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PAPER

Gold(I) complexes of water-soluble diphos-type ligands: Synthesis, anticancer activity, apoptosis and thioredoxin reductase inhibition†

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Gold(I) complexes of imidazole and thiazole-based diphos type ligands were prepared and their potential as chemotherapeutics investigated. Depending on the ligands employed and the reaction conditions complexes $[L(AuCl)_2]$ and $[L_2Au]X$ (X = Cl, PF_6) are obtained. The ligands used are diphosphanes with azoyl substituents $R_2P(CH_2)_2PR_2$ {R = 1-methylimidazol-2-yl (**1**), 1-methylbenzimidazol-2-yl (**4**), thiazol-2-yl (**5**) and benzthiazol-2-yl (**6**)} as well as the novel ligands $RPhP(CH_2)_2PRPh$ {R = 1-methylimidazol-2-yl (**3**)} and $R_2P(CH_2)_3PR_2$ {R = 1-methylimidazol-2-yl (**2**)}. The cytotoxic activity of the complexes was assessed against three human cancer cell lines and a rat hepatoma cell line and correlated to the lipophilicity of the compounds. The tetrahedral gold complexes $[(\mathbf{3})_2Au]PF_6$ and $[(\mathbf{5})_2Au]PF_6$ with intermediate lipophilicity ($\log D_{7.4} = 0.21$ and 0.25) showed significant cytotoxic activity in different cell lines. Both compounds induce apoptosis and inhibit the enzymes thioredoxin reductase and glutathione reductase.

Introduction

Since the authorization of cisplatin in 1978, the interest in and development of metal-based drugs prospers consistently. Still, cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, and its analogs, especially oxaliplatin and carboplatin, are basic chemotherapeutics in combination therapy.[1,2] The therapeutic effect of cisplatin is based on DNA metallation and the formation of cisplatin adducts with DNA is thought to trigger apoptosis.[3,4] The major draw-back for a successful chemotherapy is acquired resistance towards the applied drug in the course of therapy.[5] Resistance to a specific chemotherapeutic drug can by circumvented by using drugs, which address alternative cellular targets. There is much recent evidence that mitochondria play a critical role in the regulation of apoptosis, making them an attractive target for the design of new anticancer drugs.[6–8] Gold complexes have been investigated for potential anti-tumour properties[9,10] and gold(I) phosphane derivatives have shown the most reproducible and significant activity in murine tumour models *in vivo*.[11] Many complexes of the general formula [(R_3P)Au(thiolate)] related to auranofin exhibit promising antitumor potential, some even in the case of cisplatin resistant cell lines.[12–16] More recently, also tetrahedral gold(I) complexes of chelating bisphosphanes have shown promising activity in murine tumour models[17] and some anticancer gold(I)–phosphane complexes exhibit significant autophagy-inducing properties in cancer cells.[18]

Gold(I) complexes like $[(dppe)_2Au]^+$ (dppe = 1,2-bis-(diphenylphosphino)ethane) and analogue compounds belong to the group of delocalised lipophilic cations (DLCs),[19–21] which can pass cellular membranes and accumulate in mitochondria.[7,22] The elevated membrane potential of cancerous cells can be used for selective drug accumulation as it leads to increased uptake and retention of lipophilic cations.[23,24] Despite the promising cytotoxicity profile of $[(dppe)_2Au]^+$, preclinical studies showed that $[(dppe)_2Au]^+$ has severe adverse effects, *e.g.* cardiac, hepatic and vascular toxicity, all correlated to the highly lipophilic nature of the compound.[25,26]

In order to circumvent the lipophilicity related side effects, more hydrophilic diphos complexes were developed.[27,28] In the group of Berners-Price diphos-type ligands were used where the phenyl rings of dppe were replaced by pyridinyl substituents leading to more selective compounds.[29–33] It was shown that the balance between lipophilicity and hydrophilicity of the gold compounds

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is a very important parameter in optimizing biodistribution, activity and selectivity of the drugs. Especially the selectivity of gold(I)bisphosphane drugs for cancer over normal tissue could be improved by optimizing the lipophilic–hydrophilic balance of the complexes by ligand design. Additionally, when compared to gold(I) complexes with monodentate phosphane ligands like auranofin these tetrahedral bischelated gold(I) complexes are much more stable towards ligand exchange in the presence of serum proteins, thiols and disulfides. Therefore, the rational design of gold complex properties is critical for their future as potential antitumor drugs.[34,35]

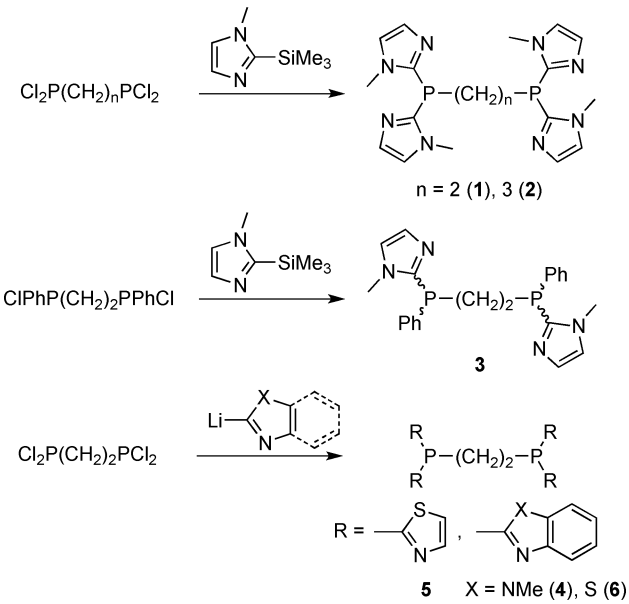
A potential cellular target for anticancer metallo-drugs alternative to DNA, are the seleno-enzymes of the thioredoxin reductase (TrxR) family, which play a crucial role in the regulation of the cellular redox state.[36–39] A cytosolic (TrxR1) and a mitochondrial form (TrxR2) are known and reduce the 12 kDa disulfid protein thioredoxin (Trx) to the corresponding dithiolic form.[40] Inhibition of the active site of TrxR is a common for cytotoxic metal-based drugs.[41–44] An increase in Trx and TrxR activities is correlated to circumvention of apoptosis and acceleration of tumour growth, while in turn efficient inhibition of TrxR leads to apoptosis of cancer cells.

Recently we reported on the cytotoxic potency of gold(I) chlorido complexes with water soluble imidazolylphosphanes.[45] In this work we studied structural and biological properties of a series of linear and tetrahedral coordinated gold(I) complexes $[(L)(AuCl)_2]$ and $[(L)_2Au]X$ with imidazolyl- and thiazolyl-based water soluble diphos-type ligands. The ligands are used to tune the solubility and lipophilicity of the gold complexes, which, in turn, have marked influence on their cytotoxic activity against different cell lines.

