Dalton Transactions

Cite this: *Dalton Trans.*, 2011, **40**, 2006

www.rsc.org/dalton

PAPER

Structure, solution chemistry, antiproliferative actions and protein binding properties of non-conventional platinum(II) compounds with sulfur and phosphorus donors†‡

Carolin Mügge,§[a] Claudia Rothenburger,§[a] Antje Beyer,[a] Helmar Görls,[a] Chiara Gabbiani,[b] Angela Casini,[c] Elena Michelucci,[d] Ida Landini,[e] Stefania Nobili,[e] Enrico Mini,[e] Luigi Messori*[b] and Wolfgang Weigand*[a]

Received 14th July 2010, Accepted 8th December 2010
DOI: 10.1039/c0dt00845a

Twelve Pt(II) complexes with *cis*-PtP_2S_2 pharmacophores (where P_2 refers to two monodentate or one bidentate phosphane ligand and S_2 is a dithiolato ligand) were prepared, characterized and evaluated as potential antiproliferative agents. The various compounds were first studied from the structural point of view; afterward, their solubility properties as well as their solution behaviour were analyzed in detail. Antiproliferative properties were specifically evaluated against A2780 human ovarian carcinoma cells, either resistant or sensitive to cisplatin. For comparison purposes similar studies were carried out on four parent *cis*-dichloro bisphosphane Pt(II)complexes. On the whole, the *cis*-PtP_2S_2 compounds displayed significant antiproliferative properties while the *cis*-PtP_2Cl_2 (*cis*-dichloro bisphosphane Pt(II)) compounds revealed quite poor biological performances. To gain further insight into the molecular mechanisms of these bisphosphane Pt(II) compounds, the reactions of selected complexes against the model protein cytochrome c were investigated by ESI-MS and their adduct formation explored. A relevant reactivity with cyt c was obtained only for *cis*-PtP_2Cl_2 compounds, whereas *cis*-PtP_2S_2 compounds turned out to be nearly unreactive. The obtained results are interpreted and discussed in the frame of the current knowledge of anticancer platinum compounds and their structure–activity-relationships. The observation of appreciable antiproliferative effects for the relatively inert *cis*-PtP_2S_2 compounds strongly suggests that these compounds will undergo specific activation within the cellular environment.

Introduction

Platinum compounds play a crucial role in modern anticancer chemotherapy. However, in spite of the numerous studies now available on this issue and the thousands of compounds tested so far, only very few platinum drugs have successfully reached clinical use.[1,2] Strikingly, the approved platinum compounds share some common structural features such as the presence of two NLG (non leaving groups, typically nitrogen ligands) and two LG (leaving groups, *e.g.* halides or carboxylates) in the *cis* position.[3,4] This type of configuration allows, in principle, bidentate coordination to adjacent nucleobases of the DNA double helix.[5] Remarkably, cytotoxic platinum complexes are in most cases *prodrugs* and their activation is a normal prerequisite for biological activity. Activation is normally attained through the release of one or more labile ligands.[6]

The above observations have prompted scientists since early times to try to establish the precise SAR (structure–activity-relationships) for anticancer platinum compounds that might be useful for the design of new active molecules. These rules were first reported by Hoeschele *et al.*[7] and rapidly became very popular within the scientific community. Now, as a far greater number of platinum compounds are available with known chemical and biological profiles, it is evident that those original rules suffer many exceptions and limitations. Indeed, numerous "rule-breaker" platinum-based drugs have been prepared and

[a]*Institute of Inorganic and Analytical Chemistry, Friedrich-Schiller-University Jena, August-Bebel-Strasse 2, 07743, Jena, Germany. E-mail: wolfgang.weigand@uni-jena.de; Fax: (+49) 3641 948102*
[b]*Laboratory of Metals in Medicine, Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019, Sesto Fiorentino, Firenze, Italy. E-mail: luigi.messori@unifi.it; Fax: (+39) 055 4573385*
[c]*Institut des Sciences et Ingéniere Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL) CH-1015, Lausanne, Switzerland*
[d]*Mass Spectrometry Center (CISM), University of Florence, Via U. Schiff 6, 50019, Sesto Fiorentino, Firenze, Italy*
[e]*Department of Preclinical and Clinical Pharmacology, University of Florence, Viale Pieraccini 6, 50139, Firenze, Italy*

† This paper is dedicated to Dr. Janina Altman on the occasion of her 80th birthday.

‡ Electronic supplementary information (ESI) available: $^{31}P\{^1H\}$ spectra of **7** and **8**; molecular structures of **6–10**; crystallographic data for **6–10**; ESI-MS spectra of cyt c interacting with compounds **1–4**. CCDC reference numbers: 776102 for **6**, 776103 for **7**, 776104 for **8**, 776105 for **9** and 776106 for **10**. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0dt00845a

§ These Authors contributed equally to this work.

characterized and yet they manifest relevant cytotoxic effects;[8] *e.g. trans*-[$PtCl_2Py_2$],[9] or the multinuclear compound BBR3464.[10] However, the originally devised scheme may still be considered as a useful starting point for the design of cytotoxic platinum compounds.

Upon careful analysis of the existing literature, we realized that the substitution of the original donors in cisplatin (N and Cl) by either phosphorus or sulfur atoms or both has not been investigated exhaustively. While several transition metal complexes bearing phosphane ligands were investigated,[11–15] only a few studies concerned the biological characterization of platinum compounds where the two ammonia ligands were replaced by phosphanes. In recent years, a series of additional compounds of this kind have been prepared and characterized by Longato *et al.*[16–21] Some related phosphane platinum compounds were prepared and characterized over the last years in other research laboratories worldwide.[22–30]

The established opinion concerning platinum phosphane complexes, mostly arising from older studies,[31] is that they are nearly devoid of antiproliferative activity. However, this feature is often due to a poor solubility of the phosphane platinum compounds and is still controversial and debated.

Recently Romerosa, Bergamini *et al.* proposed to reconsider that issue by associating phosphanes with other bioactive ligands.[32,33] In their studies, the authors showed that Pt(II) complexes containing PPh_3 and the N^7-coordinated anionic ligand 8-MTT (8-(methylthio)theophyllinate) produce a remarkable antiproliferative activity on some cancer cell lines, which decreases when PPh_3 is replaced by the more hydrophilic PTA ligand. On both cisplatin-sensitive T2 and cisplatin-resistant SKOV3 cell lines, the complex *cis*-[$PtCl(8\text{-}MTT)(PPh_3)_2$] manifested an activity comparable to cisplatin, whereas the analogous complex *cis*-[$PtCl(8\text{-}MTT)(PTA)_2$], containing PTA ligands instead of PPh_3, displayed only a residual activity. Interesting results were subsequently obtained with Pt(II) complexes simultaneously bearing both a PPh_3 and a PTA ligand.

