold based anticancer agents has been achieved, based in part on a mechanistic understanding of the pharmacological effects of classical antitumor drugs. There are quite a number of results indicating that gold coordination compounds might be developed into future drugs, but it will be a long time before their pharmacological potential can be realised. It will take even longer to have a suitable candidate turned into a clinically acceptable drug. The future development of medicinal inorganic chemistry of Au(I) and Au(III) and their coordination complexes requires an understanding of the physiological processing of gold complexes, to provide a rational basis for the design of new gold-based drugs.

Here we report the synthesis and characterisation of novel platinum(II) and gold(I) complexes with iminophosphine ligands and the examination of their cytotoxic properties.

2. Experimental

2.1. Materials

All solvents were purified prior to use by standard literature procedures and were stored under nitrogen in Teflon sealed storage bottles. All reagents were purchased from Aldrich and used without further purification. The ligands L1–L5 [23], $[PtCl_2(COD)]$ [24,25], $[PtCl_2(DMSO)_2]$ [26–28] and [Au(tht)Cl] [29] were prepared as described in the literature.

2.2. Physical measurements

^{1}H NMR spectra were recorded in DMSO-d_6 at room temperature on either a Varian Mercury-300 or a Varian Unity-400 spectrometer operating at 300.13 and 75.5 MHz or 400.13 and 100.6 MHz. Chemical shifts are reported relative to TMS on delta (δ) scale in parts per million (ppm). The splitting of proton resonances in the reported ^{1}H NMR spectra are defined as s = singlet, d = doublet, t = triplet, m = multiplet, w = weak and br broad. Melting points were determined on a Reichert-Jung Thermovar hotstage microscope and are uncorrected. Infrared spectra (KBr pellets) were recorded on a Thermo Nicolete FT-IR instrument and are reported using the following abbreviation: s = strong, m = medium, strong and br = broad. Microanalyses were determined using a Fisons EA 1108 CHNO-S instrument. Mass spectra (MS) were recorded on a Waters atmosphere pressure ionization quadruple time-of-flight (API Q-TOF) Ultima (electronspray ionization (ESI), 70 eV) and/or SA VG70-SEQ (Fast atom bombardment (FAB), 7 kV) instrument.

2.3. Synthesis

2.3.1. General procedure for the preparation of platinum(II) complexes (**1**–**5**)

To a solution of the appropriate ligand (**L1**–**L5**) in dry CH_2Cl_2 (10 ml) was added an equimolar amount of $[PtCl_2(COD)]$ or $PtCl_2(DMSO)_2]$ dissolved in dry CH_2Cl_2 (10 ml). The reaction was allowed to stir at room temperature for 4 h before reducing the solvent to *ca* 5 ml and precipitating the products using hexane. The products were filtered, washed with Et_2O and dried under vacuum.

2.3.2. Spectroscopic and analytical data for **1**

Yellow powder. Yield: 76%. M.p.: 201–203 °C. *Anal.* Calc. for $C_{31}H_{32}Cl_2NPPt$: C, 52.03; H, 4.51; N, 1.96. Found: C, 52.19; H, 4.74; N, 1.99%. MS (EI, *m*/*z*): 678.34, $[M-Cl]^+$. IR (KBr) (ν_{max}/cm^{-1}): 1624 s ν(C N). ^{1}H NMR ($CDCl_3$): δ_H (ppm): 8.95 (d, 1H, *J* = 111.3 Hz) 8.18 (dd, 1H, *J* = 4.2 Hz, *J* = 9.0 Hz) 7.90 (m, 2H) 7.60 (m, 10H) 7.18 (m, 4H) 3.01 (m, 2H) 1.24 (d, 6H, *J* = 6.8 Hz) 0.83 (d, 6H, *J* = 6.8 Hz). ^{31}P NMR ($CDCl_3$; ppm) 5.83 [*J* (^{195}Pt–^{31}P): 3661 Hz].

2.3.3. Spectroscopic and analytical data for **2**

Light yellow powder. Yield: 65%. M.p.: 180–183 °C. *Anal.* Calc. for $C_{26}H_{22}Cl_2NPPt$: C, 48.38; H, 3.44; N, 2.17. Found: C, 48.19; H, 3.12; N, 2.47%. MS (EI, *m*/*z*): 609.36, $[M-Cl]^+$. IR (KBr) (ν_{max}/cm^{-1}): 1632 s ν(C N). ^{1}H NMR ($CDCl_3$): δ_H (ppm): 7.79 (m, 1H) 7.60 (t, 1H, *J* = 7.4 Hz) 7.47 (t, 1H, *J* = 7.4 Hz) 7.07 (t, 1H, *J* = 8.6 Hz) 6.90 (t, 1H, *J* = 7.7 Hz) 6.49 (d, 1H, *J* = 7.2 Hz) 4.13 (t, 1H, *J* = 12.8 Hz). ^{31}P NMR ($CDCl_3$; ppm) 8.09 [*J* (^{195}Pt–^{31}P): 3668 Hz].

2.3.4. Spectroscopic and analytical data for **3**

Yellow powder. Yield: 76%. M.p.: 168–169 °C. *Anal.* Calc. for $C_{25}H_{21}Cl_2N_2PPt$: C, 46.45; H, 3.27; N, 4.33. Found: C, 46.19; H, 3.12; N, 4.72%. MS (EI, *m*/*z*): 610.91, $[M-Cl]^+$. IR (KBr) (ν_{max}/cm^{-1}): 1630 s ν(C N). ^{1}H NMR ($CDCl_3$): δ_H (ppm): 9.19 (m, 1H) 8.64 (m, 1H) 8.53 (d, 1H, *J* = 4.3 Hz) 8.05 (dd, 1H, *J* = 4.1 Hz, *J* = 6.9 Hz) 7.92 (t, 1H, *J* = 7.5 Hz) 7.84 (t, 1H, *J* = 7.4 Hz) 7.60 (m, 2H) 7.41 (m, 3H) 7.09 (m, 5H) 5.80 (d, 2H, *J* = 18.7 Hz). ^{31}P NMR ($CDCl_3$; ppm) 8.44 [*J* (^{195}Pt–^{31}P): 3469.9 Hz].