Results and discussion

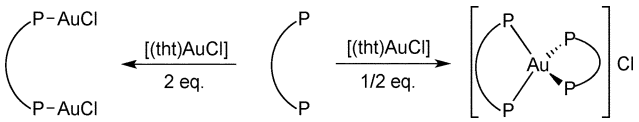
Recently we have investigated synthetic routes towards a series of azoyl substituted diphosphane ligands and reported on the preparation of compounds bis(di-1-methylimidazol-2-ylphosphino)ethane (**1**), bis(di-1-methylbenzimidazol-2-ylphosphino)ethane (**4**), bis(dithiazol-2-ylphosphino)ethane (**5**) and bis(dibenzthiazol-2-ylphosphino)ethane (**6**).[46] The synthesis of **4**, **5** and **6** involves reaction of the corresponding heteroaromatic compound with an slight excess of *n*-BuLi followed by $Cl_2P(CH_2)_2PCl_2$. The analogous imidazole-based compound **1** was synthesised by reaction of 1-methyl-2-trimethylsilylimidazole with $Cl_2P(CH_2)_2PCl_2$. This procedure was applied in this work to synthesise the analogous imidazolyl derivative of dppp (1,3-bis(diphenylphosphino)propane), 1,3-bis(1-methylimidazol-2-ylphosphono)propane (**2**), as well as the asymmetric ligand bis(1-methylimidazol-2-ylphenylphosphino)ethane (**3**). The starting material $Cl_2P(CH_2)_3PCl_2$ was synthesised according to the procedure published by Berven *et al.* which involves reaction of 1,3-dibromopropane with triethylphosphite, subsequent reduction with $LiAlH_4$ and chlorination using triphosgene.[47]

1,2-Bis(chlorophenylphosphione)ethane, $ClPhP(CH_2)_2PPhCl$, was prepared according a modified synthesis by Long and Jones. Dppe was treated with lithium in tetrahydrofuran. PCl_3 was added *in situ* to the orange solution of $LiPhP(CH_2)_2PPhLi$ to give $ClPhP(CH_2)_2PPhCl$.[48] The chlorophosphanes react with freshly distilled 1-methyl-2-trimethylsilylimidazole to give the two novel imidazole-based ligands **2** and **3** in good yields (Scheme 1).



Scheme 1 Synthesis of ligands **1**–**6**.

The complexes $[(L)(AuCl)_2]$ and $[(L)_2Au]Cl$ were prepared by reaction of stoichiometric quantities of the corresponding ligand and [(tht)AuCl] (tht = tetrahydrothiophene) in dichloromethane or chloroform (Scheme 2). Within this series, the tetrahedral complexes $[(\mathbf{4})_2Au]Cl$ and $[(\mathbf{6})_2Au]Cl$ could not be prepared by this procedure. The MALDI MS spectra of all complexes $[(L)(AuCl)_2]$ show the signal for the ion $[(L)Au_2Cl]^+$ and the spectra of all complexes $[(L)_2Au]Cl$ the ion $[(L)_2Au]^+$ as the basis peak, respectively.



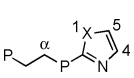
Scheme 2 Synthesis of the gold(I) complexes $[(L)(AuCl)_2]$ and $[(L)_2Au]Cl$.

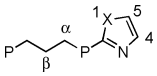
The ^1H and $^{31}P\{^1H\}$ NMR chemical shifts of the ligands and the resulting complexes are summarised in Table 1 and are very similar to those of the pyridinyl analogs published by Berners-Price *et al.*[33]

Upon coordination the ^{31}P NMR resonance is shifted 30–50 ppm towards lower field. The ^{31}P NMR resonances of complexes $[(L)_2Au]X$ with the dppe-type ligands are found at about 5 ± 2 ppm to higher field compared to the $[(L)(AuCl)_2]$ complexes. The corresponding complexes of the dppp-type ligand **2** show different resonance at –10 ppm for $[(\mathbf{2})(AuCl)_2]$ and –37 ppm for $[(\mathbf{2})_2Au]Cl$. In the ^1H NMR spectra the signal of the CH_2-protons of the ethylene bridge of ligands **1**, **3** and **5** are shifted in the corresponding complexes $[(L)_2Au]X$ and $[(L)(AuCl)_2]$. Upon coordination this signal is shifted slightly more towards lower field in the complexes $[(L)(AuCl)_2]$ than in the corresponding complexes $[(L)_2Au]X$.

The asymmetric ligand **3** shows two signals in dmso-d_6 for the *rac* and *meso* isomers in the $^{31}P\{^1H\}$ spectrum at –38.3 and

Table 1 ^{1}H and ^{31}P NMR data for ligands (L) **1**, **2**, **3**, **4**, **5**, **6** as well as their complexes $[(L)(AuCl)_2]$ and $[(L)_2Au]X$





	$\delta(^{1}H)$					
δ/ppm	$NCH_3(1)$	H^4	H^5	$CH_2(H^\alpha)$	$CH_2(H^\beta)$	$\delta(^{31}P)$
1[a]	3.67	7.27	7.34	2.62	—	−54[a]
[(**1**)$(AuCl)_2$][b]	3.79	7.20	7.60	3.28	—	−12[b]
[(**1**)$_2$Au]Cl[a]	3.44	7.32	7.43	3.02	—	−17[b]
2[a]	3.57	7.10	7.23	2.59	1.63	−53[a]
[(**2**)$(AuCl)_2$][b]	3.73	7.19	7.57	3.08	2.07	−10[b]
[(**2**)$_2$Au]Cl[b]	3.57	7.19	7.38	2.77	1.78	−37[b]
3[b]	3.75	7.10	7.30	2.08	—	−38.3
				2.35		−38.5
[(**3**)$(AuCl)_2$][c]	3.81	7.13	7.26	2.77	—	5.5[b]
						6.7[b]
[(**3**)$_2$Au]Cl[d]	3.05	—[e]	—[e]	2.58	—	4.2[b]
4[c]	3.72	—	—	3.09	—	−45[c]
[(**4**)$(AuCl)_2$][c]	4.14	—	—	3.66	—	−6[c]
5[c]	—	7.58	8.06	2.73	—	−23[c]
[(**5**)$(AuCl)_2$][b]	—	8.32	8.32	3.32	—	10[b]
[(**5**)$_2$Au]Cl[c]	—	7.67	7.89	3.09	—	3[c]
6[c]	—	—	—	3.01	—	−15[c]
[(**6**)$(AuCl)_2$][c]	—	—	—	3.77	—	16[c]

Measured in[a] MeOD-d_4, [b] dmso-d_6, [c] $CDCl_3$ or [d] D_2O. [e] The signals for the imidazolyl- and phenyl-protons overlap to give a multiplet.

−38.5 ppm and in the ^{1}H NMR spectrum for the protons of the $-(CH_2)_2-$ bridge at 2.08 and 2.35 ppm, respectively. Reaction of **3** with 2 equivalents of [(tht)AuCl] yielded a 1 : 1.5 equilibrium mixture of the *rac*- and *meso*-isomers of [(**3**)$(AuCl)_2$] with signals in $^{31}P\{^{1}H\}$ NMR spectrum in dmso-d_6 at 6.7 and 5.5 ppm, respectively. Possible equilibria between these compounds and [(**3**)$_2$Au]Cl has been investigated by NMR titrations of [(tht)AuCl] with ligand **3** in $CDCl_3$ and dmso-d_6 (see ESI†). Such solvent-dependent equilibria have been observed for many other diphosphane complexes.[49,50] At low L : Au ratios (L : Au ≤ 2) [(**3**)$(AuCl)_2$] is the predominant species, whereas at high L : Au ratios only [(**3**)$_2$Au]Cl is observed (δ_P = 4.2 ppm). The *rac*- and *meso*-isomers of [(**3**)$(AuCl)_2$] could be separated conveniently by their different solubility and stability in chloroform. The chloroform soluble compound was identified as [(**3**)$(AuCl)_2$]. Suitable crystals for X-ray crystallography of this compound were obtained by slow diffusion of *c*-pentane into a chloroform solution of [(**3**)$(AuCl)_2$].