Even more recently, Osella *et al.* showed that PtP_2Cl_2 (dichloro bisphosphane Pt(II)) compounds bearing *R*-(+)- or *S*-(−)-BINAP (2,2′-bis(diphenylphosphane)-1,1′-binaphthyl) as a bisphosphane ligand exhibit antiproliferative properties towards A2780 ovarian carcinoma and HCT116 colon adenocarcinoma cell lines.[34] When compared to analogous dichloro platinum(II) complexes bearing *R*-(+)- or *S*-(-)-DABN (1,1′-binaphthyl-2,2′-diamine) as the ligand, they proved to be less cytotoxic whilst retaining a comparable cytotoxicity against both cisplatin-sensitive and resistant A2780 cell lines. All compounds were shown to form adducts with G-quadruplexes in telomeres, a factor that is believed to positively affect antitumor activity.[35]

Thioplatin, on the other hand, provides a quite unique example of a PtS_4 pharmacophore, with the Pt(II) centre being surrounded by four sulfur atoms. The two chelating units can be opened by protonation to form a reactive diaqua complex, which is eventually able to form DNA-crosslinks.[36,37]

The simultaneous coordination of two phosphorous and two sulfur donors to the same Pt(II) centre might lead to not-easily-predictable results and, hopefully, to an innovative Pt(II) chemistry. For example, Miranda *et al.* reported on Pt(II) compounds with a *trans*-PtP_2S_2 moiety and revealed promising antiproliverative properties with this system.[38,39] Indeed, both P and S donors are, in principle, capable of forming relatively strong coordinative bonds with Pt(II),[40] the P–Pt bond presumably being the more inert. In addition, both P and S are believed to induce conspicuous *trans* effects.[41,42]

These arguments led us to synthesize and characterize a series of novel platinum compounds with the above-mentioned structural features (Fig. 1). The compounds were investigated concerning their behaviour in solution and their reactivity with the model protein cyt c (cytochrome c) and were also screened for their antiproliferative properties *in vitro*. In particular, we designed a first series of compounds possessing a *cis*-type PtP_2S_2 pharmacophore (where P_2 refers to two monodentate or one bidentate phosphane ligand and S_2 is a dithiolato ligand). For comparison purposes, a few previously described and investigated

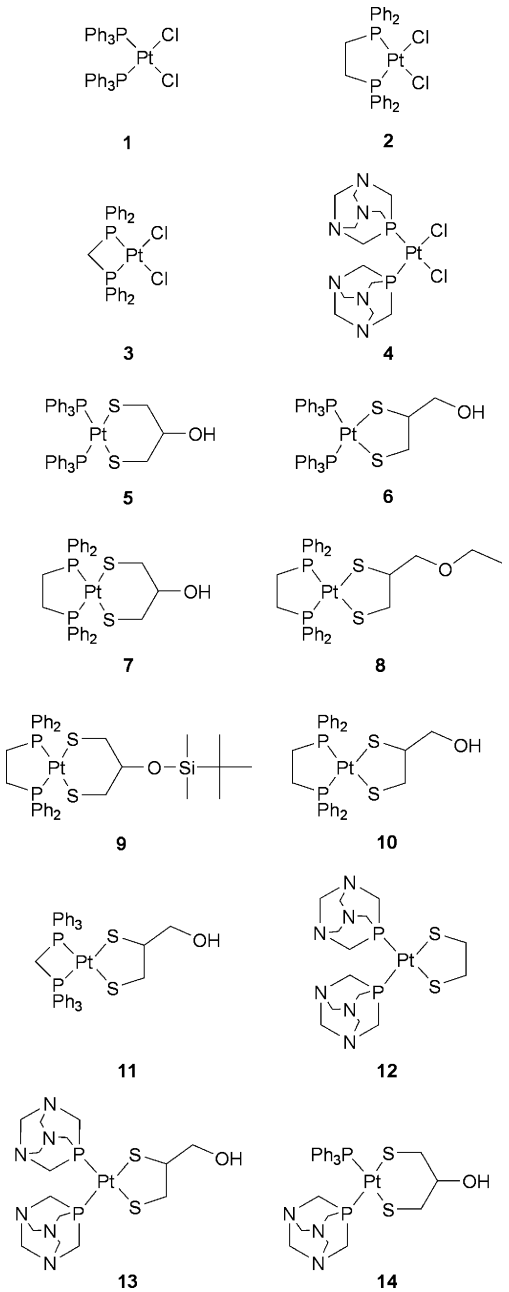

Fig. 1 Compounds **1**–**14**.

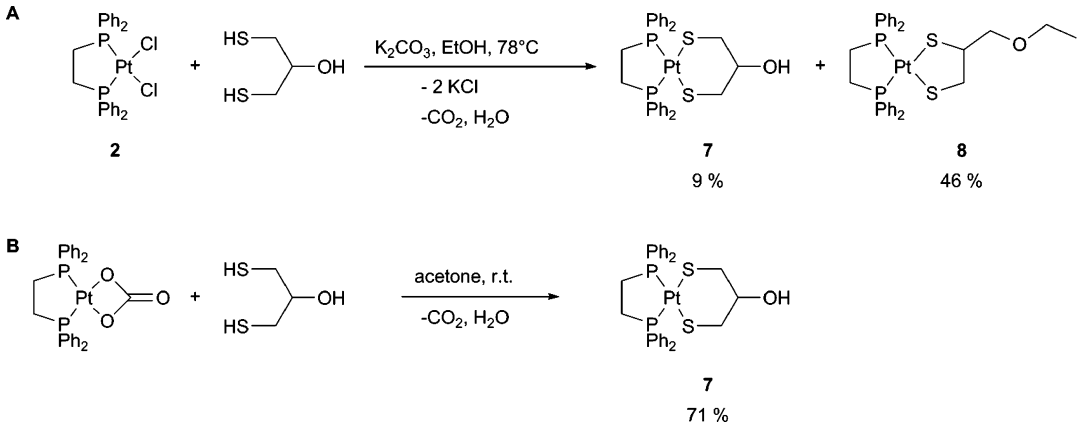

Fig. 2

platinum compounds with a *cis*-PtP_2Cl_2 pharmacophore were taken into consideration as well.

The phosphane ligands were chosen on the basis of precise structural and functional reasons. The chelating dppm (1,2-bis(diphenylphosphino)methane) and dppe (1,3-bis(diphenylphosphino)ethane) ligands as well as the monodentate PPh_3 may serve as stable, lipophilic ligands. Instead, by coordinating one or two PTA (1,3,5-phosphatriazaadamantane) ligands to the platinum centre, hydrophilic complexes are typically generated.[10] The sulfur donor sites were designed to build up to five- or six-membered chelating units with a variety of functional groups, *i.e.* CH_2, OH, OEt, O-TBDMS (*tert*-butyldimethylsilyl).

Results and discussion

The panel of bisphosphane platinum compounds

The bisphosphane Pt(II) complexes that were primarily considered for this study are represented in Fig. 1. Four compounds bearing a *cis*-PtP_2Cl_2 pharmacophore were prepared and characterized (**1–4**). Subsequently, a series of Pt(II) complexes sharing a common *cis*-type PtP_2S_2 scaffold (**5–14**) were synthesized.

Synthesis and structural characterization

The bisphosphane dithiolato Pt(II) complexes **5–16** were prepared *via* substitution reaction of the respective dichloro or carbonato bisphosphane Pt(II) complexes (*e.g.* **1–4**) with the corresponding dithiols.[43,44]

Interestingly, treatment of the dppe complex **2** with 1,3-dimercaptopropane-2-ol in the presence of K_2CO_3 in ethanol under reflux conditions for 6 h afforded not only the expected symmetrical product **7**, but also **8** as the main product (Reaction **A**, Fig. 2). The reasons for this are the rearrangement processes occurring during the synthesis. These observations were not apparent for the syntheses of similar bisphosphane dithiolato Pt(II) complexes, *e.g.* **5** or **14**. A possible mode of action and the factors influencing this rearrangement are the subject of further investigations.

Compounds **7** and **8** could be separated *via* column chromatography ($CHCl_3$–THF 10 : 0.5). The formation of the products can easily be surveyed *via* $^{31}P\{^1H\}$ NMR spectroscopy. In contrast to **7**, compound **8** exhibits an AB spin system, whereas complex **7** gives a single resonance with platinum satellites (Fig. S1‡). Furthermore, the complexes were characterized by single-crystal X-ray diffraction analysis (Fig. 3).

The variation of the reaction conditions or the use of diverse solvents, for instance the use of methanol or the addition of water, cannot avoid the formation of the unexpected asymmetrical product **8**, but do reduce the formation. In contrast, by using *cis*-[(dppe)Pt(CO_3)] as the starting material only the expected product **7** is generated in a high yield of 71% after purification processes (Reaction **B**, Fig. 2). *Cis*-[(dppe)Pt(CO_3)] has the advantage of exhibiting high solubility in common organic solvents in contrast to the dichloro complex **2**, which is scarcely soluble. For the substitution reaction with dithiols, starting from bisphosphane carbonato compounds, no additional base is necessary because the coordinated carbonato ligand readily deprotonates the thiol groups. Another method to suppress the formation of unfavourable side products was found by introducing a base-stable silyl protecting group, namely TBDMS, to selectively yield the *O*-protected compound **9** (Fig. 1).