2.3.5. Spectroscopic and analytical data for **4**

Dark yellow powder. Yield: 72%. M.p.: 198–199 °C. *Anal.* Calc. for $C_{24}H_{20}Cl_2NOPPt$: C, 48.83; H, 3.77; N, 2.28. Found: C, 48.69; H, 3.52; N, 2.52%. MS (EI, *m*/*z*): 599.91, $[M-Cl]^+$. IR (KBr) (ν_{max}/cm^{-1}): 1633 s ν(C N). ^{1}H NMR ($CDCl_3$): δ_H (ppm): 9.06 (m, 1H) 8.02 (m, 1H) 7.88 (td, 2H, *J* = 7.4 Hz, *J* = 19.3 Hz) 7.60 (t, 2H, *J* = 7.2 Hz) 7.49 (t, 4H, *J* = 6.4 Hz) 7.22 (ddd, 6H, *J* = 15.4 Hz, *J* = 23.5 Hz, *J* = 30.5 Hz) 6.55 (m, 2H) 5.80 (d, 2H, *J* = 13.0 Hz). ^{31}P NMR ($CDCl_3$; ppm) 5.66 [*J* (^{195}Pt–^{31}P): 3777.6 Hz].

2.3.6. Spectroscopic and analytical data for **5**

Pale yellow powder. Yield: 72%. M.p.: 201–203 °C. *Anal.* Calc. for $C_{24}H_{20}Cl_2NPPtS$: C, 44.25; H, 3.09; N, 2.15; S, 4.92. Found: C, 44.19; H, 3.12; N, 2.42; S, 5.03%. MS (EI, *m*/*z*): 616.01, $[M-Cl]^+$. IR (KBr) (ν_{max}/cm^{-1}): 1631 s ν(C N). ^{1}H NMR ($CDCl_3$): δ_H (ppm): 8.73 (s, 1H) 7.86 (m, 1H) 7.60 (m, 1H) 7.44 (m, 1H) 7.24 (dd, 1H, *J* = 1.2 Hz, *J* = 5.1 Hz) 7.06 (ddd, 1H, *J* = 1.6 Hz, *J* = 7.2 Hz, *J* = 10.6 Hz) 6.77 (dd, 1H, *J* = 3.4 Hz, *J* = 5.1 Hz) 6.50 (dd, 1H, *J* = 1.0 Hz, *J* = 3.4 Hz) 4.77 (t, 1H, *J* = 7.4 Hz). ^{31}P NMR ($CDCl_3$; ppm) 8.06 [*J* (^{195}Pt–^{31}P): 3758.5 Hz].

2.3.7. General procedure for the preparation of gold(I) complexes (**6**–**9**)

To the appropriate ligand (**L1, L3**–**L5**) in dry CH_2Cl_2 (10 ml) was added [Au(tht)Cl] also in dry CH_2Cl_2 (10 ml) in a ratio of 1:0.9. The reaction was allowed to stir at room temperature for *ca* 2 h before reducing the solvent to *ca* 5 ml and precipitating out the products with hexane, filtering under gravity and washing the precipitate with dry Et_2O and drying under vacuum for 4 h.

2.3.8. Spectroscopic and analytical data for **6**

Pale yellow powder. Yield: 73%. M.p.: 200–201 °C. *Anal.* Calc. for $C_{31}H_{32}AuClNP$: C, 54.60; H, 4.73; N, 2.05. Found: C, 54.88; H, 4.57; N, 1.97%. MS (EI, *m*/*z*): 449.31, $[M-Cl-Au]^+$. IR (KBr) (ν_{max}/cm^{-1}): 1628 s ν(C N). ^{1}H NMR ($CDCl_3$): δ_H (ppm): 8.90 (s, 1H) 8.41 (dd, 1H, *J* = 4.5 Hz, *J* = 7.2 Hz, ArH) 7.60 (t, 1H, *J* = 7.5 Hz, ArH) 7.41 (m, 11H, ArH) 6.94 (m, 3H, ArH) 6.79 (dd, 1H, *J* = 7.8 Hz, *J* = 13.2 Hz)

2.47 (td, 2H, J = 6.8 Hz, J = 13.7 Hz, –*CH*Me$_2$) 0.89 (d, 12H, J = 6.8 Hz, –CH*Me*$_4$). ^{31}P NMR ($CDCl_3$; ppm) 26.64.

2.3.9. Spectroscopic and analytical data for ***7***

Off white powder. Yield: 53%. M.p.: 215–217 °C. *Anal.* Calc. for $C_{25}H_{21}AuClN_2P$: C, 49.00; H, 3.45; N, 4.57. Found, C, 49.22; H, 3.23; N, 4.54%. IR (KBr) (ν_{max}/cm^{-1}): 1630 s ν(C N). ^{1}H NMR ($CDCl_3$): δ_H (ppm): 8.64 (s, 1H) 8.33 (d, 1H, J = 4.5 Hz) 8.28 (d, 1H, J = 5.8 Hz) 8.04 (m, 1H, ArH) 7.78 (m, 1H) 7.55 (m, 1H, ArH) 7.33 (m, 1H) 7.17 (m, 1H, ArH) 7.02 (dd, 1H, J = 5.0 Hz, J = 7.9 Hz, ArH) 6.79 (dt, 1H, J = 8.3 Hz, J = 13.6 Hz, ArH) 4.70 (s, 1H, ArH) 4.60 (s, 2H). ^{31}P NMR ($CDCl_3$; ppm) 31.38.

