Dalton Transactions

Cite this: *Dalton Trans.*, 2012, **41**, 3287

www.rsc.org/dalton

PAPER

Cytotoxic gold compounds: synthesis, biological characterization and investigation of their inhibition properties of the zinc finger protein PARP-1†

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Received 12th October 2011, Accepted 8th December 2011
DOI: 10.1039/c2dt11913g

The new gold(III) complexes: [Au{2-(2′-pyridyl)imidazolate}Cl_2] and [Au{2,6-bis(2′-benzimidazolate) pyridine}$(OCOCH_3)$] and the mono- and binuclear gold(I) complexes: [Au{2-(2′-pyridyl)imidazole}(PPh_3)](PF_6), [Au(2-phenylimidazolate)(DAPTA)] (DAPTA = 3,7-diacetyl-1,3,7-triaza-5-phosphabicyclo [3.3.1]nonane), [$(PPh_3Au)_2$(2-R-imidazolate)](PF_6) (R = 2-C_5H_4N, Ph) have been synthesized and characterized. The structure of the [$(PPh_3Au)_2$ {2-(2′-pyridyl)imidazolate)](PF_6) complex was also characterized by X-ray crystallography. The antiproliferative properties of the complexes were assayed against human ovarian carcinoma cell lines, either sensitive (A2780) or resistant to cisplatin (A2780cisR), human mammary carcinoma cells (MCF7) and non-tumorigenic human kidney (HEK293) cells. Most of the studied compounds showed important cytotoxic effects. Interestingly, the compounds containing the 2-(2′-pyridyl)imidazolate ligand showed selectivity towards cancer cells with respect to the non-tumorigenic ones, with the dinuclear compound [$(PPh_3Au)_2$ {2-(2′-pyridyl)imidazolate)](PF_6) being the most active. Some compounds were also screened for their inhibitory effect of the zinc-finger protein PARP-1, essential for DNA repair and relevant to the mechanisms of cancer cell resistance to cisplatin. Interaction studies of the compounds with the model protein ubiquitin were undertaken by electrospray ionization mass spectrometry (ESI MS). The results are discussed in relation to the putative mechanisms of action of the cytotoxic gold compounds.

Introduction

Transition metal complexes have proved to possess outstanding anticancer activities and several reviews summarized recent progress in the field.[1–3] In this research area, gold compounds occupy a relevant position constituting a promising class of experimental anticancer metallodrugs.[4–10] It is worth mentioning that gold compounds have a long and important tradition in medicine, the so-called *Chrysotherapy* that derives its name from Chryses, a golden-haired heroine in Greek mythology. Such a tradition dates back to ancient China (2500 B.C.) and flourished especially in Europe during the late Middle Ages and the Renaissance. In the early years of modern pharmacology, gold compounds were widely used for the treatment of several diseases, particularly as anti-infective and anti-tubercular agents.[11] Despite extensive clinical studies carried out during that pioneering time, gold compounds have found rather limited medical applications and are presently used only for the treatment of severe rheumatoid arthritis.[12] This is probably the result of their relevant systemic toxicity (*e.g.* nephrotoxicity) and the poor chemical stability of some of the tested compounds. For example, auranofin, a linear bidentate gold(I) complex with triethylphosphine and a thiosugar as ligands, which is in clinical use as an antiarthritic drug, is also a potent cytotoxic agent *in vitro*,[13] but proved to be active *in vivo* only in mice inoculated with lymphocytic P388 leukaemia.[14]

To date, several Au(I) and Au(III) compounds with profoundly different molecular structures have been developed and tested as anticancer agents, and initial structure-function relationships have been outlined. Au(I) complexes with phosphine and carbene ligands,[15,16] Au(III) dithiocarbamate compounds,[17] Au(III) porphyrinates[18] and organogold(III) compounds[19] have been among the most widely investigated *in vitro* and *in vivo*. Within this frame, in the last few years, we have particularly focused on Au(III) complexes in which the metal centre is stabilised by the presence of at least two nitrogen ligands, as in the case of the substituted 2,2′-bipyridine derivatives showing promising antiproliferative effects *in vitro*.[20–22]

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†CCDC reference number 848506. For crystallographic data in CIF or other electronic format see DOI: 10.1039/c2dt11913g

For the various families of gold compounds markedly distinct molecular mechanisms were recently invoked, such as a direct mitochondrial mechanism involving thioredoxin reductase inhibition in the case of the gold(I) complexes,[23–25] as well as interactions with various thiolate/selenolate containing proteins/enzymes.[26] The influence on some apoptotic proteins—*i.e.* MAPKs and Bcl-2—was also reported for gold(III) porphyrins,[27] as well as proteasome inhibition for gold(III) dithiocarbamates.[28] Analysis of cancer cell growth inhibition data of a series of gold(III) compounds in comparison to 110 standard agents with known mechanisms of action suggest that numerous molecular mechanisms are involved, including the inhibition of cyclin-dependent kinases or histone deacetylases.[29] Recently, we also reported on the potent inhibition of the zinc finger protein poly-(adenosine diphosphate (ADP)-ribose) polymerase 1 (PARP-1) by Au(I) and Au(III) complexes.[30] PARPs are essential proteins involved in cancer resistance to chemotherapies, and play a key role in DNA repair by detecting DNA strand breaks and catalysing poly(ADP-ribosylation).[31,32] Information on the reactivity of the gold complexes with the PARP-1 zinc-finger domain was also obtained by high-resolution mass spectrometry, and an excellent correlation between PARP-1 inhibition in protein extracts and the ability of the complexes to bind to the zinc-finger motif (in competition with zinc) was established.