For the synthesis of compounds **7** and **9** it was emphasized that the use of the carbonato Pt(II) complex as the starting material increased the yield of the compounds. However, it requires an additional synthetic step, resulting in an overall less covenient synthesis pathway that would only be applied when necessary.

All the bisphosphane dithiolato Pt(II) complexes **5–14** were characterized by multinuclear NMR spectroscopy, MS spectrometry and elemental analyses. Additionally, the bisphosphane dithiolato Pt(II) complexes **6–10**, bearing two PPh_3 or the dppe ligands, were characterized with single-crystal X-ray diffraction analysis (Fig. 3 and ESI Fig. S2–S4, Table S1‡).

In the molecular structure of compound **7**, the Pt(II) ion possesses a square planar geometry defined by the two sulfur atoms of the chelating 2-hydroxypropane-1,3-dithiolato dianion and two P atoms of the bidentate dppe ligand. The metal atom is protruded from the least-squares plane of the P_2S_2 donor set by 0.073 Å. The six-membered platinum cycle Pt–S1–C1–C2–C3–S2 possesses a twist-conformation with a disorder at the hydroxyl group bound to C2. The OH function can be situated either above or below the Pt–S1···C2···S2 plane. The five-membered ring formed by the coordination of the dppe ligand to the Pt(II) centre exhibits an envelope conformation. The two Pt–P distances

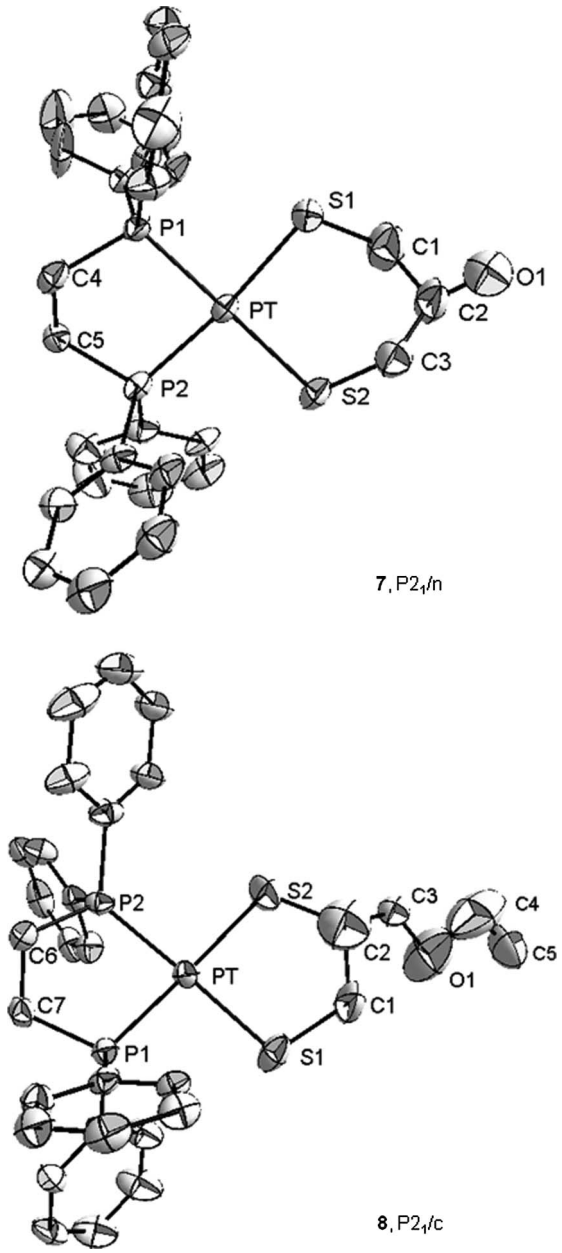

Fig. 3 Molecular structures of compounds **7** (top) and **8** (bottom). The compounds show disorders at C2, respectively. For clarity, only one motive is depicted in the pictures. Thermal ellipsoids are given at a 50% probability level.

(Pt–P1 = 2.250(2) Å, Pt–P2 = 2.250(2) Å) are equal and in good accordance with other dppe dithiolato Pt(II) compounds.[45] The bite angle for P1–Pt–P2 (85.79(9)°) is very similar to other compounds of this type and typical for the dppe ligand.

In **8**, the Pt(II) ion is also coordinated by two S and two P atoms in a slightly distorted square planar geometry. The coordination of the dithiol to the Pt(II) centre leads to the formation of a five-membered ring Pt–S1–C1–C2–S2. Similarly to **7**, the structure exhibits disorders at C2 and C3, presumably due to the existence of an asymmetric centre at C2. For this reason, the distances and angles in this moiety cannot be discussed in detail. A five-membered ring with envelope conformation is formed through the coordination of the dppe ligand to the Pt(II) centre. The coordination distances (Pt–P1 = 2.2527(13) Å, Pt–P2 = 2.2520(13) Å) as well as the bite angle P1–Pt–P2 (85.35(5)°) are in good accordance to complex **7**.

Solution chemistry

The behaviour in solution of the novel Pt(II) complexes was analyzed using UV-vis spectrophotometry. Typically, the solubility in aqueous media turned out to be a critical issue for these bisphosphane Pt(II) complexes, as compounds **1–11** and **14** are poorly soluble in aqueous solutions. Yet, significant differences in the solubility could be ascertained with the PTA containing compounds **12** and **13**, being soluble in water in concentrations of at least 10^{-2} M.

To overcome the problem of poor water solubility, most of the complexes (**1–11** and **14**) were initially dissolved in DMSO, although compounds **12** and **13** were dissolved in water. The resulting stock solutions of all complexes were diluted in 50 mM reference phosphate buffer at pH 7.4 as described in the experimental section. Under these conditions, the samples analyzed by spectrophotometry appeared to be stable for 24 h.

These results inspired the procedure of the antiproliferative tests reported below. The various compounds were added as small aliquots of a concentrated DMSO solution, in total amounts that did not lead to precipitation in the culture medium. The absence of precipitation was ensured by microscopic inspection of the samples throughout the incubation process.

To get further initial insight into the possible reactivity in solution of the reported PtP_2S_2 complexes, hydrolysis studies were carried out on compound **10** by NMR spectroscopy. Freshly prepared solutions of **10** in DMSO/aqueous phosphate buffer (pH 7.4; 1 : 4 ratio) were examined through $^{31}P\{^1H\}$ NMR spectroscopy at different temperatures (37 °C, 90 °C) over several days.

$^{31}P\{^1H\}$ NMR spectroscopy is a convenient method to analyze the hydrolysis of the compound and has been applied by others earlier (Fig. 4).[24,25,29] The chemical shift is typical of the nature of the phosphane ligand. The platinum satellites not only confirm the coordination of P to the Pt(II) centre, but also the coupling constants give information on the *trans* influence of the ligand coordinated *trans* to P. Any shifting of the P signal would therefore show a change in the chemical environment of the P atom. The loss of platinum satellites would additionally indicate a cleavage of the phosphane ligand from the Pt(II) centre. A change in the $^1J_{P\text{–}Pt}$ coupling constant would indicate that the phosphane ligand is still coordinated, whilst substitution takes

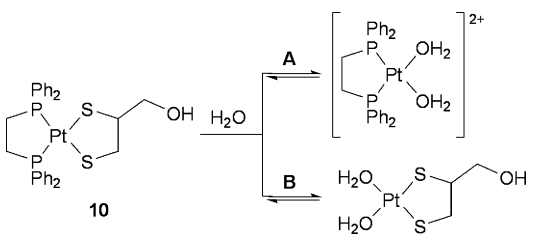

Fig. 4 Possible aquation products of **10** in aqueous solution. $^{31}P\{^1H\}$ NMR spectra would exhibit characteristic effects: **A**, change in the $^1J_{P\text{–}Pt}$ coupling constant states substitution *trans* to P; **B**, loss of platinum satellites indicates cleavage of phosphane ligand from the complex.

place at the sulfur-donor sites. Since the spin system is given by the shape of the spectrum, the distinction between symmetrically or unsymmetrically substituted complexes is possible and gives further information on the aquation process.