2.3.10. Spectroscopic and analytical data for ***8***

Pale yellow powder. Yield: 59%. M.p.: 220–221 °C. *Anal.* Calc. for $C_{24}H_{20}AuClNOP$: C, 46.90; H, 3.35; N, 2.33. Found: C, 46.77; H, 3.48; N, 2.09%. MS (EI, m/z): 563.87, $[M-Cl]^+$. IR (KBr) (ν_{max}/cm^{-1}): 1634 s ν(C N). ^{1}H NMR ($CDCl_3$): δ_H (ppm): 8.54 (d, 1H, J = 1.4 Hz) 7.82 (dd, 2H, J = 4.8 Hz, J = 7.1 Hz, ArH) 7.37 (m, 3H, ArH) 7.17 (m, 2H, ArH) 7.13 (dd, 4H, J = 0.8 Hz, J = 1.8 Hz, ArH) 6.75 (dd,2H, J = 7.8 Hz, J = 13.1 Hz) 6.13 (dd, 1H, J = 1.9 Hz, J = 3.1 Hz) 5.91 (d, 1H, J = 3.2 Hz) 4.53 (s, 2H). ^{31}P NMR ($CDCl_3$; ppm) 30.51.

2.3.11. Spectroscopic and analytical data for ***9***

Off-white powder. Yield: 55%. M.p.: 230–232 °C. *Anal.* Calc. for $C_{24}H_{20}AuClNPS$: C, 46.65; H; 3.26; N, 2.27; S, 5.19. Found: C, 46.80; H, 3.42; N, 2.45; S, 4.97%. MS (EI, m/z): 581.38, $[M-Cl]^+$. IR (KBr) (ν_{max}/cm^{-1}): 1635 s ν(C N). ^{1}H NMR ($CDCl_3$): δ_H (ppm): 8.59 (d, 1H, J = 1.4 Hz) 8.35 (m, 1H, ArH) 7.84 (m, 2H, ArH) 7.68 (dd, 3H, J = 4.6 Hz, J = 7.2 Hz, ArH) 7.39 (m, 2H, ArH) 7.17 (m, 4H, ArH) 7.04 (dd, 2H, J = 1.1 Hz, J = 5.1 Hz, ArH) 6.96 (dd, 1H, J = 1.1 Hz, J = 5.1 Hz) 6.77 (m, 1H) 6.55 (m, 1H) 4.74 (s, 2H). ^{31}P NMR ($CDCl_3$; ppm) 31.93.

2.4. X-ray crystallography

Single crystals for X-ray data collections regarding **1** and **6** were grown by the slow evaporation of CH_2Cl_2 solutions of their complexes at room temperature. X-ray intensity data were collected on a Nonius Kappa-CCD diffractometer with 1.5 kW graphite monochromated Mo Kα radiation. The structures were solved by direct methods using SHELXS-97 and refined employing full-matrix least-squares with the program SHELXL-97 [30,31] refining on F^2.

2.5. Biological studies

2.5.1. MTT assay

1500 cells were seeded per well in 90 μl DMEM in 96 well plates. Cells were incubated for 24 h, then test samples were plated at a range of concentrations in 10 μl media, with a final concentration of 0.2% DMSO. After 48 h of incubation, the cells were observed under a phase contrast microscope and the general appearance of the cells together with confluency status and presence of precipitate if any was recorded. 10 μl of MTT reagent was added per well at the end of the experiment, and the plates incubated for 4 hours at 37 °C. 100 μl of Solubilisation solution was then added to each well, and plates were incubated at 37 °C overnight. After 16 h, the plates were read at 595 nm on an Anthos microplate reader 2001.

2.5.2. Western blotting

Cells prepared for western blotting were routinely plated at 500 000 cells/60 mm dish. Cells were incubated for 24 h, then treated with the indicated concentrations of compounds for the indicated times. Cell lysates from cells treated with 2× IC_{50} for complexes **1**, **4**, **6**, **8** were harvested at 12, 24 and 48 h and analysed by Western blot. p38 was used as a loading control. Treatment of cells with 10 μM doxorubicin served as a positive control. These results are representative of two independent experiments.

2.5.3. Cell cycle analysis

Cells were seeded in 60 mm dishes at 0.25×10^6 cells per dish. Following an overnight settling period, compounds were added to culture media (to avoid the loss of cycling cells that had detached during division). At appropriate time points, cells were harvested by trypsinisation (taking care to collect all washes to avoid discarding floating cells), resuspended in 1 ml PBS, and counted. 9 ml of ice cold 100% EtOH was then added to each sample, and samples were stored at −20 °C for up to 2 weeks. Cells were then centrifuged out of EtOH, rinsed several times in PBS, and 5×10^6 cells/ml were incubated in 50 μg/ml RNAse A in PBS for 30 min at room temperature. Cell Cycle Staining solution was added to bring the cell concentration to 1×10^6 cells/ml, and following a 30 min incubation in the dark, cells were analysed on a Beckman Coulter FACSCalibur flow cytometer. Analysis of cell cycle results was carried out using ModFit 3.0 (Verity Software House).

