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Anti-cancer gold(I) phosphine complexes: Cyclic trimers and tetramers containing the P-Au-P moiety

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ABSTRACT

We report the application of cationic tri- and tetra-nuclear gold(I) phosphine complexes $[Au_3(\mu\text{-dppen})_3]X_3$ and $[Au_4(\mu\text{-dppa})_4]X_4$ (X = OTf, PF_6) [OTf = trifluoromethanesulfonate, dppen = *trans*-1,2-bis(diphenylphosphino) ethene, dppa = bis(diphenylphosphino)acetylene] for cancer treatment. The results of cytotoxicity tests on four different cancer cells [prostate (DU145), cervical (HeLa), breast (MDAMB-231) and fibro sarcoma (HT1080)] indicate these complexes possess remarkable tumor cell growth inhibitory effects and high selectivity towards cancer cells. The anti-tumor mechanism of the tri- and tetra-nuclear gold(I) complexes has also been investigated.

1. Introduction

Cisplatin and other platinum-based metal complexes such as carboplatin and oxaliplatin are the most widely used and efficient anti-cancer drugs for the treatment of testicular, ovarian and bladder cancer [1,2]. However, the clinical success of cisplatin and its derivatives is compromised due to inevitable serious side effects, such as nephrotoxicity and neurotoxicity, ototoxicity and development of drug resistance [3]. Therefore, interest in medicinal inorganic chemistry has continued to grow with the emergence of gold complexes as alternatives to cisplatin, as many gold(I) and gold(III) compounds exhibited potent tumor cell growth inhibitory properties with much lower toxicity and fewer side effects [4,5]. In particular, there has been substantial interest in the chemistry of Au(I) complexes showing biological activity with potential medicinal applications after the discovery of auranofin, a gold(I) phosphine compound which was developed initially as an anti-rheumatic agent, displayed potent anti-cancer properties towards different cancer cell lines [6–9] and has been approved by the FDA for phase II clinical trials in cancer therapy (http://clinicaltrials.gov/ct2/show/NCT01419691). Although auranofin showed excellent *in vitro* cytotoxicity, it's *in vivo* efficacy was limited due to decreased stability in the presence of natural thiols [10]. Consequently, there is need to develop more stable, efficacious gold(I) phosphine complexes, particularly those containing the P-Au-P motif which have been shown to have marked biological activities, including anti-bacterial, anti-fungal and anti-cancer properties [11–13].

While monodentate tertiary phosphine ligands have found broad application in the development of anti-cancer gold complexes [14–20], the use of diphosphine ligands has yet to be fully exploited. The first report on the use of diphosphine ligands in developing anti-cancer gold complexes dates back to 1987, when Mirabelli et al. prepared a portfolio of gold complexes containing bis(diphenylphosphino)alkanes, shown in Fig. 1 [21]. Since then, complexes of the type $[\{PR_2(CH_2)_nPR_2\}Au_2Cl_2]$ have been investigated further, including modifications to the diphosphine ligands [22,23] and the preparation of mixed ligand gold complexes containing diphosphine and *N*-heterocyclic carbene bridging ligands [24]. The anti-tumor activity of the tetrahedrally coordinated Au(I) diphosphine complexes $[Au(dppe)_2]Cl$ [dppe = bis(diphenylphosphino)ethane] [25] and $[Au(d2pypp)_2]Cl$ [d2pypp = 1,3-bis(di-2-pyridylphosphino)propane] [26], in which the diphosphine ligands bind to the gold atom in a chelate mode, have also been investigated. Strategies to alter the chelate ring size, and variations in counter ion and ligand substituents have also been used to enhance the properties of these types of complexes [27]. Motivated by the significant potential of gold(I) complexes in cancer drug discovery, herein we report the *in vitro* anti-cancer activity and an intensive biological investigation of cyclic high nuclearity gold(I) complexes of *trans*-1,2-bis(diphenylphosphino)ethene and bis(diphenylphosphino)acetylene.

2. Experimental

2.1. Synthesis of gold complexes

The gold complexes $[Au_3(\mu\text{-dppen})_3]X_3$ [X = OTf (**1**), PF_6 (**2**)] and $[Au_4(\mu\text{-dppa})_4]X_4$ [X = OTf (**3**), PF_6 (**4**)] (Fig. 2)

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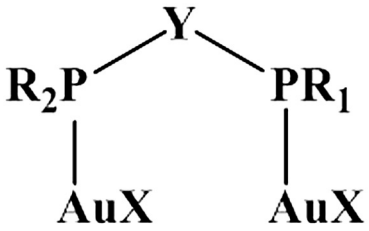

R_1, R_2= alkyl, aryl; X = halide; Y = alkane

Fig. 1. Gold complexes containing bis(diphenylphosphino)alkanes.

[OTf = trifluoromethanesulfonate, dppen = *trans*-1,2-bis(diphenylphosphino)ethene, dppa = bis(diphenylphosphino)acetylene] were prepared as described in the literature [28]. Briefly, reaction of [Au(tht)$_2$]X (tht = tetrahydrothiophene; X = OTf, PF_6) with an equimolar amount of *trans*-dppen or dppa afforded the desired air- and moisture-stable complexes **1–4**.