Therefore, relying on the promising results obtained for both Au(III) complexes and Au(I) phosphine compounds, we have synthesized new Au(III) and Au(I) derivatives bearing monodentate, bidentate or tridentate nitrogen donor ligands such as 2-phenylimidazole, 2-(2′-pyridyl)imidazole and, 2,6-bis(benzimidazol-2-yl)pyridine, as well as phosphane groups, namely triphenylphosphine and 3,7-diacetyl-1,3,7-triaza-5-phosphabicyclo[3.3.1]nonane (DAPTA) (Chart 1). Interestingly, the DAPTA ligand is known to increase the water solubility of the resulting metal compounds, and recently Au(I)-DAPTA complexes with thionate or alkynyl ligands showed promising results as anticancer agents.[33,34] The new gold complexes were screened *in vitro* for their antiproliferative effects on human ovarian and breast cancer cell lines and on non-tumorigenic cells in order to evaluate their selectivity profiles. The most promising compound out of the cytotoxicity screening was further tested for PARP-1 inhibition on protein extracts from cancer cells. Information on the possible protein-bound metallo-fragments was obtained by electrospray ionization mass spectrometry (ESI MS).

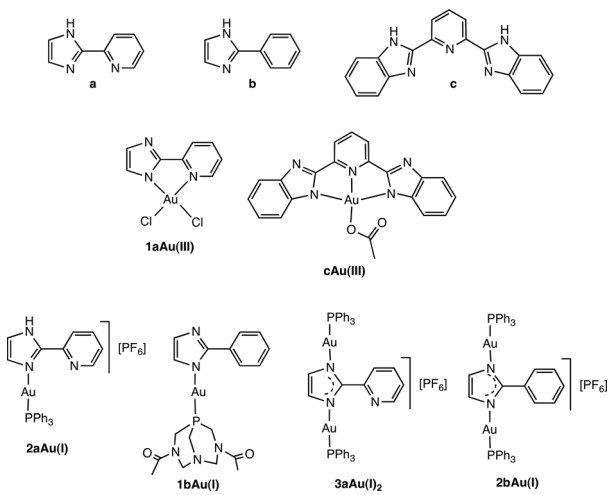

Chart 1 Ligands and gold complexes presented in this study.

Results and discussion

The Au(III) complexes **1aAu(III)** and **cAu(III)** with bidentate 2-(2′-pyridyl)imidazole (**a**) and tridentate 2,6-bis(benzimidazol-2-yl)pyridine (**c**) nitrogen donor ligands, respectively, which are able to stabilize the Au(III) centre in a typical square-planar geometry, were synthesized according to previously reported procedures for Au(III) bipyridine derivatives.[21] Mono- and dinuclear Au(I) complexes **2aAu(I)**, **1bAu(I)**, **3aAu(I)$_2$** and **2bAu(I)$_2$** were obtained by reaction of the ligands 2-(2′-pyridyl)imidazole (**a**) and 2-phenylimidazole (**b**) with one or two equivalents of the appropriate Au(I)–phosphine precursor, as reported in the experimental section. The obtained compounds are shown in Chart 1.

Interestingly, the Au(I) derivatives, with the exception of **2aAu(I)**, were soluble in water, while the Au(III) complexes were poorly soluble. Single crystals of the dinuclear complex **3aAu(I)$_2$** were obtained by slow diffusion of diethyl ether into a dichloromethane solution and were analysed by X-ray diffraction. An ORTEP diagram of compound **3aAu(I)$_2$** is shown in Fig. 1, with the most relevant bond distances and angles. Crystal data and other crystallographic parameters are reported in the experimental section (Table 2) and in the available supplementary material†. In **3aAu(I)$_2$** the cation $[(PPh_3Au)_2\{2\text{-}(2'\text{-pyridyl})\text{imidazolate}\}]^+$ lies on a crystallographic 2-fold axis (.2.) passing through the middle of the 2-(2′-pyridyl)imidazolate which bridges two Au(I) metal centres. The geometry around the metal is mostly linear, as shown by the P–Au–N angle 177.7(5)°. The Au–P and Au–N bond lengths, 2.242(5) and 2.05(2) Å respectively, can be easily compared with values found for similar compounds.[35,36]