3. Results and discussion

3.1. Synthesis and characterisation of Pt(II) and Au(I) complexes

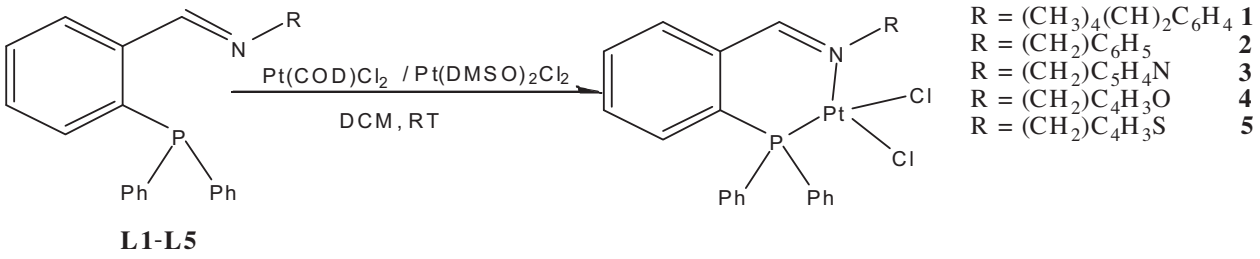
The platinum(II) iminophosphine complexes **1**–**5** were obtained in reasonable yields (65–76%) by reacting [$PtCl_2$(COD)] or [$PtCl_2$(DMSO)$_2$] with L_1–L_5 ligands in a 1:1 molar ratio at room temperature in CH_2Cl_2 (Scheme 1). The platinum compounds were isolated as pale to dark yellow powders and are air stable.

The gold(I) iminophosphine complexes were obtained by reacting [Au(tht)Cl] with L1 and L3–L5 at room temperature (Scheme 2). These were isolated as off white to pale yellow powders which are also air stable.

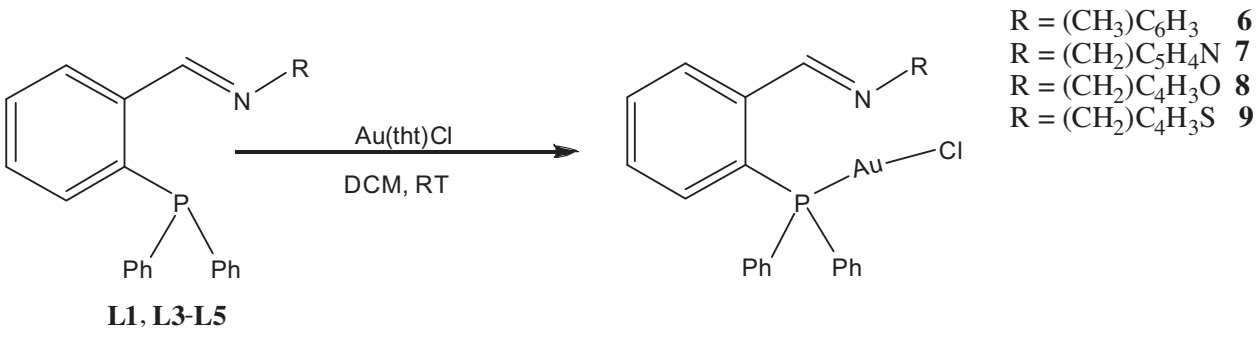
Both platinum and gold complexes are soluble in $CHCl_3$, CH_2Cl_2 and DMSO, less soluble in MeCN and insoluble in MeOH or EtOH.

The platinum complexes **1**–**5** displayed sharp singlets flanked by platinum satellites in their ^{31}P NMR spectra. The ^{31}P NMR spectra of the platinum complexes provides a sensitive probe for the structures of complexes. The one-bond coupling $^1J(^{195}Pt-^{31}P)$ is characteristic of these complexes and those which contain Cl atoms *trans* to phosphorus atoms have been found to possess coupling constants greater than 3500 Hz [32]. ^{31}P NMR data for the complexes **6**–**10** show a downfield shift of between 19.76 and 21.86 ppm on complexation of the ligands to the metal centre, with the most significant shift arising from formation of complex **10** indicating that the P centre of the ligand is coordinated to the metal. The coordination chemical shift observed for complexes **6**–**10** is comparable to that reported previously [33]. There is a strong similarity in chemical shift observed among all the complexes *e.g* δ_p values for the platinum complexes are almost identical, indicating that the substituents on the aldehyde constituent of the iminophosphine ligand have little influence on the value of the shift. This observation is also consistent with previous reports of similar types of ligands [33]. The signal due to the P centre in the platinum complexes is shifted *ca* 20 ppm upfield. This trend was also noted previously for platinum complexes of the [P, N, O] ligand 2-[$Ph_2PC_6H_4CH{=}N]C_6H_3OH$ [33]. The presence of Pt satellites in the spectra of complexes **1**–**5** is further evidence of coordination by the P centre.

The most informative peak in the IR spectra of iminophosphine complexes of platinum is that of the C=N group occurring in the 1600 cm^{-1} region. Comparison of the position of this peak in complexes **1**–**5** with that of the corresponding ligands **L1**–**L5** shows a shift to lower wavenumbers on complexation of between 5 and



Scheme 1. Synthesis of platinum(II) dichloride complexes.



Scheme 2. Synthesis of gold(I) chloride complexes.

9 cm^{-1}. This shift is in agreement with previous observations on metal complexes of similar types of ligands [35,37,39].

The positive-ion EI-MS spectra of all complexes contained molecular ion peaks at *m/z* 678.34 (**1**), 609.36 (**2**), 610.92 (**3**), 599.91 (**4**) and 616.01 (**5**). The fragmentation patterns arising from the loss of a Cl was evident in each spectrum of the proposed structures.

3.2. X-ray diffraction studies

To further confirm the integrity of these complexes, crystals suitable for X-ray structure determination were obtained for complexes **1** and **6**. Crystals were grown from dichloromethane at room temperature. Details of the crystal parameters, data collection and refinements were listed in Table 1.

The molecular structures for complexes **1** and **6** with atomic numbering scheme are shown in Figs. 1 and 2, respectively. Selected bond lengths and angles are given in Tables 2 and 3.

The platinum complex is of distorted square planar geometry with the P–Pt–N angle at 89.80(5)° and the corresponding angle between the chloride ligands has also been reduced to 87.92(2)°. As phosphorus ligands have higher *trans* influence than amine ligands, the Pt–Cl bond *trans* to P is slightly elongated compared to the chloride bound *cis* to P [36].