The tested compound manifested an appreciable stability and there were neither evident signs of aquation processes nor of significant chemical transformations, similar to the inertness of Pt(II) complexes with tri-n-butylphosphine reported by Kozelka *et al.*[24] Only after heating a solution of **10** to 90 °C in DMSO/phosphate buffer medium for 5 d, the emergence of additional signals in the $^{31}P\{^{1}H\}$ NMR spectra suggested the formation of several products. All the surveyed main signals exhibited platinum satellites with varying coupling constants, suggesting that the bisphosphane ligand stays bound to the Pt centre whilst the sulfur donor sites might be substituted through several ligands. Due to the variety of the formed products, most likely due to some decomposition, it was impossible to isolate and characterize the formed compounds separately.

To ensure that the presence of chloride ions does not significantly influence the hydrolysis of the compounds, the behaviour of **10** was also tested in the presence of 0.9% NaCl. After 72 h at 37 °C, no reaction was visible in the $^{31}P\{^{1}H\}$ NMR spectra, indicating that neither the P nor the S donors are replaced by chloride.

These results lead to the assumption that the Pt(II) compounds with the PtP_2S_2 pharmacophore bear an activation mechanism different from the "classical" drugs cisplatin and analogues, since the latter compounds were proven to hydrolyze under physiological-like conditions prior to binding to DNA.[46] Yet, binding of the presented compounds to DNA must not be precluded, as Messere *et al.* showed that bisphosphane Pt(II) complexes that wouldn't undergo ligand exchange still readily bound to DNA fragments.[29]

Cytotoxic activity

The antiproliferative effects of the panel of Pt(II) compounds were carefully determined *in vitro* against the mentioned A2780 ovarian carcinoma human cell lines, either sensitive (A2780/S) or resistant (A2780/R) to cisplatin, according to established protocols. The cell growth inhibition was measured after 72 h of exposure to the substances. The resulting IC_{50} values are shown in Table 1.

Out of an initial screening of the whole panel of 14 substances, 8 compounds manifested relevant antiproliferative actions, with most of the IC_{50} values in the low μM range. Those were then analyzed in more detail. Among them, 7 compounds were characterized by a PtP_2S_2 pharmacophore. The remaining compounds with an $IC_{50} > 50$ μM were classified as inactive. Remarkably, the two water soluble PtP_2S_2 compounds **12** and **13** turned out to be inactive. Additionally, most PtP_2Cl_2 compounds were discarded, with the only exception being (dppm)$PtCl_2$ **3**. Among the inactive compounds were also **1**, which had been tested on A2780 cell lines recently with comparable results[34] and **2**, said to be ineffective *in vivo* against L1210/0 leukemia earlier.[22]

Table 1 IC_{50} values determined for the most active compounds in the A2780 cisplatin sensitive (S) and resistant (R) cell lines

Compound	IC_{50} (μM)		r^a
	A2780/S	A2780/R	
3	12.0 ± 2.8	19.7 ± 2.7	1.6
5	5.1 ± 1.5	7.7 ± 1.5	1.5
6	3.4 ± 1.2	5.9 ± 0.3	1.7
7	4.6 ± 1.8	8.5 ± 1.3	1.8
8	3.6 ± 1.8	8.0 ± 0.2	2.2
9	5.8 ± 1.5	17.5 ± 3.1	3.0
10	4.6 ± 1.8	8.3 ± 1.4	1.8
11	2.8 ± 1.4	6.3 ± 3.0	2.3
cisplatin	1.3 ± 0.3	22.2 ± 4.9	17.1

[a] r, resistance ratio.

It is apparent that a number of compounds with a PtP_2S_2 scaffold (namely **5–11**) show relevant growth inhibition effects on both cell lines, all being more cytotoxic than cisplatin on the A2780/R cells. Notably, **3**, the only PtP_2Cl_2 complex that was tested, resulted to be only moderately active in line with the behaviour of related PtP_2Cl_2 compounds.[17,27,28,34]

Although all the investigated compounds are less active in the cisplatin resistant phenotype A2780/R than in its cisplatin sensitive counterpart A2780/S, cross-resistance ratios (*r*) are markedly lower (1.5–3.0) than that of cisplatin (17.1) (Table 1). This observation may suggest that these Pt(II) compounds might partially overcome some of the mechanisms of resistance to cisplatin.

The observation of a significant cytotoxic activity for various PtP_2S_2 compounds, in spite of their apparent chemical inertness (*vide supra*) suggests that these compounds may undergo activation under biologically relevant conditions (*i.e* in the cellular milieu) and thus cause an appreciable cellular damage through platinum coordination to essential biomolecules. It remains to be established whether these compounds retain, as cisplatin does, the ability to bind DNA and to cause the characteristic, often lethal, DNA intrastrand lesions, or follow a different mode of action.

Reactions with cytochrome c

To further characterize the reactivity of the investigated Pt(II) compounds with biomolecules and their mechanisms of interaction, a few representative compounds of the above panel were reacted with the model protein cyt c and the resulting products were analyzed through mass spectrometric methods, using established protocols.[47–50] These experiments were performed with the dichloro complexes **1–4**, as well as with the complexes bearing a 2,3-dimercaptopropanole ligand **6**, **10**, **11** and **13**, in order to investigate the influence of both the nature of the phosphane ligand and the presence of Cl or S in the complexes.

In the case of the dithiolato compounds **6**, **10**, **11** and **13**, protein platination is very scarce, nearly undetectable, after incubation for 24 and 96 h at 37 °C (data not shown). These findings are in agreement with the relevant chemical inertness of these compounds (*vide supra*).

In contrast, the formation of relevant amounts of adducts was observed in the case of the PtP_2Cl_2 complexes. Experiments were performed at 3 : 1 metal complex : cyt c molar ratios, as described in the experimental section. Representative deconvoluted ESI-MS spectra are shown in Fig. 5 as well as in the ESI‡.

Cis-[$(PPh_3)_2PtCl_2$] **1** resulted to be the least reactive PtP_2Cl_2 compound with cyt c. In fact, incubation for 24 h at 37 °C led to a weak signal (3% r.i.) at 13077.5 Da assigned to a cyt c-Pt$(PPh_3)_2$ adduct (Fig. S5 in the ESI‡).