The structures of the complexes [(L)$(AuCl)_2$] (L = **1**, **3**, **4**, **6**) and [(**3**)$_2$Au]PF_6 were determined by single crystal X-ray diffraction. In the molecular structure of [(**3**)$_2$Au]PF_6 the gold atom is coordinated tetrahedral by the two bidentate ligands in a κ^2P,P fashion (Fig. 1). The metric parameters of [(**3**)$_2$Au]PF_6 are within the range reported for other monomeric bis-chelated tetrahedral $[(L)_2Au]^+$ complexes (Table 2).[49] In this structure a phenyl and 1-methylimidazole ring connected to the same phosphorous atom are disordered in a 6 : 4 ratio. In one case, four of the imidazole ring atoms overlap with four of the phenyl ring carbon atoms, in the other case, all atoms are separated. Due to this disorder, not all atoms could be refined anisotropically and none of the hydrogen atoms were added.

The gold atoms in the solid-state structures of [(**1**)$(AuCl)_2$], [(**3**)$(AuCl)_2$], [(**4**)$(AuCl)_2$] and [(**6**)$(AuCl)_2$] are coordinated linearly by a phosphorus and chlorine atom (Fig. 2 and 3). The metric parameters are within the range found in other diphos complexes of this type, *e.g.* [(dppe)$(AuCl)_2$].[51]

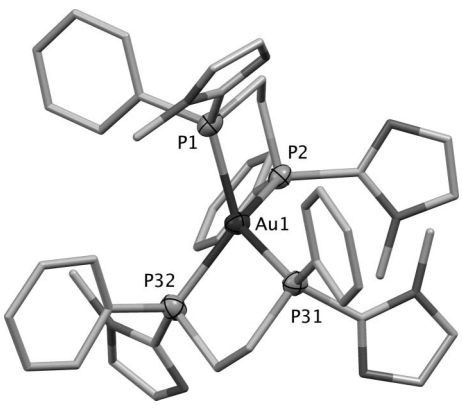


Fig. 1 Molecular structure of $[(3)_2Au]^+$ in [(**3**)$_2$Au]PF_6. Displacement ellipsoids are drawn at the 50% level and C and N atoms are drawn as capped sticks. H-atoms, minor components of the disordered phenyl/methylimidazole groups, the counter ion and co-crystallised solvent molecules are omitted for clarity. Selected bond lengths [Å] and angles [°]: Au1–P1 2.4301(9), Au1–P2 2.4197(9), Au1–P31 2.4130(9), Au1–P32 2.4198(9), P1–Au1–P2 84.94(3), P1–Au1–P31 120.26(3), P1–Au1–P32 127.30(4), P2–Au1–P31 121.54(3), P2–Au1–P32 122.04(3), P31–Au1–P32 85.44(3).

The structures of these complexes vary considerably in their PCCP torsion angles (from 180° to 64.4°). In the complexes [(**1**)$(AuCl)_2$], [(**4**)$(AuCl)_2$] and [(**6**)$(AuCl)_2$] an anti-conformation is found, since in all cases, the middle of the PC–CP bond is situated on an center of inversion. On the other hand in the solid state of [(**3**)$(AuCl)_2$] intramolecular aurophilic contacts (d_{Au-Au} = 3.122 Å)

Table 2 Distribution coefficients of bisphosphane ligands and corresponding gold(I) complexes

Ligand	L	$[(L)(AuCl)_2]$	$[(L)_2Au]X$
1	−0.73 ± 0.03	−1.05 ± 0.03	−1.73 ± 0.03
2	−0.74 ± 0.05	−1.51 ± 0.09	−1.38 ± 0.01
3	1.65 ± 0.03	0.79 ± 0.08	0.25 ± 0.03
5	0.73 ± 0.01	n.d.[a]	0.21 ± 0.02

[a] n.d. not determined, insoluble in water/n-octanol.

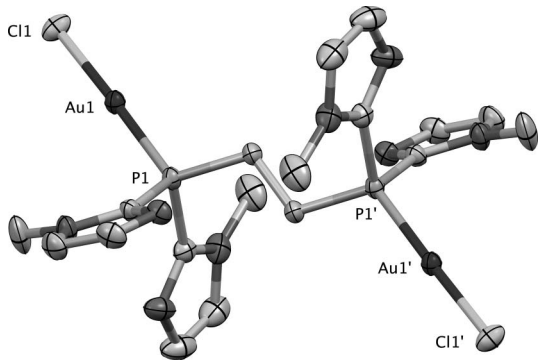


Fig. 2 Molecular structure of $[(\mathbf{1})(AuCl)_2]$. Projection along the crystallographic *b*-axis. Displacement ellipsoids are drawn at the 50% level and H-atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: Au1–P1 2.2284(12), Au1–Cl1 2.2848(12), P1–Au1–Cl1 177.04(4), P1–C1–C1a–P1a 180.00.

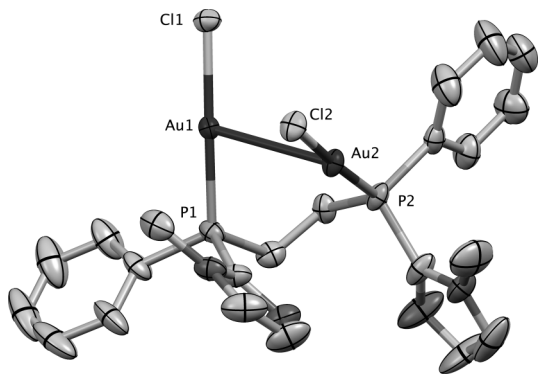


Fig. 3 Molecular structure of $[(\mathbf{3})(AuCl)_2]\cdot 0.5EtOH\cdot 0.4CH_2Cl_2$. The configurations of the stereocenters at the phosphorus atoms are of opposed sign. Displacement ellipsoids are drawn at the 50% level. Solvent molecules and H-atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: Au1–P1 2.2345(17), Au1–Cl1 2.2970(15), Au2–P2 2.2388(16), Au2–Cl2 2.2984(16), Au1–Au2 3.1218(3), P1–Au1–Cl1 179.25(7), P2–Au2–Cl2 176.54(7), P1–C– C–P2 64.4(7).

lead to a *gauche* conformation. Complex $[(\mathbf{3})(AuCl)_2]$, with the asymmetric ligand **3**, crystallised as an ethanol/dichloromethane solvate in the space group $P2_1$ and racemic twinning was found.