The antiproliferative properties of the new gold complexes were assayed by monitoring their ability to inhibit cell growth using the MTT assay (see Experimental section). Cytotoxic activity of the compounds was determined after exposing for 72 h the human ovarian cancer A2780 cell line, and its cisplatin-resistant variant (A2780cisR), as well as the human breast cancer cell line MCF7, in comparison to cisplatin and auranofin. The results are summarized in Table 1. Most of the compounds turned out to be good cytotoxic agents with the only exceptions of the mononuclear Au(III) and Au(I) complexes **1aAu(III)** and **1bAu(I)**, with IC_{50} values > 50 μM. In general the cationic derivatives are the most effective among the tested compounds. In particular, the dinuclear Au(I) compounds **3aAu(I)$_2$** and

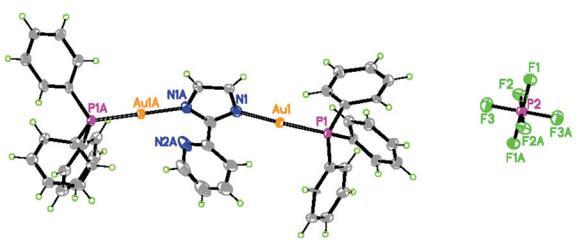

Fig. 1 ORTEP diagram of the cation of **3aAu(I)$_2$**. Ellipsoids are drawn at 50% probability level. Selected bond lengths (Å) and angles (°): Au1–P1 2.242(5), Au1–N1 2.05(2), N1–Au1–P1 177.7(5).

Table 1 IC_{50} values of the Au complexes described in this study against human ovarian carcinoma cell lines cisplatin sensitive (A2780) and resistant (A2780cisR), human breast cancer cells (MCF7), and the non-tumorigenic cell line HEK293, compared to cisplatin and auranofin

	IC_{50} (μM)[a]			
Compound	A2780	A2780cisR	MCF7	HEK293
1aAu(III)	61.9 ± 17.3	52.9 ± 16.2	80.3 ± 7.3	217.9 ± 67.8
2aAu(I)	7.2 ± 0.6	4.1 ± 0.2	12 ± 4.2	9.6 ± 0.9
3aAu(I)$_2$	1.1 ± 0.01	0.5 ± 0.2	5.13± 0.5	6.4 ± 2.4
1bAu(I)	93.7 ± 3.7	69.4 ± 12.3	149.8 ± 0.4	53.7 ± 13.2
2bAu(I)$_2$	2.1 ± 0.7	1.9 ± 0.7	3.3 ± 0.9	2.6 ± 1.0
cAu(III)	9.7 ± 1.3	6.8 ± 1.0	15 ± 3.1	5.8 ± 1.5
Auranofin	1.25± 0.5	1.5± 0.3	1.7± 0.4	4.62 ± 1.1
Cisplatin	1.9 ± 0.6	35 ± 7.0	20 ± 3.0	—

[a] Mean ± SE of at least three determinations.

2bAu(I)$_2$, bearing two $AuPPh_3$ units, are even more effective antiproliferative agents than cisplatin. These two compounds are comparable to auranofin, being active in the low micromolar range on both A2780 and A2780cisR cell lines, as well as on the MCF7 cells. Among the derivatives with the 2-phenylimidazole ligand, the mononuclear one (**1bAu(I)**), in which Au(I) is bound to DAPTA, is *ca.* 40-fold less effective than the dinuclear analogue with triphenylphosphines (**2bAu(I)$_2$**). Finally, **2aAu(I)**, and **cAu(III)** have IC_{50} values comparable to cisplatin for the A2780 cell line, but are at least 5–7-fold more effective towards the A2780cisR variant. These results, attesting the compounds ability to overcome cisplatin resistance, strongly support the hypothesis of their different mechanism of action with respect to cisplatin.

In order to assess the compounds' selectivity for cancerous cells with respect to normal cell lines, the gold complexes were also screened for their antiproliferative effects on the non-tumorigenic human embryonic kidney cells HEK293. Interestingly, while auranofin is still highly cytotoxic, the new gold complexes show different activity profiles. In most cases the cytotoxicity is comparable for the cancerous and normal cell lines. However, a general trend was found for the complexes containing the 2-(2′-pyridyl)imidazole/imidazolato ligand (**a**): the latter are less cytotoxic towards the HEK293 (and MCF7), and more active on the ovarian cancer cell lines. In particular **3aAu(I)$_2$** is *ca.* 12-times more toxic on the A2780cisR (IC_{50} *ca.* 0.5 μM) than on the HEK293 cells (IC_{50} *ca.* 6.4 μM).