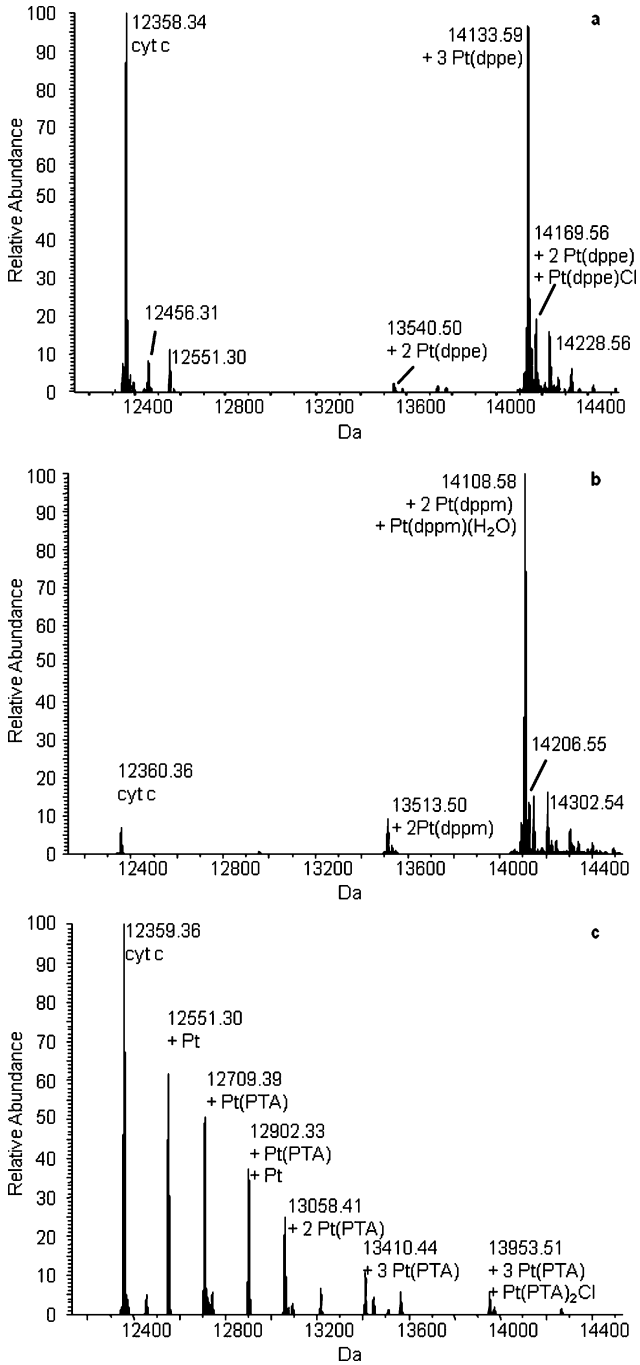

Fig. 5 Deconvoluted ESI-MS spectra of (a) **2**, (b) **3** and (c) **4**, incubated with cyt c at a metal complex : protein ratio of 3 : 1 at 37 °C, recorded after 96 h.

Representative spectra for compound **2** are shown in Fig. 5a and S6‡. After 24 h incubation, signals around 14170.6 Da can be assigned to [cyt c + 2 Pt(dppe) + Pt(dppe)Cl] species and less intense signals at 14133.6 Da can be attributed to [cyt c + 3 Pt(dppe)] adducts (Fig. S6‡). In addition, low r.i. signals at 13540.5 Da could be attributed to [cyt c + 2 Pt(dppe)] adducts. With time, the signals of the platinated cyt c species markedly increase, and the peaks of the adducts containing the $[(dppe)PtCl]^+$ fragment decrease in favour of the bidentate $[Pt(dppe)]^{2+}$ adducts (Fig. 5a). Fig. S7‡ shows the theoretical and observed isotope patterns of the [cyt c + 3 Pt(dppe)] adduct at charge state +8.

Cis-[(dppm)$PtCl_2$] **3** shows a similar behaviour, with three Pt fragments bound to cyt c. Moreover, the platinated cyt c species become the predominant ones in solution over the unbound protein after 96 h (Fig. 5b). In detail, the peak at 14108.6 Da in the spectrum corresponds to the [cyt c + 2 Pt(dppm) + Pt(dppm)H_2O] species, indicating that one Pt(II) has substituted a chlorido ligand with a water molecule prior to adduct formation. Fig. S8‡ shows the theoretical and observed isotope patterns of the [cyt c + 2 Pt(dppm) + Pt(dppm)H_2O] adduct at charge state +8.

Finally, the PTA-containing complex **4** exhibits a peculiar behaviour with respect to the above-mentioned compounds and has the least tendency to form 3 : 1 Pt:protein adducts. After incubating the protein with a stoichiometric amount of compound, the [cyt c + Pt(PTA)] species at 12709.4 Da are predominant in the sample (Fig. S9‡). By increasing the metal complex : protein ratio up to 3 : 1, a mixture of adducts is formed, showing protein binding of free Pt^{2+} ions as well as a mixture of platinum centres with varying PTA content (Fig. 5c). The obtained results demonstrate that among the phosphane ligands under investigation, the PTA group is the most labile platinum ligand.

In general, for the three most reactive compounds, adducts with cyt c bear one or more bound Pt-phosphane moieties. This means that the release of the original chloro ligand takes place upon protein binding, whilst the phosphane ligand remains bound to the platinum centre in most cases, with the exception of the PTA containing complex. In the case of **2**, the presence of monodentate $[(dppe)PtCl]^+$ fragments is observed at 24 h incubation time, which then convert into bidentate adducts upon chloride loss.

Overall, the reactivity of the PtP_2Cl_2 compounds with cyt c are similar to those previously observed for cisplatin and other established platinum drugs,[47] although all the tested PtP_2Cl_2 complexes are much less cytotoxic. It is possible to hypothesize that protein interactions might account for the reduced antiproliferative effects of the chloride derivatives in the reported series.

Concluding remarks

In spite of the intense investigations carried out so far on platinum compounds as potential anticancer agents, only relatively few studies have considered Pt(II) complexes with phosphane ligands in the place of amines. This is possibly due to the poor solubility in aqueous solutions of platinum phosphane complexes and to the old idea that the replacement of amine with phosphane ligands in antitumor Pt(II) compounds irreversibly leads to nearly complete loss of biological activity. However, this latter concept never achieved any conclusive confirmation and was recently questioned by several authors. The state of the art on this specific issue was revisited in detail by Osella *et al.* in a very recent paper.[34]

In the present study we have prepared and characterized some novel Pt(II) complexes bearing the unusual PtP_2S_2 pharmacophore. Both P and S ligands are known to be strong ligands for the Pt(II) centre; thus the resulting complexes are expected to manifest a high degree of stability (both thermodynamic and kinetic). The obtained PtP_2S_2 compounds were thoroughly characterized from the structural point of view and the hypothesized structures confirmed. Moreover, their solution behaviour was analyzed in detail and the extreme stability of the PtP_2S_2 scaffold towards

aquation could be demonstrated. Only under very drastic solution conditions, some initial aquation of a representative PtP_2S_2 complex was observed.

The novel PtP_2S_2 compounds were subsequently evaluated for their antiproliferative potential against the standard A2780 ovarian carcinoma human cell lines, either sensitive or resistant to cisplatin. For comparison reasons, even a few representative PtP_2Cl_2 compounds were tested. Very surprisingly, in spite of their proved chemical inertness and of the expected poor biological actions, relevant antiproliferative effects were detected for PtP_2S_2 compounds, with some of them being only slightly less effective than cisplatin. Interestingly, the measured cross-resistance ratios of PtP_2S_2 compounds are markedly lower than that of cisplatin. This observation suggests that these Pt(II) compounds might partially overcome some of the mechanisms of resistance to cisplatin. Far lower antiproliferative effects were instead evidenced for PtP_2Cl_2 compounds. No antiproliferative activity was found for the water-soluble PtP_2S_2 compounds bearing PTA ligands.

Afterward, four selected PtP_2S_2 complexes were challenged against the model protein cyt c in comparison with a few representative PtP_2Cl_2 compounds to disclose their possible interaction modes at the molecular level. Reactions were monitored through an established ESI-MS procedure. Mass spectra revealed a very poor reactivity of PtP_2S_2 compounds with cyt c whereas PtP_2Cl_2 compounds turned out to be far more reactive and to produce extensive protein platination.

Thus, the reported data prove unambiguously that novel PtP_2S_2 compounds are very inert both alone, in aqueous solution, and in their reactions with the model protein cyt c in line with expectations. On the other hand, the observation of appreciable cytotoxic effects for several of them against both A2780 cell lines strongly suggests that these compounds may undergo activation within the cellular milieu, as chemical activation of the platinum(II) centre is indeed an essential prerequisite for the antiproliferative actions. These mechanisms of activation will be the subject of further investigation.