A similar geometrical arrangement of the ligands was observed by McIsaac et al. [33] for novel iminophosphino rhodium(I) complexes including $[Rh(K^2\text{-}o\text{-}Ph_2PC_6H_4CH{=}N\text{-}2,6\text{-}iPr_2C_6H_3)(\mu\text{-}Cl)]_2$. In comparison to the related compound $o\text{-}Ph_2PC_6H_4CH{=}NC_6H_4\text{-}OMe\text{-}o$ [36], both iminophosphines have a very similar arrangement with planar C(14)–C(19)═N(20)—C(21) imino units with an *E* configuration with respect to the diphenylphosphine group. These structures are in contrast to $o\text{-}Ph_2PC_6H_4CH{=}NC_6H_4OH\text{-}o$ [38] where the nitrogen is closer to the phosphorus atom as the molecule assumes a *Z* configuration. The N(1)–C(13) double bond of complex **1** is 1.287(2) Å is well within the range observed for these types of compounds [34]. There is little variation observed in the bond lengths of the ligand upon chelation to Pt(II), for the free ligand the length is 1.2668 (19) Å. The ligand backbone carbon-carbon distances for the complex are comparable with that of the free ligand. The average Pt–N and Pt–P bond lengths of 2.0421(18) and 2.2128(6) Å, respectively are in the range expected for iminophosphine platinum(II) complexes [40]. The torsion angle Pt(1)–P(1)–C(15)–C(14) = −35.5(2)° indicates that the $=CHC_6H_4-$ unit lies below the $PtCl_2(P,N)$ plane.

The crystallographic asymmetric unit contains two independent molecules which are essentially identical structurally. The main differences concern the arrangement of the phenyl groups. No unusual bond angles or distances were observed and the complex **6** showed a virtually linear P–Au–Cl system (bond angle of 177.17°). Williams et al. report the preparation and characterisation of three new complexes of gold(I) with bidentate P–N ligands where the ligand binds to gold(I) either in a mono- or bi-dentate fashion to form the corresponding gold(I) complexes [41]. For the $C_{23}H_{24}AuClNP$ complex they report bond lengths of 2.2916 and 2.2380 Å for the Cl–Au and P–Au bonds and the complex had a linear P–Au–Cl geometry with a bond angle of 177°.

3.3. Biological evaluation

A preliminary investigation into the antitumour activity of several metal-containing complexes prepared during the course of this study was carried out in the form of cytotoxicity assays on two separate cancer cell lines–human oesophageal cancer cell lines WHCO1 and KYSE450.

3.3.1. In vitro anticancer activity

The in vitro anticancer activity data of the platinum and gold complexes (**1**–**9**) against WHCO1 and KYSE450 cell lines is presented in Table 4. The cytotoxicity of several platinum complexes was examined. In WHCO1 cell lines complex **3** had the highest activity with an IC_{50} value of 5.49 μM and the least active was complex **1** with an IC_{50} value of 9.47 μM. In comparison to cisplatin which has an IC_{50} of 13–15 μM in WHCO1 cell lines, all the novel

Table 1
Selected crystallographic and refinement data for complexes **1** and **6**.

Complex	**1**	**6**
Empirical formula	$C_{31}H_{32}Cl_2NPPt$	$C_{32}H_{35}AuClNP$
Formula weight	715.54	705.00
T (K)	173(2)	173(2)
λ (Å)	0.71073	0.7103
Crystal system	monoclinic	triclinic
Space group	$P21/n$	$P1$
a (Å)	12.0686(7)	13.0315(3)
b (Å)	13.4007(7)	13.3638(2)
c (Å)	17.8182	19.3489(5)
α (°)	90	96.358(2)
β (°)	105.8190(10)	99.2290(10)
γ (°)	90	116.1910(10)
V (Å^3)	2772.6(3)	2921.15(11)
Z	4	4
D_{calc} (mg/ml)	1.714	1.603
Absorption coefficient (mm^{-1})	5.333	5.205
$F(000)$	1408	1396
Crystal size (mm)	0.22 × 0.11 × 0.09	0.21 × 0.18 × 0.12
θ (°)	1.83–29.60	2.31–26.38
Limiting indices	$-16 \leqslant h \leqslant 16$, $-18 \leqslant k \leqslant 18$, $-24 \leqslant l \leqslant 24$	$-16 \leqslant h \leqslant 16$, $-16 \leqslant k \leqslant 16$, $-24 \leqslant l \leqslant 24$
Reflections collected	40326	119370
Independent reflections (Rint)	7788 (0.0000)	11941 (0.0534)
Completeness of theta maximum and minimum transmission	29.6 (99.9%)	26.38 (99.8%)
Refinement method	full-matrix least-squares on F^2	full-matrix least-squares on F^2
Data/restraints/parameters	7788/0/326	11941/9/604
Goodness-of-fit (GOF) on F^2	1.059	1.050

platinum complexes described here displayed significant activity against oesophageal cancer cell lines.

The gold(I) complexes exhibited very good activity against both cancer cell lines. Complexes **8** and **9** were the most active with IC_{50} values of 3.41 and 3.61 μM.

3.3.2. Effect of compounds on DNA damage and induction of apoptosis

Since the morphology of the WHCO1 cells treated with complexes **1**, **4**, **6** and **8** clearly showed that the complexes were killing the oesophageal cancer cells, we investigated the effect of these complexes on DNA damage and the induction of apoptosis in the cancer cells. Phosphorylation of H2AX was used as a marker of Double Strand Breaks (DNA). H2AX by itself cannot repair the damage, but it acts as a recruiter, and collects other repair site of damage to initiate repair. The results show that complexes **1** and **6** very strongly induce the phosphorylation of H2AX, suggesting that these complexes induce DNA damage in the form of DSB's. Our Western blot shows that complexes **4** and **8** also induce DNA damage although to a lesser extent than in complexes **1** and **6**.