Following our recent results that indicate some cytotoxic gold compounds are efficient inhibitors of the zinc-finger protein PARP-1, we tested two compounds, namely the most active and selective on cancer cells **3aAu(I)$_2$**, and the least active **1bAu(I)**, for PARP-1 inhibition on A2780 and A2780cisR cell extracts. The PARP-1 activity was evaluated after incubation of protein cell extracts with the compounds for 1 h at room temperature. Fig. 2 shows the residual PARP-1 activity in protein extracts treated with the complexes at a fixed concentration. Interestingly, while the poorly cytotoxic compound **1bAu(I)** is practically ineffective as a PARP-1 inhibitor in all cases, the active dinuclear complex **3aAu(I)$_2$** is able to efficiently inhibit PARP-1 in A2780 cell extracts reducing its activity to *ca.* 24%. However, **3aAu(I)$_2$** has no effect on PARP-1 activity in cell extracts from the A2780cisR variant, indicating that other proteins are competing

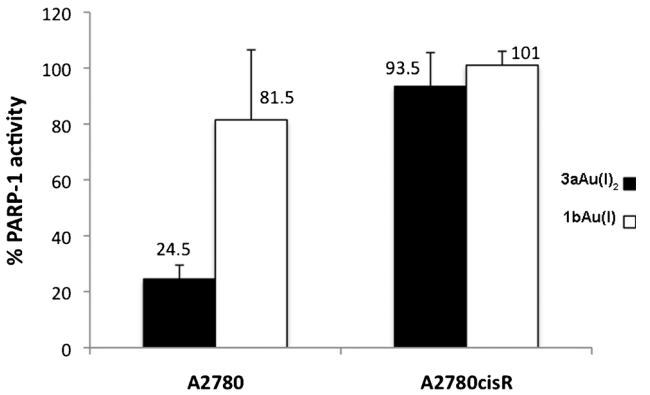

Fig. 2 PARP-1 activity levels in A2780 and A2780cisR cellular extracts. PARP-1 activity was measured in homogenates (50 μg of protein) treated with the compounds (50 μM) over 1 h at room temperature. Data are the mean ± SD of at least three experiments each performed in triplicate.

with PARP-1 for gold binding. In other words, the specific protein content/composition of each cancer cell line orients the reactivity of the compound towards different targets. In the specific case of the A2780 cell lines, previously reported proteomic studies evidenced a few proteins to be differentially expressed in the cisplatin resistant variant compared to the sensitive one, and five of them were considered responsible for platinum resistance.[37] Interestingly, glutathione-S-transferase (GST), a known target for metallodrugs, was one of the five protein candidates.

In order to shed light on the reactivity of the Au complexes we investigated their interactions with ubiquitin (Ub), used as a model protein, by ESI MS following established protocols.[38,39,40] Thus, three molar equivalents of **3aAu(I)$_2$** were added to an aqueous solution of Ub buffered at pH 7.4 (see Experimental for details). Fig. 3 shows the mass spectrum of the Ub–**3aAu(I)$_2$** sample recorded after 1 h incubation. In the spectrum, Ub was identified as one of the main peaks at 952.49 *m*/*z* (*ca.* 8565 Da), and gold-containing species were observed at 1003.54 *m*/*z* (*ca.* 9023 Da) corresponding to an Ub–$AuPPh_3$ adduct in which the original 2-(2′-pyridyl)imidazolato ligand is absent. The observed reactivity is similar to the one described

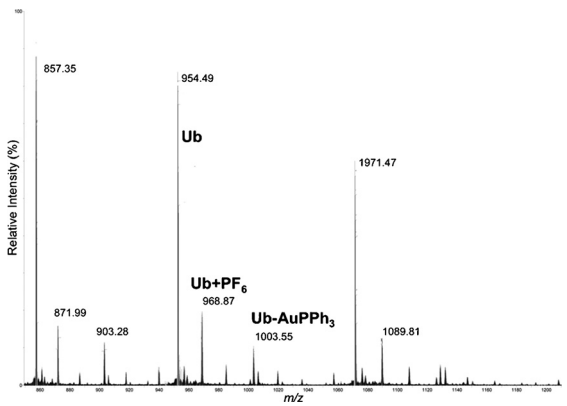

Fig. 3 ESI mass spectrum (+10, +9 and +8 ions) of Ub incubated with **3aAu(I)$_2$** (metal complex : Ub ratio = 3 : 1) in $(NH_4)_2CO_3$ buffer (pH 7.4) for 1 h at 37 °C.

for the reference compound auranofin, which has also been reported to release the thioglucose ligand upon protein binding, while maintaining the phosphine moiety.[41,42]

Experimental section

Materials and methods

Chemicals were obtained from commercial suppliers and used as received. The ligand 3,7-diacetyl-1,3,7-triaza-5-phosphabicyclo [3.3.1]nonane (DAPTA) was prepared as previously reported.[43] Cisplatin and $NaAuCl_4$ were purchased from Sigma-Aldrich, Auranofin, Au(III)acetate and 2-phenylimidazole (**2**) from AlfaAesar. Solvents used in synthesis were dried using standard procedures. The identity and purity (≥95%) of the gold complexes were unambiguously established using elemental analysis, mass spectrometry and NMR. Elemental analyses were performed on a Carlo Erba EA 1110 CHN instrument. ESI-MS spectra were obtained in acetonitrile on a Thermo Finnigan LCQ Deca XP Plus quadrupole ion-trap instrument operated in positive ion mode over a mass range of *m*/*z* 150–2000. The ionization energy was set at 30 V and the capillary temperature at 200 °C. ^{1}H, and ^{31}P NMR spectra were recorded at 25 °C with a Bruker Avance 400 spectrometer operating at 400.13 and 161.98 MHz, respectively. Chemical shifts are given in ppm relatively to internal TMS (^{1}H), and external H_3PO_4 (^{31}P). Numbering schemes of the protons are as in the drawings below.