The synthesized tetrahedral gold(I) complexes $[(diphos)_2Au]^+$ are more hydrophilic compared to the highly lipophilic and toxic parent compound $[Au(dppe)_2]^+$ ($\log P = 1.41$).[29] The nature of the substituents, the length of the spacer between the phosphorous atoms and the structure of the resulting complexes finely tune solvent interactions and their hydrophilicity/lipophilicity covers a wide range with distribution coefficients ($\log D_{7.4}$) between −1.73 and +0.79 (Table 2). The distribution coefficients of the ligands as well as the values for the corresponding gold(I) complexes are summarized in Table 4. Due to the poor solubility of the benzannulated ligands **4** and **6** in n-octanol and water distribution coefficients of these ligands as well as the corresponding complexes were not determined.

Table 3 IC_{50} values (μM) of $[(L)_2Au]Cl$ and $[(L)(AuCl)_2]$ against human ovarian carcinoma cell lines sensitive (A2780 sens.) or resistant to cisplatin (A2780 cis.)[a,b]

	$[(L)_2Au]X$		$[(L)(AuCl)_2]$	
Ligand	A2780 sens.	A2780 cis.	A2780 sens.	A2780 cis.
Cisplatin	1.32	13.4	1.32	13.4
1	28.9	34.6	30.1	32.9
2	21.6	41.2	24.0	37.8
3	0.398	0.812	0.575	18.8
4	n.a.[c]	n.a.[c]	2.34	11.8
5	0.446	2.18	2.63	6.76
6	n.a.[c]	n.a.[c]	6.55	>10

[a] cisplatin as reference compound. [b] pIC_{50} ± error are found in the ESI.† [c] n.a. = not available.

Table 4 IC_{50} values (μM) of $[(L)_2Au]^+$ against human leukaemia (K562), human colon carcinoma (Hct116) and rat hepatoma cell lines (H4IIE)[a]

Compound	K 562	Hct116	H4IIE
Cisplatin	18.0	37.5	34.8
$[(\mathbf{3})_2Au]PF_6$	>10	21.4	38.3
$[(\mathbf{5})_2Au]Cl$	0.93	11.0	4.94

[a] pIC_{50} ± error are found in the ESI.†

Table 5 Relative activities of $[(L)_2Au]^+$ in different cell lines referenced to cisplatin ($IC_{50\ Cisplatin}/IC_{50\ compound}$)

Compound	A2780 sens.	A2780 cis.	K 562	Hct116	H4IIE
$[(\mathbf{3})_2Au]PF_6$	3.32	16.5	< 1.8	1.75	1.20
$[(\mathbf{5})_2Au]Cl$	2.96	6.15	19.2	3.41	6.02

The gold(I) compounds were screened for their cytotoxicity against cisplatin sensitive and resistant A2780 human ovarian cancer cell lines (Table 3). The gold complexes show IC_{50} values ranging from 0.4 to 40 μM. Especially, complexes bearing the thiazolyl ligand **5** and the asymmetric imidazolyl ligand **3**, respectively show considerably higher cytotoxic activity than cisplatin. Complex $[(\mathbf{3})_2Au]PF_6$ shows highest cytotoxicity in both cisplatin sensitive and resistant A2780 cell line (Tables 3 and 5). Complex $[(\mathbf{5})_2Au]Cl$ also shows a higher cytotoxic activity than cisplatin in both A2780 cell lines, but here partial resistance is observed. Interestingly complex $[(\mathbf{3})(AuCl)_2]$ shows a significant difference in the cytotoxicity towards the cisplatin sensitive and resistant A2780 cell lines, with a selectivity in the order found for cisplatin, whereas the other complexes of the type $[(L)(AuCl)_2]$ show much lower selectivity.

Within the series of gold(I) complexes $[(\mathbf{3})_2Au]PF_6$ and $[(\mathbf{5})_2Au]Cl$ as well as $[(\mathbf{3})(AuCl)_2]$ exhibit highest antitumour activity against the human ovarian cancer cell line A2780sens.

The high activity correlates with the intermediate lipophilicity of these compounds. These complexes are significantly more lipophilic (log$D_{7.4}$ = +0.25, +0.21 and +0.79) than the water-soluble complexes [(**1**)$_2$Au]Cl and [(**2**)$_2$Au]Cl (log$D_{7.4}$ = −1.73 and −1.38) and more hydrophilic than the highly toxic parent compound [Au(dppe)$_2$]$^+$ (log$P$ = +1.41). Consequently, an optimal balance of the lipophilic and hydrophilic nature of those complexes is one of the most important factors for antitumor activity as was previously shown for analogous pyridinyl-based complexes by Berners-Price.[35] Only the complexes with an intermediate lipophilicity (between 0.21 and 0.79) show promising cytotoxicity, while the more hydrophilic complexes are inactive against these cell lines.

The most active compounds [(**3**)$_2$Au]PF$_6$ and [(**5**)$_2$Au]Cl were further screened for their cytotoxicity against human leukaemia (K562), human colon carcinoma (Hct116) and rat hepatoma (H4IIE) cell lines (Table 4). Here, the two complexes show some significant differences in their antitumor activities, especially against the leukaemia cell line K562. While the thiazole-based complex [(**5**)$_2$Au]Cl shows cytotoxcity against all tested cell lines, complex [(**3**)$_2$Au]PF$_6$ only exhibits high antitumor activity against the ovarian cancer cell lines, considerable less against the human colon carcinoma and rat hepatoma cell line and almost no cytotoxicity against the leukaemia cell line K562. This clearly demonstrates, that this compound is not toxic in general but shows selectivity towards different cancer cell lines.

Cell death can be induced by apoptosis and necrosis. Apoptosis is the process of programmed cell death whereas necrosis is the premature death of cells and living tissue. The mode of cell death induction of compounds [(**3**)$_2$Au]PF$_6$ and [(**5**)$_2$Au]Cl in H4IIE cells was determined using a DNA fragmentation assay.[52–54] Fig. 4 shows gel electrophoresis chromatograms of the DNA fragments after incubation of H4IIE cells at 37 °C for 24 h with different concentrations of [(**3**)$_2$Au]PF$_6$ and [(**5**)$_2$Au]Cl, respectively. Both compounds show the characteristic ladder pattern starting at 2.5 μM, which indicates apoptosis.

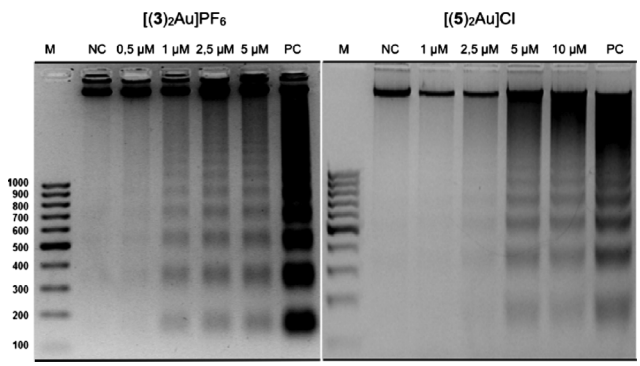


Fig. 4 Gel electrophoresis of DNA treated with various concentrations of a) [(**3**)$_2$Au]PF$_6$ and b) [(**5**)$_2$Au]Cl after 24 h incubation on H4IIE cells at 37 °C (M = marker, NC = negative control, PC = positive control).