2.2. Stability experiments

The stability of the complexes in phosphate buffered saline (PBS) was monitored using Ultraviolet-Visible (UV–Vis) absorption spectroscopy (Fig. S1). The UV–Vis absorption spectra of the gold complexes were recorded on a Perkin-Elmer Lambda 19 or a Varian Cary 50 UV–Visible spectrophotometer. Stock solutions of the metal complexes were freshly prepared in dimethyl sulfoxide (DMSO) and diluted to 25 μM in phosphate buffered saline (PBS). The absorption spectra of 25 μM solutions of gold complexes in PBS were monitored over time from 0 h to 72 h. ^{1}H (300 MHz) and ^{31}P(121 MHz) NMR spectra were measured with a Bruker Avance 300 spectrometer at room temperature in DMSO-*d*6 and monitored over time to investigate their stability in DMSO.

2.3. Cell culture

Human fibro sarcoma (HT1080) and keratinocytes (HaCaT) cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Cervical (HeLa) and Prostate (DU145) cells were generously provided by Professor Joseph Trapani (Peter MacCullum Cancer Centre, Melbourne) and Professor Roger Dadly (Monash University, Melbourne), respectively. DU145 and HeLa cells were grown in Roswell Park Memorial Institute (RPMI) 1640 (GIBCO) media whereas HT1080 and HaCaT were cultured in Dulbecco's Modified Eagle Medium (DMEM-GIBCO) media; in both cases the media were supplemented with 1% penicillin-streptomycin and 10% FBS (foetal bovine serum). All cells were maintained in a humidified atmosphere incubator containing 95% air and 5% CO_2 at 37 °C. Cells were harvested with 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA, Life Technologies) for subculture and plating for drug treatments when they reached 80% confluence. For all the assays, stock solutions of the gold complexes were prepared in DMSO (10 mM) and the final treatment concentrations (100 M–0.01 μM) were made in complete growth medium.

2.4. MTT assay

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay is a standard method for the determination of cell viability and proliferation. This assay is based on the reduction of a yellow coloured MTT solution to purple formazan crystals by the active mitochondria of viable cells. Thus, the amount of produced formazan crystals is proportional to the number of viable cells. In this assay, DU145, HeLa, HT1080, MDAMB-231 and HaCaT cells were seeded in a 96 well plate at a density depending on their doubling times and plates were incubated overnight at 37 °C. Later, cells were treated with different concentrations (100, 10, 1, 0.1 and 0.01 μM) of complexes **1–4** for 72 h. 1% DMSO in complete medium was used as a control. After the treatment, the medium containing test compounds was removed and 100 μL of MTT solution (0.5 mg/mL in serum-free medium) was added to each well and further incubated for 4 h in the dark at 37 °C. Then, the excess unreacted MTT solution was removed and the produced formazan crystals were solubilised by adding 100 μL of DMSO. The absorbance of each well was recorded using a SpectraMax plate reader at a wavelength of 570 and 630 nm and the IC_{50} values were calculated using the Probit software [29]. The results are presented as means of three independent experiments.

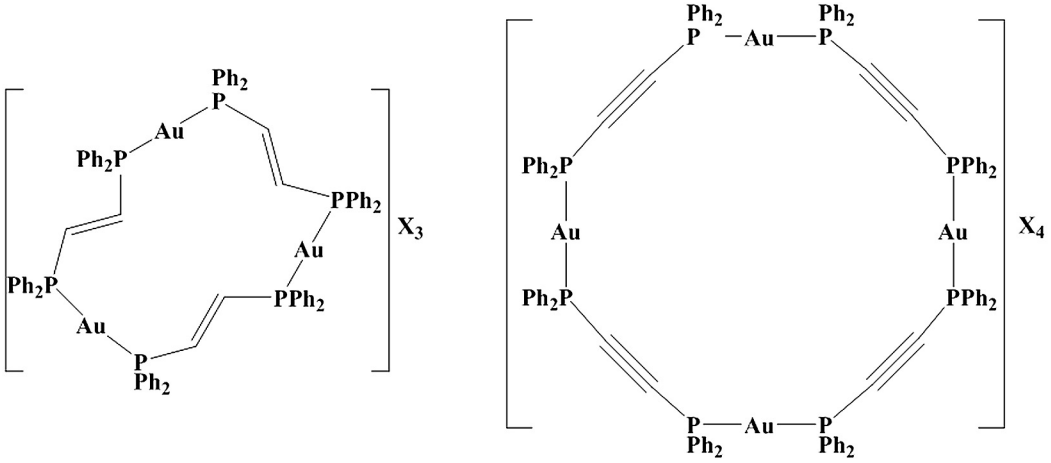

Fig. 2. [Au_3(μ-dppen)$_3$]X_3 and [Au_4(μ-dppa)$_4$]X_4 [X = OTf, PF_6] complexes.