Considering that treatment with these complexes resulted in DSB's we determined whether these compounds also induced apoptosis in treated cells. PARP cleavage was investigated by Western blot analysis in order to determine the mode of cell death induced by the complexes **1**, **4**, **6**, and **8**. From the results, there is very little evidence that treatment of the WHCO1 cells with complexes **1**, **4**, **6** and **8** resulted in PARP cleavage and hence apoptosis. The system that we used to detect PARP cleavage was clearly working since we could detect the PARP cleavage in cells treated with Doxorubicin.

3.3.3. Effects of complexes *1*, *4*, *6* and *8* on the cell cycle

The complexes **1**, **4**, **6** and **8** exhibited significant DNA damage as evidenced by the phosphorylation of histone H2AX we then further explored the effect of the complexes on the cell cycle as it is well documented that DNA damage triggers cell cycle arrest in cells. From the results it appears that the compounds do not affect the cell cycle significantly except for complex **6**. Cattaruzza et al report that gold(III)-dithiocarbamato derivatives cause cell damage, slightly affecting the cell cycle, thus suggesting a different mechanism of action from established platinum-based compounds [42]. The lack of a sub-G1 peak in the cell analysis is most likely due to the extended processing of cells for this assay (multiple washes and spins), during which the population may have been lost. The cell-cycle disturbances were associated with inhibition of cell proliferation in agreement with results reported by Martin et al [43] in their cell-cycle disturbance studies. This suggests that the reduction in G1-phase cells was as a result of the accumulation of cells a fact that is attributed to induction of apoptosis by compounds in test. The cells were trapped in G1-phase and therefore paused

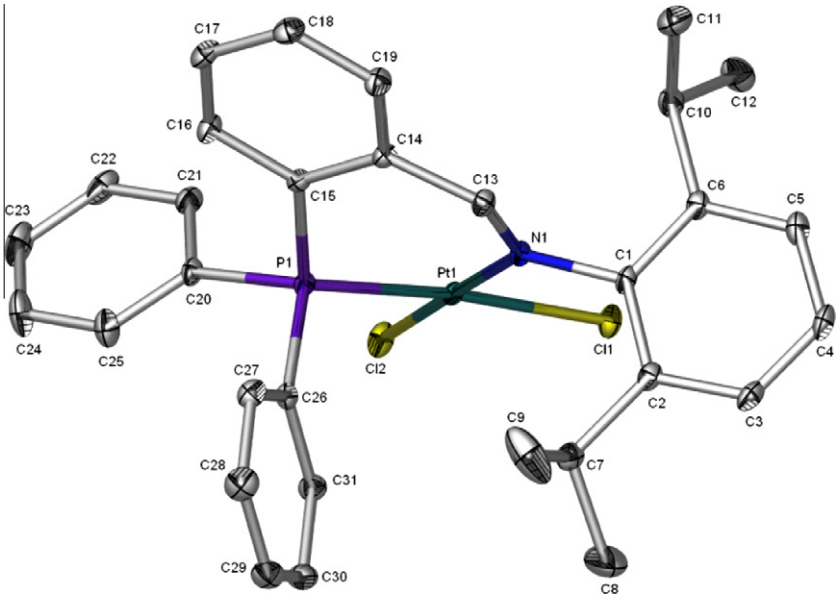


Fig. 1. The ORTEP plot of the molecular structure of **1** showing the atomic numbering. All the H atoms are omitted for clarity and are presented with ellipsoidal model with probability level 35%.

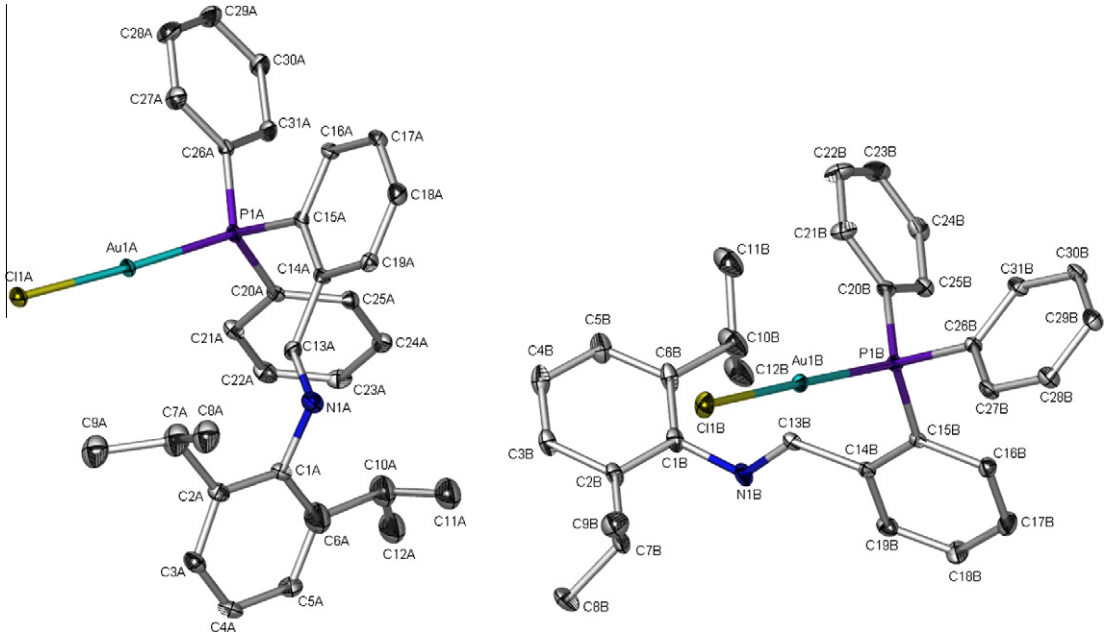


Fig. 2. The Ortep plot of the molecular structure of **6** showing the atomic numbering. There are two crystallographic independent molecules labelled as A and B in the asymmetric unit. All non-hydrogen atoms were presented with ellipsoidal model with probability level 25%. There is one ethanol solvent molecule and all hydrogen atoms are omitted for clarity.