The potential of the complexes [(L)$_2$Au]Cl (L = **1**, **2**, **5**) and [(**3**)$_2$Au]PF$_6$ to inhibit the activity of the disulfide reductases TrxR and GR was studied on the isolated enzymes using an established procedure (DTNB reduction assay, Table 6).[55] Generally [(**1**)$_2$Au]Cl, [(**2**)$_2$Au]Cl and [(**5**)$_2$Au]Cl displayed strong inhibitory activities with EC$_{50}$ values for TrxR comparable to that of other bioactive gold(I) species.[12,55] The activity of GR was also inhibited significantly but with lower efficiency. In complex [(**3**)$_2$Au]PF$_6$ two heteroaryl ligands were replaced by phenyl resulting in a significant decrease of activity against both enzymes.

Table 6 Inhibition of thioredoxin reductase (TrxR) and glutathione reductase (GR)[a]

Compound	IC$_{50}$ (TrxR)/μM	IC$_{50}$ (GR)/μM	IC$_{50}$(GR)/IC$_{50}$(TrxR)
[(**1**)$_2$Au]Cl	0.14	0.60	4
[(**2**)$_2$Au]Cl	0.12	1.13	10
[(**3**)$_2$Au]PF$_6$	1.22	4.23	3
[(**5**)$_2$Au]Cl	0.14	0.46	3

[a] pIC$_{50}$ ± error are found in the ESI.†

The most potent compounds [(**3**)$_2$Au]PF$_6$ and [(**5**)$_2$Au]Cl are of immediate lipophilicity (log$D_{7.4}$ = 0.25 and 0.21) and inhibit both GR and TrxR. Although complexes [(**1**)$_2$Au]Cl and [(**2**)$_2$Au]Cl are as potent TrxR inhibitors as [(**5**)$_2$Au]Cl, their cytotoxic activity is much lower. For [(**5**)$_2$Au]Cl this is consistent with the proposed mechanism of action for gold(I) compounds of the type [(L)$_2$Au]$^+$, as these lipophilic cations have to cross membranes before they can interact with cytosolic or mitochondrial TrxR as cellular targets. [(**3**)$_2$Au]PF$_6$ shows in some cell lines the highest cytotoxicity (A2780sens. and cis.), although the inhibition of TrxR is poor. This could indicate a different mode of action for this compound in these cell lines.

Conclusion

We prepared a series of gold(I) complexes [(diphos)(AuCl)$_2$] and [(diphos)$_2$Au]X with azol-based diphos type ligands. Therefore two novel imidazole-based ligands bis(di-1-methylimidazol-2-ylphosphino)propane (**2**) and bis(1-methylimidazol-2-ylphenylphosphino)ethane (**3**), have been prepared. The *rac* and *meso* isomers of [(**3**)(AuCl)$_2$] could be separated due to their different solubility in chloroform. In the solid state structure of [(**3**)(AuCl)$_2$] intramolecular aurophilic contacts are found. The complexes [(diphos)(AuCl)$_2$] and [(diphos)$_2$Au]X have distribution coefficients (log$D_{7.4}$) between −1.73 and 0.79 and are more hydrophilic than the highly toxic parent compound [Au(dppe)$_2$]Cl. Of the number complexes screened, only the bis-chelated gold(I)-complexes [(**3**)$_2$Au]PF$_6$ and [(**5**)$_2$Au]Cl with intermediate lipophilicity (log$D_{7.4}$ = 0.21 und 0.25) showed high cytotoxcities. But there are differences in the selectivity of these two complexes. While complex [(**5**)$_2$Au]Cl showed a similar cytotoxicity towards all tested cell lines, complex [(**3**)$_2$Au]PF$_6$ is inactive against the leukaemia cell line K562. Therefore the complexes [(**3**)$_2$Au]PF$_6$ and [(**5**)$_2$Au]Cl do not share a common toxic moiety. This observation could be confirmed by proving the occurrence of apoptosis.

As reported recently by Rackham *et al.* bischelated gold(I) phosphine complexes can be effective inhibitors of TrxR and act as antimitochondrial agents.[56] Here we showed that complexes of this type are also able to inhibit the activity of GR but with lower potency. The main difference between the two proteins is the presence of a selenocysteine–cysteine bridge in the active site of TrxR, which is replaced by a cysteine–cysteine bridge in GR. The preferential inhibition of TrxR over GR might be a consequence

of this difference and has also been observed with other gold species.[10,36]

Experimental section

The compounds [(tht)AuCl], 1-methyl-2-trimethylsilylimidazole, ligands **1**, **4**, **5** and **6** were prepared according to literature procedures.[46] The preparations were carried out in Schlenk tubes under an atmosphere of dry nitrogen using anhydrous solvents purified according to standard procedures. The metal complexes were prepared using wet solvents. All chemicals were used as purchased. ^{1}H and ^{31}P{^{1}H}NMR spectra were recorded on a Bruker DRX 200 and ^{13}C{^{1}H} NMR spectra on a Bruker DRX 500 spectrometer. The ^{1}H and ^{13}C{^{1}H} NMR spectra were calibrated against the residual proton signals and the carbon signals of the solvents as internal references ($CDCl_3$: $\delta_H = 7.30$ ppm and $\delta_C = 77.0$ ppm; MeOD-d_4: $\delta_H = 3.31$ ppm and $\delta_C = 49.1$ ppm, dmso-d_6: $\delta_H = 2.50$ ppm and $\delta_C = 39.5$ ppm; D_2O: $\delta_H = 4.79$) while the ^{31}P{^{1}H} NMR spectra were referenced to external 85% H_3PO_4. The signals are referred to as singlet (s), doublet (d), virtual triplets (vt), multiplets (m) and broad signals (br). The MALDI mass spectra were recorded on a Bruker Ultraflex MALDI-TOF mass spectrometer and ESI mass spectra with a Finnigan LCQ Deca Ion-Trap-API mass spectrometer, The elemental composition of the compounds was determined with a Perkin Elmer Analysator 2400 at the Institut für Pharmazeutische und Medizinische Chemie, Heinrich-Heine-Universität Düsseldorf.