Overall, the herein reported results are rather intriguing and make a global interpretation of the biological profile of platinum phosphane compounds very complex and hard to achieve. The biological behaviour of this class of compounds had been generalized and oversimplified in early times of platinum compounds research leading to the (erroneous) idea that platinum phosphane compounds, on the whole, should be classified as biologically ineffective. Remarkably, recent and promising results coming from various laboratories worldwide, and the present investigation, point out that phosphane ligands may offer new and unexplored opportunities in the discovery and development of new anticancer platinum compounds. Even very inert complexes such as those bearing a PtP_2S_2 pharmacophore, such as reported here, may manifest interesting antitumor profiles.

Experimental

General

All syntheses involving air-sensitive compounds were carried out using standard vacuum-line and Schlenk techniques under an Argon atmosphere. All chemicals and solvents used were obtained from commercial suppliers and were used as received. The following Pt(II) complexes were prepared according to literature procedures: *cis*-[$(Ph_3P)_2PtCl_2$] **1**,[51] *cis*-[(dppe)$PtCl_2$] **2**,[51] *cis*-[(dppe)$PtCO_3$],[52] *cis*-[(dppm)$PtCl_2$] **3**,[53] *cis*-[$(PTA)_2PtCl_2$] **4**[54] and *cis*-[(Ph_3P)(PTA)$PtCl_2$].[54]

Structural and purity determination

NMR spectra were recorded with Bruker Avance 200 (operating at 200.13, 50.33 and 81.01 MHz for ^1H, ^{13}C and ^{31}P, respectively) and Bruker Avance 400 (operating at 400.13, 100.63 and 162.00 MHz for ^1H, ^{13}C and ^{31}P, respectively) instruments at room temperature. Deuterated chloroform was used as the NMR solvent and ^1H and $^{13}C\{^1H\}$ NMR spectra were referenced to residual solvent resonances (7.26 ppm and 77.16 ppm, respectively), whereas $^{31}P\{^1H\}$ NMR spectra were referenced to external H_3PO_4. The mass spectra were recorded using SSQ 10 or MAT95XL, using ESI- or DEI-ionization. IR spectra (KBr pellets) were recorded on a Perkin Elmer System 2000 FT-IR spectrometer. Elemental analyses (C, H, N, S) are the results of single measurements and were carried out with LECO CHNS-932. They confirmed all compounds to be pure to a degree of ≥95% purity.

Crystal structure determination

Single crystals were obtained from CH_2Cl_2–pentane. The intensity data for the compounds were collected on a Nonius KappaCCD diffractometer using graphite-monochromated Mo-Kα radiation. Data were corrected for Lorentz and polarization effects but not for absorption effects.[55,56] Crystallographic data as well as structure solution and refinement details are summarized in Table S1‡. The structures were solved by direct methods (SHELXS[57]) and refined by full-matrix least squares techniques against F_o^2 (SHELXL-97[57]). All hydrogen atoms were included at calculated positions with fixed thermal parameters. All non-disordered, non-hydrogen atoms were refined anisotropically[57] and XP (SIEMENS Analytical X-ray Instruments, Inc.) was used for structure representations.

Synthesis of the Pt(II) compounds

***cis*-Bis(PPh_3)$_2$(2-hydroxypropane-1,3-dithiolato)platinum(II) 5.** 1,3-Dimercaptopropane-2-ol (27 mg, 0.22 mmol) was added to a suspension of **1** (158 mg, 0.20 mmol) and K_2CO_3 (55 mg, 0.40 mmol) in EtOH (50 mL). After stirring at r.t. for 15 h, the mixture was heated to 78 °C for 6 h. The solvent was removed under reduced pressure and the crude product was extracted with a $CHCl_3$–H_2O mixture, the organic layer was subsequently dried with Na_2SO_4, filtered and then the solvent was removed under reduced pressure. Purification was carried out on silica gel using a gradient (CH_2Cl_2/acetone 10 : 0.5). The collected fraction (R_F = 0.2) was dried under vacuum and crystallized in CH_2Cl_2–pentane to give **5** (90 mg, 54%) as a yellow substance (decomp. 197 °C). Found: C 55.0, H 4.53, S 7.6. Calcd for $C_{39}H_{36}OP_2PtS_2$ (841.86): C 55.6, H 4.3, S 7.6. IR (KBr): ν_{max}/cm^{-1} 3435 (ν_{OH}), 3053 ($\nu_{CH,Ph}$), 2905 ($\nu_{as,s\,CH2}$), 1480 ($\nu_{C=C}$), 1435 ($\nu_{P\text{-}Ph}$), 744 ($\nu_{C\text{-}S}$). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.42 (12 H, m, Ph), 7.26 (8 H, m, Ph), 7.13 (12 H, m, Ph), 4.23–4.34 (1 H, m, C*H*), 3.10–3.30 (2 H, m, S–CH_2), 2.72–2.97 (2 H, m, S–CH_2). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$, 25 °C): δ 134.8 (m, Ph), 130.9 (m, Ph), 130.1 (m, Ph), 127.5 (m, Ph), 71.0 (s, *C*H), 32.8 (s w/Pt satellites, $^2J_{C,Pt}$ 16.1 Hz,

S–CH_2). $^{31}P\{^1H\}$ NMR (81 MHz, $CDCl_3$, 25 °C): δ 27.3 (s w/Pt satellites, $^1J_{PPt}$ 2863 Hz). MS (ESI): m/z (%) = 864.0 $(M+Na)^+$.

***cis*-Bis(PPh_3)$_2$(3-hydroxypropane-1,2-dithiolato)platinum(II) 6.** Prepared as described for **5** from 2,3-dimercaptopropane-1-ol (27 mg, 0.22 mmol), **1** (158 mg, 0.20 mmol) and K_2CO_3 (55 mg, 0.40 mmol) in EtOH (50 mL) and H_2O (1 mL). The purification was carried out on silica gel using a gradient (CH_2Cl_2/acetone 10 : 0.25). The collected fraction (R_F = 0.1) was dried under vacuum, crystallized in CH_2Cl_2–pentane to give **6** (111 mg, 60%) as a yellow substance (decomp. 196 °C). Found: C 52.3, H 4.2, S 6.95. Calcd for $C_{39}H_{36}OP_2PtS_2{\cdot}CH_2Cl_2$ (926.79): C 51.8, H 4.1, S 6.9. IR (KBr): ν_{max}/cm^{-1} 3436 (ν_{OH}), 3053 ($\nu_{CH,Ph}$), 2922 ($\nu_{as,s\ CH2}$), 1481 ($\nu_{C=C}$), 1435 ($\nu_{P\text{-}Ph}$), 744 ($\nu_{C\text{-}S}$). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.41 (12 H, m, Ph), 7.26 (8 H, m, Ph), 7.14 (12 H, m, Ph), 3.73–3.78 (1 H, m, CH_2–O), 3.52–3.58 (1 H, m, CH_2–O), 3.02–3.24 (1 H, m, CH), 2.89–2.99 (1 H, m, S–CH_2), 2.53–2.74 (1 H, m, S–CH_2), 2.23 (1 H, m, OH). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$, 25 °C): δ 134.7 (m, Ph), 131.0 (m, Ph), 130.2 (m, Ph), 127.5 (m, Ph), 65.3 (virt. t, $^3J_{CPt}$ 18.7 Hz, CH_2–O), 55.8 (dd, $^3J_{CP(A)}$ 11.1 Hz, $^3J_{CP(B)}$ 12.6 Hz, CH), 40.6 (dd, $^3J_{CP(A)}$ 12.2 Hz, $^3J_{CP(B)}$ 14.4 Hz, S–CH_2). $^{31}P\{^1H\}$ NMR (81 MHz, $CDCl_3$, 25 °C): δ 24.6 (AB spin system, $^1J_{PPt}$ 2855 Hz, $^2J_{PP}$ 24 Hz), 23.4 (AB spin system, $^1J_{PPt}$ 2929 Hz, $^2J_{PP}$ 24 Hz). MS (ESI): m/z 863.9 $(M+Na)^+$.