Table 2
Selected bond distances and angles for complex **1**.

Bond distances (Å)		Bond angles (°)	
Pt(1)–N(1)	2.0421(18)	N(1)–Pt(1)–P(1)	89.80(5)
Pt(1)–P(1)	2.2128(6)	N(1)–Pt(1)–Cl(2)	178.85(5)
Pt(1)–Cl(2)	2.2901(6)	P(1)–Pt(1)–Cl(2)	91.25(2)
Pt(1)–Cl(1)	2.3512(6)	N(1)–Pt(1)–Cl(1)	90.98(5)
P(1)–C(15)	1.809(2)	P(1)–Pt(1)–Cl(1)	174.20(2)
P(1)–C(26)	1.816(2)	Cl(2)–Pt(1)–Cl(1)	87.92(2)
N(1)–C(13)	1.287(3)		

Table 3
Selected bond distances and angles for complex **6**.

Bond distances (Å)		Bond angles (°)	
Molecule 1			
Au(1A)–P(1A)	2.2350(13)	P(1A)–Au(1A)–Cl(1A)	177.17(5)
Au(1A)–Cl(1A)	2.2783(14)	C(26A)–P(1A)–C(20A)	105.2(2)
P(1A)–C(26A)	1.805(6)	C(26A)–P(1A)–C(15A)	105.3(2)
P(1A)–C(20A)	1.825(6)	C(26A)–P(1A)–Au(1A)	110.80(18)
P(1A)–C(15A)	1.834(6)	C(20A)–P(1A)–Au(1A)	116.84(17)
N(1A)–C(13A)	1.231(7)		
Molecule 2			
Au(1B)–P(1B)	2.2272(13)	P(1B)–Au(1B)–Cl(1B)	178.55(6)
Au(1B)–Cl(1B)	2.2709(15)	C(26B)–P(1B)–C(20B)	106.4(2)
P(1B)–C(26B)	1.810(5)	C(26B)–P(1B)–C(15B)	106.4(2)
P(1B)–Cl(20B)	1.814(6)	C(26B)–P(1B)–Au(1B)	110.01(17)
P(1B)–C(15B)	1.828(5)	C(20B)–P(1B)–Au(1B)	115.51(19)
N(1B)–C–(13B)	1.259(7)		

their progress in the cycle and subsequently entered an indefinite phase. This is attributed to the complex binding to DNA in G1-phase and therefore disrupting its programme of passing the DNA for replication in the S-phase. This then caused the cell receptors to detect the anomaly hence triggering apoptosis. Selenium compounds are the most extensively studied cancer chemopreventive agents, and induce apoptotic death of tumor cells and the selenite-induced apoptosis involves DNA damage. Selenite has also been shown to stimulate phosphorylation of H2AX [44,45].

Table 4
IC_{50} determination for Pt(II) and Au(I) complexes (**1**–**9**) evaluated for anticancer activity in WHCO1 and KYSE450 cell lines.

Compound	IC_{50} in WHCO1 (μM)	95% CI	IC_{50} in KYSE450 (μM)	95% CI
1	9.47	8.96–10.01	2.16	1.96–2.38
2	8.45	7.99–8.94	6.00	4.91–7.35
3	5.49	3.97–7.60	3.11	2.83–3.41
4	7.24	5.89–8.90	7.58	6.29–9.12
5	6.66	5.81–7.63	7.17	6.24–8.25
6	8.42	8.28–8.58	5.23	4.73–5.79
7	4.15	3.35–5.13	6.89	5.87–8.51
8	3.41	2.39–4.88	5.87	5.29–6.51
9	3.61	2.92–4.47	5.40	4.88–5.98

All experiments were done three times and all experimental points within an experiment were done in triplicate.

4. Conclusions

New neutral Pt(II) and Au(I) complexes containing iminophosphine ligands were prepared and fully characterised. The complexes discussed were studied for their cytotoxicity on oesophageal cancer cells. The platinum and gold complexes showed moderate activity and block the proliferation of WHCO1 cells with an IC_{50} range of 2.5–9.4 μM, and IC_{50} range of 2.2–7.6 μM for the KYSE450 cell lines. The morphological changes observed showed that the complexes induce cell death, however apoptosis was not observed and cell death is occurring by some other mechanism. It might therefore be interesting to investigate whether the compounds trigger cell death in WHCO1 cells by necrosis, induce senescence or autophagy. Further analysis of cytotoxicity activities of these complexes by FACS analysis showed that the complexes do not affect the cell cycle significantly. These studies provide an insight into the action of platinum and gold complexes and demonstrates potential use of these compounds in the development of new anticancer agents.

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Appendix A. Supplementary material

Supplementary data and figures for complex **1** are available from the IUCR electronic archives (Reference: GO2029). CCDC 872768 contains the supplementary crystallographic data for complex **6**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