Bis(di-1-methylimidazol-2-ylphosphino)propane (2)

1,3-Bis(dichlorophosphino)propane (1.0 g, 4.1 mmol) was added to 1-methyl-2-trimethylsilanylimidazole (2.8 g, 0.02 mol) at 0 °C drop-wise. The suspension was stirred for 1 h at 0 °C and for 17 h at ambient temperature. The resulting $(CH_3)_3SiCl$ was removed *in vacuo* and the residue washed with n-hexane. The solid was filtered off and dried in vacuo. Yield: 1.4 g (82%). ^{1}H NMR (MeOD-d_4): δ = 1.63 (m, 2H, CH_2(CH$_2$)$_2$CH_2),2.59 (vt, 4H, (CH_2)$_2$), 3.57 (s, 12H, NCH_3), 7.10 (d, $^{3}J_{HH}$ = 1.2 Hz 4H, $H4_{im}$), 7.23 (s, 4H, $H5_{im}$). ^{31}P{^{1}H}NMR (MeOD-d_4): δ = –59 (s). ^{13}C{^{1}H} NMR (MeOD-d_4): δ = 22.6 (*C*H$_2$ (β)), 26.1 (CH$_2$ (α)), 33.9 (N*C*H$_3$), 124.4 (*C*5$_{im}$), 130.5 (*C*4$_{im}$), 144.0 (*C*2$_{im}$). MALDI MS ($CHCl_3$): m/z (%) = 429 [M+H]$^+$. $C_{19}H_{26}N_8P_2 \cdot 0.5CH_2Cl_2$(470.88): calc. C 49.74, H 5.87, N 23.8, found C 49.9, H 6.4, N 23.6. Distribution coefficient: log$D_{7.4}$ = –0.74 ± 0.09.

Bis(1-methylimidazol-2-ylphenylphosphino)ethane (3)

1,2-Bis(chloro-2-ylphenylphosphino)-ethane (0.71 g, 46 mmol) was added to 1-methyl-2-trimethylsilanylimidazole (0.73 g, 0.023 mol) at 0 °C drop-wise. The suspension was stirred for 1 h at 0 °C and 17 h at ambient temperature. The resulting $(CH_3)_3SiCl$ was removed in vacuo. The residue was dissolved in chloroform and the product was precipitated upon addition of diethyl ether. Yield: 0.31 g (33%). ^{1}H NMR (dmso-d_6): δ = 2.08–2.35 (m, 4H, (CH$_2$)$_2$), 3.57 (s, 6 H, NCH_3), 7.10 (s, 2H, H4$_{im}$), 7.30 (s, 2H, H5$_{im}$), 7.33–7.39 (m, 10H, Ph). ^{31}P{^{1}H} NMR (dmso-d_6): δ = –38.3 (s), –38.5 (s). MALDI MS ($CHCl_3$): m/z (%) = 407 [L]$^+$, 423 [LO]$^+$, 439 [LO$_2$]$^+$. $C_{22}H_{24}N_4P_2 \cdot 0.5CHCl_3$(466.09):calc. C 57.98, H 5.30, N 12.02, found C 57.7, H 5.5, N 12.3. Distribution coefficient: log$D_{7.4}$ = 1.65 ± 0.03.

Preparation of complexes [(L)(AuCl)$_2$]

[(tht)AuCl] (0.31 mmol) and the corresponding ligand (0.16 mmol) were stirred in dichloromethane at least for 2 h. The resulting precipitate was filtered, washed twice with dichloromethane or chloroform and diethyl ether and dried in vacuo.

[(1)(AuCl)$_2$]

Yield: 0.11 g (78%). ^{1}H NMR (dmso-d_6): δ = 3.28 (vt, 4H, (CH_2)$_2$), 3.79 (s, 12H, NCH_3), 7.20 (s, 4H, $H4_{im}$), 7.60 (s, 4H, $H5_{im}$). ^{31}P{^{1}H} NMR (dmso-d_6): δ = –13 (s). ESI$^+$ (MeOH): m/z (%) = 333 [L-im^{NMe}]$^+$, 612 [LAu]$^+$, 808 [LAu$_2$]$^+$, 843 [LAu$_2$Cl]$^+$. MALDI MS ($CHCl_3$): m/z (%) = 843 [LAu$_2$Cl]$^+$. $C_{18}H_{24}N_8P_2Au_2Cl_2 \cdot 2CH_2Cl_2$ (1049.08): calc. C 22.90, H 2.69, N 10.68; found C 22.8, H 2.7, N 10.7. Distribution coefficient: log$D_{7.4}$ = –1.05 ± 0.03.

[(2)(AuCl)$_2$]

Yield: 97 mg (68%). ^{1}H NMR (dmso-d_6): δ = 2.07 (s (br), 2H, CH$_2$-(CH_2)-CH$_2$), 3.08 (br, 4H, CH_2-(CH$_2$)-CH_2), 3.73 (s, 12H, NCH_3), 7.19 (s, 4H, $H4_{im}$), 7.57 (s, 4H, $H5_{im}$). ^{31}P{^{1}H}NMR (dmso-d_6): δ = –10 (s). ESI$^+$ (H$_2$O): m/z (%) = 347 [L-im]$^+$, 626 [LAu]$^+$. MALDI MS (MeOH): m/z (%) = 626 [LAu]$^+$, 857 [LAu$_2$Cl]$^+$. $C_{19}H_{26}N_8P_2Au_2Cl_2 \cdot 0.5CH_2Cl_2$ (935.72): calc. C 25.03, H 2.91, N 11.98; found C 25.3, H 2.6, N 11.6. Distribution coefficient: log$D_{7.4}$ = –1.51 ± 0.09.

[(3)(AuCl)$_2$]

Yield: 0.12 g (86%).^{1}H NMR ($CDCl_3$): δ = 2.77 (vt, 4H, (CH_2)$_2$), 3.81 (s, 6H, NCH_3), 7.10 (s, 2H, $H4_{im}$), 7.22 (s, 2H, $H5_{im}$), 7.41–7.76 (m, 10H, Ph). ^{31}P{^{1}H} NMR (dmso-d_6): δ = 5.5 (s), 6.7 (s). MALDI MS ($CDCl_3$): m/z (%) = 835 [LAu$_2$Cl]$^+$, 1705 [LAu$_2$Cl$_2$]$_2^+$. $C_{22}H_{24}N_4P_2Au_2Cl_2 \cdot 3\ CHCl_3$ (1229.38): calc. C 24.43, H 2.21, N 4.56; found C 24.3, H 2.4, N 4.7. Distribution coefficient: log$D_{7.4}$ = 0.79 ± 0.08.

[(4)(AuCl)$_2$]

Yield: 92 mg (53%). ^{1}H NMR ($CDCl_3$): δ = 3.66 (d, J = 5.44 Hz, 4H, (CH_2)$_2$), 4.14 (s, 12H, NCH_3), 7.29–7.46 (m, 12H, H_{ph}), 7.70 (d, $^{3}J_{HH}$ = 7.72 Hz, 4H, H_{ph}). ^{31}P{^{1}H} NMR ($CDCl_3$): δ = –6 (s). MALDI MS ($CHCl_3$): m/z = 1043 [LAu$_2$Cl]$^+$. $C_{34}H_{32}N_8P_2Au_2Cl_2 \cdot 2CH_2Cl_2$ (1249.32): calc. 34.61, H 2.90, N 8.97; found: C 34.4, H 2.9, N 9.0. Distribution coefficient: log$D_{7.4}$ = n.d.

[(5)(AuCl)$_2$]

Yield: 0.11 g (77%). ^{1}H NMR (dmso-d_6): δ = 3.32 (d, 4H, (CH_2)$_2$), 8.32 (s, 4H, H_{th}). ^{31}P{^{1}H} NMR (dmso-d_6): δ = 10 (s). FAB MS: m/z = 343 [L-C$_3$H$_3$NS]$^+$, 623 [LAu]$^+$, 855 [LAu$_2$Cl]$^+$. $C_{14}H_{12}N_4S_4P_2Au_2Cl_2$ (891.32): calc. C 18.87, H 1.36, N 6.29; found C 19.0, H 1.4, N 6.0. Distribution coefficient: log$D_{7.4}$ = n.d.