Synthesis

[Au{2-(2-pyridyl)imidazolate}Cl_2] (**1aAu(III)**): an aqueous solution of $NaAuCl_4$ (199 mg, 0.5 mmol, 20 mL) was added to a solution of **a** (72.6 mg, 0.5 mmol, 20 mL) and KOH (28 mg, 0.5 mmol) in acetonitrile (5 mL)/water (10 mL). The resulting suspension was stirred for 24 h at room temperature in the darkness. Afterwards, the precipitate was filtered under vacuum and the crude product recrystallized from acetone/diethyl ether to give the analytical sample as a brown solid (180 mg, 87%).

Solubility: acetone, DMSO and insoluble in chloroform, dichloromethane, water, ether and hexane.

^{1}H NMR (DMSO) δ: 9.04 (d, J = 6.0 Hz, 1H, H$^{6'}$), 8.33 (t, 1H, J = 7.8 Hz; H$^{4'}$), 8.03 (d, 1H, J = 8.0 Hz; H$^{3'}$), 7.66 (t, 1H, J = 7.0 Hz (av); H$^{5'}$), 7.41 (s, 1H; H^5), 7.33 (s, 1H; H^4). Anal. Calcd. for $C_8H_6AuCl_2N_3$ (412.03 g mol^{-1}): C 23.32; H 1.47; N 10.20. Found: C 23.40; H 1.36; N 10.13. ESI-MS (H_2O/acetone/CH_3CN, positive mode) exact mass for $C_8H_6N_3AuCl_2$ (410.96): found *m*/*z* 411.97 [AuLH]$^+$ (calc. *m*/*z* 411.97).

[Au{2-(2-pyridyl)imidazole}(PPh_3)]PF_6 (**2aAu(I)**): $AgPF_6$ (126 mg, 0.5 mmol) was added to a dichloromethane solution of (PPh_3)AuCl (373 mg, 0.5 mmol) and the resulting mixture stirred for 10 min until the precipitation of AgCl was completed. Then the filtered solution was added dropwise to a dichloromethane solution of **a** (78 mg, 0.5 mmol, 20 mL), and stirred for 2 h at room temperature, in the darkness. The solution was filtered through Celite and concentrated to a small volume; addition of diethyl ether afforded a white solid. This was filtered off, washed with diethyl ether (3 × 10 mL) and dried under vacuum (253.1 mg, 59.3%). Solubility: chloroform, dichloromethane, acetone and insoluble in water, ether and hexane.

^{31}P NMR ($CDCl_3$) δ 30.6 (PPh_3), −143.6 (PF_6). ^{1}H NMR ($CDCl_3$); δ 8.30 (d, 1H, J = 7.8 Hz; H$^{3'}$), 8.19 (d, 1H, J = 5.9 Hz; H$^{6'}$), 7.96 (t, 1H, J = 7.5 Hz; H$^{4'}$), 7.55–7.68 (m, 15H, PPh_3), 7.50 (s, 1H; H^5), 7.38 (dd, 1H, J = 7.8, 5.9 Hz; H$^{5'}$), 7.27 (s, 1H; H^4). Anal. Calcd. for $C_{26}H_{22}AuF_6N_3P_2$ (749.38 g mol^{-1}): C 41.67; H 2.96; N 5.61. Found: C 41.70; H 3.11; N 5.70. ESI-MS (H_2O/acetone/CH_3CN, positive mode) exact mass for $C_{26}H_{22}AuF_6N_3P_2$ (749.09): found *m*/*z* 604.12 [AuLPPh$_3$]$^+$ (calc. *m*/*z* 604.12).

[Au_2{2-(2-pyridyl)imidazolate}$(PPh_3)_2$]PF_6 (**3aAu(I)$_2$**): $AgPF_6$ (252 mg, 1 mmol) was added to an acetone solution of (PPh_3)AuCl (604 mg, 1 mmol, 20 mL) and the resulting mixture stirred for 10 min until the precipitation of AgCl was completed. Then, the filtered solution was added dropwise to a solution of **a** (72.8 mg, 0.5 mmol) and KOH (28.1 mg 0.5 mmol) in acetonitrile (5 mL)/water (20 mL). The resulting mixture was stirred for 24 h at room temperature in the darkness. Then, the solvent was removed under vacuum and the white product crystallized from dichloromethane/diethyl ether and dried under vacuum (332 mg, 55%). Solubility: chloroform, dichloromethane, acetone, poorly soluble in water and insoluble in ether and hexane.