***cis*-Bis(dppe)(2-hydroxypropane-1,3-dithiolato)platinum(II) 7.** *Method A*: Prepared as described for **5** from 1,3-dimercaptopropane-2-ol (27 mg, 0.22 mmol), **2** (133 mg, 0.20 mmol) and K_2CO_3 (55 mg, 0.40 mmol) in EtOH (50 mL). *Method B*: 1,3-Dimercaptopropane-2-ol (27 mg, 0.22 mmol) was added to a solution of *cis*-[(dppe)Pt(CO_3)] (120 mg, 0.18 mmol) in acetone (40 mL). The mixture was stirred at room temperature for 15 h, then the solvent was removed under reduced pressure. Purification was carried out on silica gel using a gradient ($CHCl_3$–THF, 10 : 0.5), the collected fraction (R_F = 0.2) was dried under vacuum, crystallized in CH_2Cl_2–pentane to give a pale yellow substance of **7** (*Method A*: 13 mg, 9%; *Method B*: 101 mg, 71%, decomp. 206 °C). Found: C 48.6, H 4.4, S 8.9. Calcd for $C_{29}H_{30}OP_2PtS_2$ (715.70): C 48.7, H 4.2, S 9.0. IR (KBr): ν_{max}/cm^{-1} 3435 (ν_{OH}), 3051 ($\nu_{CH,\ Ph}$), 2908 ($\nu_{as,s\ -CH2}$), 1483 ($\nu_{C=C}$), 1435 ($\nu_{P\text{-}Ph}$), 750 ($\nu_{C\text{-}S}$). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.77 (8 H, m, Ph), 7.43 (12 H, m, Ph), 4.33 (1 H, m, CH), 3.15–3.33 (2 H, m, S–CH_2), 2.91–3.11 (2 H, m, S–CH_2), 2.85 (1 H, d, $^3J_{HH}$ 10.11 Hz, OH), 2.28 (4 H, m, P–CH_2). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$, 25 °C): δ 133.4 (m, Ph), 131.2 (m, Ph), 128.7 (m, Ph), 128.6 (m, Ph), 71.6 (s w/Pt satellites, $^3J_{CPt}$ 26.7 Hz, CH), 30.7 (virt. t $^2J_{CPt}$ 21.3 Hz, S–CH_2), 28.7 (m, P–CH_2). $^{31}P\{^1H\}$ NMR (81 MHz, $CDCl_3$, 25 °C): δ 48.4 (s w/Pt satellites, $^1J_{PPt}$ 2748 Hz). MS (EI): m/z 715 (M^+).

***cis*-Bis(dppe)(3-ethoxypropane-1,2-dithiolato)platinum(II) 8.** Prepared as described for **5** from 1,3-dimercaptopropane-2-ol (27 mg, 0.22 mmol), **2** (133 mg, 0.20 mmol) and K_2CO_3 (55 mg, 0.40 mmol) in EtOH (50 mL). The purification was carried out on silica gel using a gradient ($CHCl_3$–THF 10 : 0.5), the collected fraction (R_F = 0.4) was dried under vacuum and crystallized in CH_2Cl_2–pentane to give **8** (68 mg, 46%) as a pale yellow substance (decomp. 183 °C). Found: C 49.9, H 4.55, S 8.55. Calcd for $C_{31}H_{34}OP_2PtS_2$ (743.76): C 50.1, H 4.6, S 8.6. IR (KBr): ν_{max}/cm^{-1} 3053 ($\nu_{CH,Ph}$), 2969 (ν_{asCH3}), 2913 (ν_{asCH2}), 1483 ($\nu_{C=C}$), 1436 ($\nu_{P\text{-}Ph}$), 1104 ($\nu_{C\text{-}O\text{-}C}$), 750 ($\nu_{C\text{-}S}$). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.76 (8 H, m, Ph), 7.42 (12 H, m, Ph), 3.66 (1 H, t, $^3J_{HH}$ 9.9 Hz, CH_2–O), 3.47–3.52 (1 H, m, CH_2–O), 3.39–3.54 (2 H, m, CH_2–CH_3), 3.32 (1 H, m, CH), 2.72–3.04 (2 H, m, S–CH_2), 2.21–2.47 (4 H, m, P–CH_2), 1.13 (3 H, t, $^3J_{HH}$ 7.0 Hz, CH_3). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$, 25 °C): δ 133.3 (m, Ph), 131.1 (m, Ph), 130.0 (m, Ph), 128.6 (m, Ph), 73.6 (virt. t, $^3J_{CPt}$ 32.9 Hz, CH_2–O), 65.8 (s, CH_2–CH_3), 50.8 (d, $^3J_{CP}$ 10.6 Hz, CH), 39.6 (d, $^3J_{CP}$ 10.7 Hz, S–CH_2), 28.1 (m, P–CH_2), 15.3 (s, CH_3). $^{31}P\{^1H\}$ NMR (81 MHz, $CDCl_3$, 25 °C): δ 49.1 (AB spin system, $^1J_{PPt}$ 2720 Hz, $^2J_{PP}$ 12 Hz), 48.8 (AB spin system, $^1J_{PPt}$ 2778 Hz, $^2J_{PP}$ 12 Hz). MS (ESI): m/z 765.9 $(M+Na)^+$.

2-(*tert*-butyldimethylsilyloxy)propane-1,3-dithiol[58,59]. 1,3-Dimercaptopropane-2-ol (1.58 g, 12.72 mmol) and imidazole (1.98 g,19.15 mmol) were dissolved in dry DMF (5 mL) at 0 °C. The mixture was slowly dropped into a solution of *tert*-butyldimethyl-chlorosilane (TBDMSCl, 2.28 g, 15.16 mmol) in 5 mL of dry DMF, leading to a colour shift from colourless to pale pink. After stirring at r.t. for 15 h, extraction was carried out with hexane, followed by washing with 10% $NaHCO_3$ and water. The organic extracts were dried with Na_2SO_4, filtered and evaporated to dryness to yield 2-(*tert*-butyldimethylsilyloxy)propane-1,3-dithiol (2.3 g, 75% yield) as a colourless oil, which was used without further purification. IR (Nujol): ν_{max}/cm^{-1} 2939 2925 2855 ($\nu_{as,s\ CH3/CH2}$), 1362 ($\nu_{sC(CH3)3}$), 1254 ($\nu_{Si\text{-}C}$), 837 ($\nu_{Si\text{-}C}$), 743 ($\nu_{C\text{-}S}$). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.07 (6H, s, Si(CH_3)$_2$), 0.88 (9H, s, C(CH_3)$_3$), 1.38 (2H, t, SH, $^3J_{HH}$ 8.5 Hz) 2.71 (4H, m, S–CH_2), 3.80 (1H,m, CH). $^{13}C\{^1H\}$-NMR (50 MHz, $CDCl_3$): δ –4.5 (Si(CH_3)$_2$), 18.0 (C(CH$_3$)$_3$), 25.8 (C(CH_3)$_3$), 29.2 (S–CH_2), 73.8 (CH).