[(6)(AuCl)$_2$]

Yield: 94 mg (54%). ^{1}H NMR ($CDCl_3$): δ = 3.76 (d, J = 5.1 Hz, 4H, (CH_2)$_2$), 7.57–7.68 (m, 8H, H_{ph}), 8.13–8.27 (m, 8H, H_{ph}). ^{31}P{^{1}H} NMR ($CDCl_3$): δ = 16 (s). MALDI MS ($CHCl_3$): m/z = 823 [LAu]$^+$, 1055 [LAu$_2$Cl]$^+$. $C_{30}H_{20}N_4S_4P_2Au_2Cl_2 \cdot 2.5CH_2Cl_2$

(1303.88): calc. C 29.94, H 1.93, N 4.30; found C 29.8, H 1.7, N 4.3. Distribution coefficient: log$D_{7.4}$ = n.d.

Preparation of complexes [(L)$_2$Au]Cl

[(tht)AuCl] (320.59 g mol^{-1}, 0.16 mmol) and the corresponding ligand (0.31 mmol) were stirred in dichloromethane for 17 h. The volume of the solution was reduced to 1/3 and layered with diethyl ether. The resulting precipitate was filtered, washed with diethyl ether and dried in vacuo.

[(**1**)$_2$Au]Cl

Yield: 68 mg (40%). ^{1}H NMR (MeOD-d_4): δ = 3.02 (s, 8H, (CH_2)$_2$), 3.44 (s, 24H, NCH_3), 7.32 (s, 8H, $H4_{im}$), 7.43 (s, 8H, $H5_{im}$). ^{31}P{^{1}H} NMR (D$_2$O): δ = −16 (s). ESI$^+$ (MeOH): m/z (%) = 333 [L-im^{NMe}]$^+$, 612 [LAu]$^+$, 1026 [L$_2$Au]$^+$, 1062 [L$_2$AuCl]$^+$. MALD IMS (CHCl$_3$): m/z (%) = 333 [L-im^{NMe}]$^+$, 415 [L]$^+$, 612 [LAu]$^+$, 1026 [L$_2$Au]$^+$. $C_{36}H_{48}N_{16}P_4AuCl{\cdot}3CH_2Cl_2$ (1315.98): calc. C 35.60, H 4.14, N 17.03, found C 35.4, H 4.4, N 16.8. Distribution coefficient: log$D_{7.4}$ = −1.73 ± 0.03.

[(**2**)$_2$Au]Cl

Yield: 0.1 g (57%). ^{1}H NMR (dmso-d_6): δ = 1.78 (s (br), 4H, CH$_2$-(CH_2)-CH$_2$), 2.77 (vt, 8H, CH_2-(CH$_2$)-CH_2), 3.57 (s, 24H, NCH_3), 7.19 (d, $^3J_{HH}$ = 1.1 Hz,8H, $H4_{im}$), 7.38 (d, $^3J_{HH}$ = 1.1 Hz, 8H, $H5_{im}$). ^{31}P{^{1}H} NMR (D$_2$O): δ = −37 (s). ESI$^+$ (H$_2$O): m/z (%) = 347 [L-im^{NMe}]$^+$, 626 [LAu]$^+$. MALDI MS (MeOH): m/z (%) = 626 [LAu]$^+$, 1053 [L$_2$Au]$^+$. $C_{38}H_{52}N_{16}P_4AuCl{\cdot}3.5\ CH_2Cl_2$ (1386.50): calc. C 35.95, H 4.29, N 16,16; found C 35.5, H 4.2, N 16.4. Distribution coefficient: log$D_{7.4}$ = −1.38 ± 0.01.

[(**3**)$_2$Au]Cl

Yield: 0.12 g (72%). ^{1}H NMR (D$_2$O): δ = 2.58 (br, 8H, (CH_2)$_2$), 3.05 (s, 12H, NCH_3), 7.08-7.49 (m, 28H, $H_{im/Ph}$). ^{31}P{^{1}H} NMR (dmso-d_6): δ = 4.2 (s). ESI$^+$ (MeOH): m/z (%) = 603 [LAu]$^+$. MALDI MS (MeOH): m/z (%) = 407 [L+H]$^+$, 603 [LAu]$^+$, 1009 [L$_2$Au]. $C_{44}H_{48}N_8P_4AuCl{\cdot}0.5CH_2Cl_2$ (1087.69): calc. C 49.14, H 4.54, N 10.30; found C 49.1, H 4.9, N 9.8.

[(**5**)$_2$Au]Cl

Yield: 96 mg (55%). ^{1}H NMR (CDCl$_3$): δ = 3.09 (br, 8H, (CH_2)$_2$), 7.67 (d, $^3J_{HH}$ = 2.85 Hz, 8H, $H4_{th}$), 7.89 (br, 8H, $H5_{th}$). ^{31}P{^{1}H} NMR (CDCl$_3$): δ = 3 (s). ESI$^+$ (CH$_3$OH): m/z = 342 [L-th]$^+$, 623 [LAu]$^+$. MALDI MS (CHCl$_3$): m/z = 623 [LAu]$^+$, 1049 [L$_2$Au]$^+$. $C_{28}H_{24}N_8S_8P_4AuCl{\cdot}1.5CH_2Cl_2$ (1212.78): calc. C 29.22, H 2.24, N 9.24; found C 29.2, H 2.3, N 9.3. Distribution coefficient: log$D_{7.4}$ = 0.21 ± 0.02.

Preparation of [(**3**)$_2$Au]PF$_6$

[(tht)AuCl] (39 mg, 0.12 mmol) and ligand **3** (100 mg, 0.25 mmol) were stirred in dichloromethane for 15 min. To the solution NH_4PF_6 (21 mg, 0.12 mmol) in methanol was added and the mixture stirred for 15 h. The volume of the solution was reduced to 1/3 and layered with diethyl ether. The resulting precipitate was filtered, washed with diethyl ether and dried in vacuo. Yield: 0.15 g (81%). ^{1}H NMR (dmso-d_6): δ = 2.16–2.31 (br, 8H, (CH_2)$_2$), 2.92 (s, 12H, NCH_3), 6.96–7.46 (m, 28H, $H_{im/Ph}$). ^{31}P{^{1}H} NMR (dmso-d_6): δ = 4.2 (s), −143 (q, PF_6). ESI$^+$ (MeOH): m/z (%) = 603 [LAu]$^+$, 1009 [L$_2$Au]$^+$. MALDI MS (MeOH): m/z (%) = 407 [L+H]$^+$, 603 [LAu]$^+$, 1009 [L$_2$Au]$^+$. $C_{44}H_{48}N_8P_5AuF_6{\cdot}0.5CH_2Cl_2{\cdot}1.5\ H_2O$ (1224.20): calc. C 43.66, H 4.28, N 9.15; found C 43.4, H 4.69, N 9.9. Distribution coefficient: log$D_{7.4}$ = 0.25 ± 0.03.