^{31}P NMR ($CDCl_3$) δ 31.7 (PPh_3), −144.1 (PF_6). ^{1}H NMR ($CDCl_3$) δ: 8.53 (d, 1H, J = 8.0 Hz; H$^{3'}$), 8.21 (d, 1H, J = 4.8 Hz, H$^{6'}$), 7.70 (t, 1H, J = 7.8 Hz; H$^{4'}$), 7.54–7,62 (m, 30 H, 2PPh_3), 7.40 (br m, 1H; H$^{5'}$), 7.36 (s, 2H, H^4,H^5). Anal. Calcd. for $C_{44}H_{36}Au_2F_6N_3P_3$ (1207.6 g mol^{-1}): C 43.76; H 3.00; N 3.48. Found: C 43.69; H 3.00; N 3.59. ESI-MS (H_2O/acetone/CH_3CN, positive mode) exact mass for $C_{44}H_{36}Au_2F_6N_3P_3$ (1207.14): found *m*/*z* 1602.17 [AuL$(PPh_3)_2$]$^+$ (calc. *m*/*z* 1602.17).

[Au(2-phenylimidazole)(DAPTA)] (**1bAu(I)**): an aqueous solution of (DAPTA)AuCl (83.2 mg, 0.17 mmol, 20 mL), obtained from [(THT)AuCl][44] and DAPTA, was added to a solution of **b** (24.7 mg, 0.17 mmol) and KOH (9.54 mg, 0.17 mmol) in acetonitrile (5 mL)/water (20 mL). The resulting mixture was stirred for 24 h at room temperature in the darkness. Afterwards, the solvent was removed under vacuum and the beige product crystallized from chloroform/diethyl ether and dried under vacuum (40.2 mg, 40%). Solubility: water, chloroform, dichloromethane, acetone and insoluble in ether and hexane.

^{31}P NMR (DMSO) δ: −28.6 (P DAPTA); ^{1}H NMR (DMSO) δ: 8.17 (d, 2H, J = 7.2 Hz; H°), 7.38 (t, 2H, J = 7.6 Hz (av); H^m), 7.30 (t, 1H, J = 7.9 Hz; H^p), 6.99 (s, 2H, H^4,H$^{5)}$, 5.54 (d, 1H, J = 14.9 Hz; NCH_2N), 5.46 (dd, 1H, J = 14.9, 7.9 Hz, NCH_2P), 4.95 (d, 2H, J = 14 Hz, NCH_2N), 4.66 (d, 1H, J = 15.0 Hz; NCH_2P), 4.34 (d, 1H, J = 16.8 Hz; NCH_2P), 4.13 (d, 2H, J = 14.0 Hz; NCH_2N), 4.07 (s, 2H; NCH_2P), 3.81 (d, 1H, J = 15 Hz; NCH_2P), 2.05 (s, 3H; CH_3), 2.04 (s, 3H; Me). Anal. Calcd. for $C_{18}H_{23}AuN_5O_2P$ (569.13 g mol^{-1}): C 37.97; H 4.07; N 12.30. Found: C 37.88; H 4.04; N 12.15. ESI-MS (H_2O/acetone/CH_3CN, positive mode) exact mass for $C_{18}H_{23}AuN_5O_2P$ (569.13): found *m*/*z* 570.13 [AuLDAPTAH]$^+$ (calc. *m*/*z* 570.13).

[Au_2(2-phenylimidazolate)$(PPh_3)_2$]PF_6 (**2bAu(I)$_2$**): an acetone solution of [(PPh_3)Au]PF_6 (604 mg, 1 mmol, 20 mL), prepared as described above, was added dropwise to a solution of **b** (72.0 mg, 0.5 mmol) and KOH (28.1 mg, 0.5 mmol) in acetonitrile (5 mL)/water (20 mL). The resulting mixture was stirred for 24 h at room temperature in the darkness. Afterwards, the

solvent was removed under vacuum and the white product crystallized from dichloromethane/diethyl ether and dried under vacuum (216 mg, 40%). Solubility: water, chloroform, dichloromethane, acetone and insoluble in ether and hexane.

^{31}P NMR ($CDCl_3$) δ 31.6 (PPh_3), −143.9 (PF_6). ^{1}H NMR ($CDCl_3$) δ: 8.01 (d, 2H, J = 8.0 Hz; H°), 7.65–7.35 (m, 33H; H^m + H^p + H PPh_3), 7.33 (s, 2 H; H^4, H^5). Anal. Calcd. for $C_{45}H_{37}Au_2F_6N_2P_3$ (1206.6 g mol^{-1}): C 44.79; H 3.09; N 2.32. Found: C 44.65; H 3.18; N 2.19. ESI-MS (H_2O/acetone/CH_3CN, positive mode) exact mass for $C_{45}H_{37}Au_2F_6N_2P_3$ (1206.14): found m/z 1061.18 $[AuL(PPh_3)_2]^+$ (calc. m/z 1061.17).