References

[1] G. Jaouen, A. Vessieres, I.S. Butler, Acc. Chem. Res. 26 (1993) 361.
[2] R.H. Fish, G. Jaouen, Organometallics 22 (2003) 2166.
[3] N. Metzler-Nolte, Angew. Chem., Int. Ed. 40 (2001) 1040.
[4] D.M. Parkin, S.L. Whelan, J. Ferlay, H. Storm, Cancer incidence in five continents, IARC CancerBase No. 7, 2005.
[5] N. Ahmed, P. Cook, Br. J. Cancer 23 (1969) 302.
[6] D.G. Gatei, P.A. Odhiambo, D.A. Orinda, F.J. Muruka, Cancer Res. 38 (2) (1978) 303.
[7] J. Roth, A. Putman, S.L. Allen, A.F. Aslene, Cancer: Principles and Practise of Oncology, fourth ed., J.B. Lippincott Co., Philadelphia, PA, 1993.
[8] C.W. Evans, The Metastatic Cell Behaviour and Biochemistry, second ed., Chapman and Hill, London, 1991.
[9] P. Boyle, B. Levin, World Cancer, Report 15 (2008).
[10] P. Andre Ferreira, L. Joao Ferreira da Silva, M. Teresa Duarte, M. Fatima Minas da Piedade, M. Paula Robalo, G. Shrika Harjivan, Cristina Marzano, Valentina Gandin, M. Matilde Marques, Organometallics 28 (2009) 5412.
[11] E. Wong, C.M. Giandomenico, Chem. Rev. 99 (1999) 2451.
[12] L.R. Kelland, S.Y. Sharp, C.F. O'Neill, F.I. Raynaud, P.J. Beale, I.R. Judson, J. Inorg. Biochem. 77 (1999) 111.
[13] J.M. Perez, M.A. Fuertes, C. Alonso, C. Navarro-Ranninger, Crit. Rev. Oncol. Hematol. 35 (2000) 109.
[14] Y.-P. Ho, S.C.F. Au-Yeung, K.K.W. To, Med. Res. Rev. 23 (2003) 633.
[15] D. Wang, S.J. Lippard, Nat. Rev. Drug Discov. 4 (2005) 307.
[16] N.J. Wheate, J.G. Collins, Curr. Med. Chem: Anti-Cancer Agents 5 (2005) 267.
[17] B.K. Bhuyan, B.E. Loughman, T.J. Fraser, K. Day, J. Exp. Cell Res. 97 (1996) 275.
[18] T.M. Simon, D.H. Kunishima, G.J. Vilbert, A. Lorber, Cancer 44 (1979) 1965.
[19] C.K. Mirabelli, R.K. Johnson, L. Faucette, C.M. Sung, Proc. Am. Assoc. Cancer Res. 25 (1984) 367.
[20] C.K. Mirabelli, R.K. Johnson, L. Faucette, C.M. Sung, Cancer Res. 45 (1985) 32.
[21] P.J. Sadler, R.E. Sue, Met.-Based Drugs 1 (1994) 107.
[22] C.F. Shaw, Chem. Rev. 99 (1999) 2589.
[23] M.M. Mogorosi, T. Mahamo, J.R. Moss, S.F. Mapolie, J.C. Slootweg, K. Lammertsma, G.S. Smith, J. Organomet. Chem. 696 (2011) 3585.
[24] J.X. McDermott, J.F. White, G.M. Whitesides, J. Am. Chem. Soc. 98 (1976) 6521.
[25] D. Drew, J.R. Doyle, Inorg. Synth. 28 (1990) 346.
[26] T.A.K. Al-Allaf, L.J. Rashan, A.S. Abu-Surrah, R. Fawzi, M. Steimann, Transition Met. Chem. 23 (1998) 403.
[27] J.J. Price, A.N. Williamson, R.F. Schramm, B.B. Wayland, Inorg. Chem. 11 (1972) 1280.
[28] K.A. Jensen, Z. Anorg. Allg. Chem. 229 (1936) 265.
[29] R. Uson, A. Laguna, Organometallic Synthesis, in: R.B. Lang, J.J. Eish (Eds.), Elsevier, Amsterdam, 1986, p. 324.
[30] G.M. Sheldrick, SHELX97, Programme for Solving Crystal Structures, University of Gottingen, Germany, 1997.
[31] G.M. Sheldrick, SHELX97, Programme for the Refinement of Crystal Structures, University of Gottingen, Germany, 1997.
[32] G. Petocz, G. Rangits, M. Shaw, H. de Bod, D.B.G. Williams, J. Organomet. Chem. 694 (2009) 219.
[33] D.I. McIsaac, S.J. Geier, C.M. Vogels, A. Decken, S.A. Westcott, Inorg. Chim. Acta 359 (2006) 2771.
[34] P. Bhattacharyya, M.L. Loza, J. Parr, A.M. Z Slawin, J. Chem. Soc., Dalton Trans. 17 (1999) 2917.
[35] J. Parr, A.M. Z Slawin, Inorg. Chim. Acta 303 (2000) 116.
[36] M.L. Clarke, A.M.Z. Slawin, J.D. Woolins, Polyhedron 22 (2003) 19.
[37] G. Bandoli, A. Dolmella, L. Crociani, S. Antonetta, B. Crociani, Transition Met. Chem. 25 (2000) 17.
[38] J.R. Dilworth, S.D. Howe, A.J. Hutson, J.R. Miller, J. Silver, R.M. Thompson, M. Harman, M.B. Hursthouse, J. Chem. Soc., Dalton Trans. (1994) 3533.
[39] H.A. Ankersmit, B.H. Loken, H. Kooijman, A.L. Spek, K. Vrieze, G. van Koten, Inorg. Chim. Acta 252 (1996) 141.
[40] S.I. Pascu, K.S. Coleman, A.R. Cowley, M.L.H. Green, N.H. Rees, New J. Chem. 29 (2005) 385.
[41] D.B.G. Williams, T. Traut, F.H. Kriel, W.E. van Zyl, Inorg. Chem. Comm. 10 (2007) 538.
[42] L. Cattaruzza, D. Fregona, M. Mongat, L. Ronconi, A. Fassina, A. Colombatti, D. Aldinucci, Int. J. Cancer 126 (2010) 1.
[43] B. Martin, S.A. Oleksiewicz, J. Virol. 71 (1997) 1386.
[44] T. Kim, U. Jung, D.Y. Cho, A.S. Chung, Carcinogenesis 22 (2001) 559.
[45] N. Zhou, H. Xiao, T. Kunli, A.N. E-Kamal, L.F. Liu, J. Biol. Chem. 278 (2003) 29532.