X-ray crystallography

Crystallographic data were collected at 183(2) K on an Oxford Diffraction Xcalibur system with a Ruby detector using Mo-Kα radiation (λ = 0.7107 Å) that was graphite-monochromated. Suitable crystals were covered with oil (Infineum V8512, formerly known as Paratone N), mounted on top of a glass fibre and immediately transferred to the diffractometer. The program suite CrysAlis^{Pro} was used for data collection, semi-empirical absorption correction and data reduction.[57] Structures were solved with direct methods using SIR97 and were refined by full-matrix least-squares methods on F^2 with SHELXL-97.[58,59] [(**3**)(AuCl)$_2$] crystallized in the space group $P2_1$. A racemic twinning was found and refined at a ratio of 56 : 44. The asymmetric unit additionally contained 0.5 equiv. ethanol and 0.4 equiv. dichloromethane. The structure of [(**4**)(AuCl)$_2$] contained undefined residual electronic density caused by disordered solvent molecules. This was treated with the program utility SQUEEZE of the Platon program suite.[60] The structure of [(**3**)$_2$Au]PF$_6$ contained a disorder between one phenyl and one *N*-methylimidazole group bound to the same phosphorous atom. The occupancies were refined as a ratio of 6 : 4. In one case, two atoms of the imidazole and the phenyl overlaid almost perfectly. All the structures were checked for higher symmetry with help of the program Platon.[60]

Distribution coefficients (log*D*)

The n-octanol–water distribution coefficients of the compounds were determined using a shake-flask method. PBS buffered bidistilled water (100 mL, phosphate buffer, $c(PO_4^{3-})$ = 10 μM, c(NaCl) = 0.15 M, pH adjusted to 7.4 with HCl) and n-octanol (100 mL) were shaken together using a laboratory shaker (Perkin Elmer), for 72 h to allow saturation of both phases. 1 mg of each compound was mixed in 1 mL of aqueous and organic phase, respectively for 10 min using a laboratory vortexer. The resultant emulsion was centrifuged (3000 × g, 5 min) to separate the phases. The concentrations of the compounds in the organic and aqueous phases were then determined using UV absorbance spectroscopy (230 nm). LogD_{pH} was defined as the logarithm of the ratio of the concentrations of the complex in the organic and aqueous phases log$D$ = {[compound$_{(org)}$]/[compound$_{(aq)}$], the value reported is the mean of three separate determinations.

Materials, cell lines and cell culture

The human ovarian carcinoma cell line A2780 was obtained from European Collection of Cell Cultures (ECACC, Salisbury, UK). The human chronic myelogenous leukemia cell line K562 was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany). All other reagents were supplied by Sigma Chemicals unless otherwise stated. All cell lines were grown at 37 °C under humidified air supplemented with 5% CO_2 in RPMI 1640 (Invitrogen, Germany) containing 10% fetal calf

serum (PAN Biotech, Germany), 100 IU/mL penicillin, 100 μg mL^{-1} streptomycin and 2 mM glutamine. The cells were grown to 80% confluency before using them for the MTT cell viability assay.

MTT cell viability assays

The rate of cell-survival under the action of test substances was evaluated by an improved MTT assay as previously described.[61] The assay is based on the ability of viable cells to metabolize yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Applichem, Germany) to violet formazane crystals that can be detected spectrophotometrically. In brief, A2780 cells were seeded at a density of 8,000 cells/well and K562 at a density of 30,000 cells/well in 96well plates (Sarstedt, Germany). After 24 h, cells were exposed to the test compounds at concentrations of 10^{-5} M and 10^{-4} M. Incubation was ended after 72 h and cell survival was determined by addition of MTT solution (5 mg mL^{-1} in phosphate buffered saline). The formazan precipitate was dissolved in DMSO. Absorbance was measured at 544 nm and 620 nm in a FLUOstarmicroplate-reader (BMG LabTech, Offenburg, Germany). The absorbance of untreated control cells was taken as 100% viability. All tests were performed in triplicate.

DNA fragmentation analysis[52]

H4IIE cells (0.5×10^6) were plated in 30 mm cell culture dishes and allowed to attach for 48 h, then cells were incubated 24 h with the compounds. Then attached and floating cells were collected and lysed with 500 μL lysis buffer (10 mM Tris–HCl, 0.6% SDS, 10 mM EDTA) and 5 μL RNase A (10 mg mL^{-1}, DNase-free) for 40 min at 37 °C. Proteins were precipitated by addition of 125 μL NaCl (5 M) for 1 h at 4 °C, followed by centrifugation (10,000 × *g*, 15 min). The supernatant was first extracted with chloroform/phenol (250 μL/500 μL) followed by a second extraction with chloroform (650 μL). The DNA in the supernatant was precipitated with ice-cold isopropanol (1 mL) and stored overnight at −20 °C. Afterwards the samples were centrifuged (15 min, 10,000 × *g*, 4 °C) and the DNA pellet was resuspended in 30 μL TE-buffer (10 mM Tris–HCl, 1 mM EDTA in H_2O). DNA yield was measured photometrically at 260 nm. The oligonucleosomal DNA fragmentation as a characteristic feature of the apoptotic cell death was analysed *via* electrophoresis (1.75% agarose, 3.5 h at 60 V, 4 μg DNA/lane). After this the gel was stained with ethidium bromide and analysed under UV-light using the Quantity One system from Biorad (München, Germany).

Inhibition of thioredoxin-reductase

To determine the inhibition of TrxR and GR an established microplate reader based assay was performed with minor modifications.[55] For this purpose commercially available rat liver TrxR and baker yeast GR (both from Sigma-Aldrich) were used and diluted with distilled water to achieve a concentration of 2.0 U/mL. The compounds were freshly dissolved as stock solutions in DMF. To each 25 μL aliquots of the enzyme solution each 25 μL of potassium phosphate buffer pH 7.0 containing the compounds in graded concentrations or vehicle (DMF) without compounds (control probe) were added and the resulting solutions (final concentration of DMF: 0.5% v/v) were incubated with moderate shaking for 75 min at 37 °C in a 96 well plate. To each well 225 μL of reaction mixture (1000 μL reaction mixture consisted of 500 μL potassium phosphate buffer pH 7.0, 80 μL 100 mM EDTA solution pH 7.5, 20 μL BSA solution 0.05%, 100 μL of 20 mM NADPH solution and 300μL of distilled water) were added and the reaction started by addition of 25 μL of an 20 mM ethanolic DTNB solution. After proper mixing, the formation of 5-TNB was monitored with a microplate reader (Perkin Elmer Victor^{tm}X4) at 405 nm in 10 s intervals for 6 min. The increase in 5-TNB concentration over time followed a linear trend ($r^2 \geq 0.99$) and the enzymatic activities were calculated as the slopes (increase in absorbance per second) thereof. For each tested compound the non interference with the assay components was confirmed by a negative control experiment using an enzyme free solution. The IC_{50} values were calculated as the concentration of compound decreasing the enzymatic activity of the untreated control by 50% and are given as the means and error of repeated experiments.

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