[Au{2,6-bis(2-benzimidazolate)pyridine}(O_2CCH_3)] (**cAu(III)**): a solution of $Au(O_2CCH_3)_3$, (187 mg, 0.5 mmol) in acetic acid (20 mL) was added to a solution of **c** (155 mg, 0.5 mmol) in the same solvent (20 mL). The mixture was stirred for 5 h at reflux (180 °C) in the darkness. Afterwards, the solution was filtered, dried under vacuum to afford and orange solid. This was crystallized from dichloromethane/diethyl and dried under vacuum (80.3 mg, 29%). Solubility: DMSO, chloroform, dichloromethane and insoluble in water, ether and hexane.

^{1}H NMR ($CDCl_3$) δ: 8.21 (t, 1H, J = 7.8 Hz; H^4), 7.88 (d, 2H, J = 7.8 Hz; H^3,H^5), 7.71 (d, 2H, J = 8.0 Hz; $H^{7'}$,$H^{7''}$), 7.42 (d, 2H, J = 8.0 Hz; $H^{4'}$,$H^{4'}$), 7.23 (m, 4H, $H^{5'}$,$H^{6'}$,$H^{5''}$,$H^{6''}$), 2.48 (s, 3H, CH_3). Anal. Calcd. for $C_{21}H_{14}AuN_5O_2$ (565.33 g mol^{-1}): C 44.62; H 2.50; N 12.39. Found: C 44.57; H 2.52; N 12.16. ESI-MS (acetone/CH_3CN, positive mode) exact mass for $C_{21}H_{14}AuN_5O_2$ (565.08): found m/z 566.08 $[AuL(OOCCH_3)H]^+$ (calc. m/z 566.08).

X-Ray crystallography

Crystals of **3aAu(I)$_2$** were obtained by slow diffusion of diethyl ether into a saturated solution of the complex in CH_2Cl_2 and were resolved with X-ray diffraction. The data collection was measured at low temperature [100(2) K] using Mo Kα radiation on a Bruker APEX II CCD diffractometer having a kappa geometry goniometer. Data reduction was carried out by EvalCCD[45] and then corrected for absorption.[46] The solution and refinement was performed by SHELX.[47] The structure was refined using full-matrix least-squares based on F^2 with all non hydrogen atoms anisotropically defined. Hydrogen atoms were placed in calculated positions by means of the "riding" model. Disorder problems due to the imposed crystallographic symmetry and dealing with the pyridine moiety were found during the last stages of refinement. Some restraints were applied (SHELX cards: DFIX and EADP) in order to get reasonable parameters. An additional problem was discovered by TWINROTMAT/PLATON,[48] and was due to pseudo-merohedral twinning; a new HKL was then generated and used for refinement with MERG 0, HKLF 5 and two BASF parameters [0.294(6); 0.208(6)].

Cell culture and inhibition of cell growth

The human breast cancer cell line MCF7 and human ovarian cancer cell lines A2780 and A2780cisR (obtained from the European Centre of Cell Cultures ECACC, Salisbury, UK) were cultured respectively in DMEM (Dulbecco's Modified Eagle Medium) and RPMI containing GlutaMaxI supplemented with 10% FBS and 1% penicillin/streptomycin (all from Invitrogen), at 37 °C in a humidified atmosphere of 95% of air and 5% CO_2 (Heraeus, Germany). Non-tumoral human embryonic kidney cells HEK293 were kindly provided by Dr Maria Pia Rigobello (CNRS, Padova, Italy), and were cultivated in DMEM medium, added with GlutaMaxI (containing 10% FBS and 1% penicillin/streptomycin (all from Invitrogen) and incubated at 37 °C and 5% CO_2. For evaluation of growth inhibition, cells were seeded in 96-well plates (Costar, Integra Biosciences, Cambridge, MA) and grown for 24 h in complete medium. Solutions of the compounds were prepared by diluting a freshly prepared stock solution (in DMSO) of the corresponding compound in aqueous media (RPMI or DMEM for the A2780 and A2780cisR or MCF7 and HEK293, respectively). Afterwards, the intermediate dilutions of the compounds were added to the wells (100 µL) to obtain a final concentration ranging from 0 to 150 µM, and the cells were incubated for 72 h. DMSO at comparable concentrations did not show any effects on cell cytotoxicity. Following 72 h drug exposure, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to the cells at a final concentration of 0.25 mg mL^{-1} incubated for 2 h, then the culture medium was removed and the violet formazan (artificial chromogenic precipitate of the reduction of tetrazolium salts by dehydrogenases and reductases) was dissolved in DMSO. The optical density of each well (96-well plates) was quantified three times in tetraplicates at 540 nm using a multiwell plate reader (iEMS Reader MF, Labsystems, US), and the percentage of surviving cells was calculated from the ratio of absorbance of treated to untreated cells. The IC_{50} value was calculated as the concentration reducing the proliferation of the cells by 50% and is presented as a mean (± SE) of at least three independent experiments.

PARP-1 activity determinations

PARP-1 activity was determined using Trevigen's HT Universal Colorimetric PARP Assay. This assay measures the incorporation of biotinylated poly(ADP-ribose) onto histone proteins in a 96 microtiter strip well format. An aliquot of protein cell extracts (50 µg) from A2780 or A2780cisR cells was used as the enzyme source and 3-aminobenzamide (3-AB), provided in the kit, was used as a control inhibitor. The cells extracts were incubated with the compounds (10 or 50 µM) for 1 h at room temperature followed by determination of PARP-1 activity. Two cell extract controls were always performed in parallel: a positive activity control for PARP-1 without inhibitors, that provided the 100% activity reference point, and a negative control, without PARP-1 to determine background absorbance. The final reaction mixture (50 µL) was treated with TACS-Sapphire™, a horseradish peroxidase colorimetric substrate, and incubated in the dark for 30 min. Absorbance was read at 630 nm after 30 min. The data corresponds to means of at least three experiments performed in triplicate ± S.D.

ESI-MS studies

Samples were prepared by mixing 100 µM Ub (Sigma, U6253) with an excess of gold compound (3 : 1, metal : protein ratio) in

20 mM $(NH4)_2CO_3$ buffer (pH 7.4) and incubated for 1 h at 37 °C. Prior to analysis samples were extensively ultrafiltered using a Centricon YM-3 filter (Amicon Bioseparations, Millipore Corporation) in order to remove the unbound complex. ESI-MS data were acquired on a Q-Tof Ultima mass spectrometer (Waters) fitted with a standard Z-spray ion source and operated in the positive ionization mode. Experimental parameters were set as follows: capillary voltage 3.5 kV, source temperature 80 °C, desolvation temperature 120 °C, sample cone voltage 100 V, desolvation gas flow 400 L h^{-1}, acquisition window 300–2000 *m*/*z* in 1 s. The samples were diluted 1 : 20 in water and 5 μL was introduced into the mass spectrometer by infusion at a flow rate of 20 μL min^{-1} with a solution of CH_3CN/H_2O/HCOOH 50 : 49.8 : 0.2 (v : v : v). External calibration was carried out with a solution of phosphoric acid at 0.01%. Data were processed using the MassLynx 4.1 software.

Conclusions

Gold compounds have clearly emerged as an attractive new class of cytotoxic agents with potential application in cancer treatment. So far, conspicuous experimental evidence has been gathered suggesting that the pronounced antiproliferative effects caused by gold compounds most likely arise from innovative mechanisms of action in comparison to established anticancer metallodrugs. Here we report on the synthesis and antiproliferative properties of novel gold(III) complexes, [Au{2-(2-pyridyl) imidazolato}Cl_2] and [Au{2,6-bis(2-benzimidazolato)pyridine}$(OCOCH_3)$], and mono- and dinuclear gold(I) complexes bearing imidazolate and phosphane ligands. Interestingly, the mono and dinuclear gold(I) compounds **2aAu(I)** and **3aAu(I)$_2$** bearing the 2-(2-pyridyl)imidazole and PPh_3 ligands were the most cytotoxic of the series, and more selective towards cancer cell lines compared to the non-tumorigenic HEK293 cells. Most of the studied compounds were also more active than cisplatin on the A2780cisR cell line, a feature that is common to several cytotoxic gold complexes and that indicates different mechanisms of biological action with respect to classical Pt(II) drugs.

Notably, not many gold complexes have been reported showing greater selectivity for tumour cells *versus* normal cells. Among them a bis-chelated Au(I) bidentate phosphine complex of the water soluble ligand 1,3-bis(di-2-pyridylphosphino) propane (d2pypp), namely [Au(d2pypp)$_2$]Cl, was shown to selectively induce apoptosis in breast cancer cells but not in normal cells.[49] Similarly, Au(I) complexes with modified diphosphine ligands (*e.g.* bidentate pyridylphosphines) were designed and tested on both cancer cells and isolated rat hepatocytes, the latter taken as non-tumorigenic cell models,[50,51] and showed selectivity between different cell types.

In order to identify possible biological targets for the highly cytotoxic and selective dinuclear compound **3aAu(I)$_2$** we evaluated its PARP-1 inhibition properties on cell extracts from A2780 and A2780cisR cell lines, in comparison to the non-cytotoxic compound **1bAu(I)**. Compound **3aAu(I)$_2$** was able to inhibit PARP-1 activity only in extracts from A2780 cells, while **1bAu(I)** was ineffective in both cases. These results confirm the relevance of zinc finger proteins such as PARP-1 as possible targets for cytotoxic gold compounds, but also indicate that when establishing the selectivity of a compound for a possible biological target it is essential to consider the overall specific cellular context under investigation. In other words, different cell lines may express different proteins at different relative concentrations, as well as presenting variegate intracellular environments where the reactivity and selectivity of a metallodrug can be importantly modulated.

Acknowledgements

The authors thank EU COST D39 action for stimulating discussions. MS thanks the Institute of Chemical Sciences and Engineering (EPFL) for providing her with a Master fellowship. AC thanks the Swiss National Science Foundation (AMBIZIONE project n° PZ00P2-136908/1) and the University of Groningen (Rosalind Franklin fellowship) for financial support. Authors thank Dr Maria Rigobello for providing the HEK cells.

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