***cis*-Bis(dppe)(2-(*tert*-butyldimethylsilyloxy)propane-1,3-dithiolato)platinum(II) 9.** Prepared as described for **7** (*Method B*) from 2-(*tert*-butyldimethylsilyloxy)propane-1,3-dithiol (52 mg, 0.22 mmol) and *cis*-[(dppe)Pt(CO_3)] (120 mg, 0.18 mmol) in acetone (40 mL). The purification was carried out on silica gel using a gradient (CH_2Cl_2–acetone, 10 : 0.2). The collected fraction (R_F = 0.4) was dried under vacuum and crystallized in CH_2Cl_2–pentane to give **9** (114 mg, 76%) as a pale yellow substance (decomp. 201 °C). Found: C 50.85, H 5.5, S 7.6. Calcd for $C_{35}H_{44}OP_2PtS_2Si$ (829.96): C 50.65, H 5.3, S 7.7. IR (KBr): ν_{max}/cm^{-1} 3053 ($\nu_{CH,Ph}$), 2956 (ν_{asCH3}), 2926 (ν_{asCH2}), 2855 ($\nu_{sCH3/CH2}$), 1471 ($\nu_{C=C}$), 1436 ($\nu_{P\text{-}Ph}$), 1254 ($\nu_{Si\text{-}C}$), 835 ($\nu_{Si\text{-}C}$), 748 ($\nu_{C\text{-}S}$). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.79 (8 H, m, Ph), 7.42 (12 H, m, Ph), 4.05–4.17 (1 H, m, CH), 2.77–3.32 (4 H, m, S–CH_2), 2.28 (4 H, m, P–CH_2), 0.82 (9 H, s, C(CH_3)$_3$), –0.02 (6 H, s, Si(CH_3)$_2$). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$, 25 °C): δ 133.5 (m, Ph), 131.1 (m, Ph), 129.3 (m, Ph), 128.6 (m, Ph), 76.4 (s w/Pt satellites, $^3J_{CPt}$ 21.0 Hz, CH), 31.8 (s w/Pt satellites, $^2J_{CPt}$ 20.9 Hz, S–CH_2), 28.6 (m, P–CH_2), 26.0 (s, C(CH_3)$_3$), 18.3 (s, C(CH$_3$)$_3$), –4.6 (s, Si(CH_3)$_2$). $^{31}P\{^1H\}$ NMR (81 MHz, $CDCl_3$, 25 °C): δ 47.9 (s w/Pt satellites, $^1J_{PPt}$ 2738 Hz). MS (ESI): m/z 851.9 $(M+Na)^+$.

***cis*-Bis(dppe)(3-hydroxypropane-1,2-dithiolato)platinum(II) 10.** Prepared as described for **5** from 2,3-dimercaptopropane-1-ol (27 mg, 0.22 mmol), **2** (133 mg, 0.20 mmol) and K_2CO_3 (55 mg, 0.40 mmol) in MeOH (50 mL). Purification was carried out on silica gel using a gradient (CH_2Cl_2–acetone, 10 : 0.5), the collected fraction (R_F = 0.3) was dried under vacuum and crystallized in a

CH_2Cl_2–pentane mixture to give **10** (120 mg, 79%) as a pale yellow substance (decomp. 199 °C). Found for C 46.5, H 4.1, S 8.2, Cl 4.5. Calcd for $C_{29}H_{30}OP_2PtS_2{\cdot}0.5CH_2Cl_2$ (758.17): C 46.7, H 4.1, S 8.5, Cl 4.7. IR (KBr): ν_{max}/cm^{-1} 3435 (ν_{OH}), 3051 ($\nu_{CH,Ph}$), 2916 ($\nu_{as,s\,CH2}$), 1483 ($\nu_{C=C}$), 1435 ($\nu_{P\text{-}Ph}$), 749 ($\nu_{C\text{-}S}$). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.83 (4 H, m, Ph), 7.67 (4 H, m, Ph), 7.45 (6 H, m, Ph), 7.42 (6 H, m, Ph), 3.53–3.67 (2 H, m, C*H*$_2$–O), 3.26 (1 H, m, C*H*), 2.75–2.92 (2 H, m, S–C*H*$_2$), 2.19–2.53 (5 H, m, P–C*H*$_2$, OH). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, 25 °C): δ 133.6 (m, Ph), 133.0 (m, Ph), 131.3 (m, Ph), 130.0 (m, Ph), 128.7 (m, Ph), 65.3 (virt. t, $^3J_{CPt}$ 19.8 Hz, *C*H$_2$–O), 54.6 (d, $^3J_{CP}$ 8.9 Hz, *C*H), 39.2 (d, $^3J_{CP}$ 10.1 Hz, S–*C*H$_2$), 28.3 (m, P–*C*H$_2$). ^{31}P{^{1}H} NMR (81 MHz, $CDCl_3$, 25 °C): δ 49.0 (AB spin system, $^1J_{PPt}$ 2720 Hz, $^2J_{PP}$ 12 Hz), 48.9 (AB spin system, $^1J_{PPt}$ 2778 Hz, $^2J_{PP}$ 12 Hz). MS (ESI): *m*/*z* 737.8 (M+Na)$^+$.

***cis*-Bis(dppm)(3-hydroxypropane-1,2-dithiolato)platinum(II) 11.** 2,3-Dimercaptopropane-1-ol (21 mg, 0.17 mmol) and K_2CO_3 (41 mg, 0.30 mmol) was added to a solution of **3** (98 mg, 0.15 mmol) in CH_2Cl_2 (30 mL). The mixture was stirred at room temperature for 15 h, followed by extraction with water (3 × 20 mL). The organic layer was dried with Na_2SO_4, filtered and the solvent was removed under reduced pressure. The purification was carried out on silica gel using a gradient (CH_2Cl_2–acetone 10 : 0.25), the collected fraction (R_F = 0.2) was dried under vacuum and crystallized from CH_2Cl_2–pentane to give **11** (70 mg; 67%) as a yellow substance (decomp. 204 °C). Found: C 48.1, H 3.85, S 8.85. Calcd for $C_{28}H_{28}OP_2PtS_2$ (701.68): C 47.9, H 4.0, S 9.1. IR (KBr): ν_{max}/cm^{-1} 3433 (ν_{OH}), 3051 ($\nu_{CH,Ph}$), 2922 ($\nu_{as,s\,CH2}$), 1483 ($\nu_{C=C}$), 1436 ($\nu_{P\text{-}Ph}$), 733 ($\nu_{C\text{-}S}$). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.80 (8 H, m, Ph), 7.40 (12 H, m, Ph), 4.51 (2 H, t w/Pt satellites, $^2J_{HP}$ 10.5 Hz, $^3J_{HPt}$ 42.5 Hz, P–C*H*$_2$–P), 3.61–3.73 (2 H, m, C*H*$_2$–O), 3.21 (1 H, m, C*H*), 2.69–2.86 (2 H, m, S–C*H*$_2$), 2.34 (1 H, dd, $^3J_{HH(1)}$ 3.82 Hz, $^3J_{HH(2)}$ 3.88 Hz, O*H*). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, 25 °C): δ 132.8 (m, Ph), 131.5 (m, Ph), 129.0 (m, Ph), 65.1 (s w/Pt satellites, $^3J_{CPt}$ 21.1 Hz, *C*H$_2$–O), 54.4 (dd, $^3J_{CP(A)}$ 6.0 Hz, $^3J_{CP(B)}$ 5.9 Hz, *C*H), 45.1 (t, $^1J_{CP}$ 30.8 Hz, *C*H$_2$), 39.1 (s, S–*C*H$_2$). ^{31}P{^{1}H} NMR (81 MHz, $CDCl_3$, 25 °C): δ –40.8 (AB spin system, $^1J_{PPt}$ 2348 Hz, $^2J_{PP}$ 70 Hz), –39.8 (AB spin system, $^1J_{PPt}$ 2329 Hz, $^2J_{PP}$ 70 Hz). MS (ESI): *m*/*z* 723.8 (M+Na)$^+$.

***cis*-Bis(PTA)$_2$(ethane-1,2-dithiolato)platinum(II) 12.** Ethane-1,2-dithiol (20 mg, 0.21 mmol) and K_2CO_3 (55 mg, 0.40 mmol) were added to a solution of **4** (114 mg, 0.20 mmol) in EtOH (50 mL). The mixture was heated to 78 °C for 6 h. Upon cooling down to room temperature the product precipitated as pale yellow powder. After filtration the product was washed with a small portion of H_2O, EtOH and Et_2O. The product **12** was dried under vacuum (115 mg, 96%). Found C 27.7, H 4.7, N 13.6, S 10.5. Calcd for $C_{14}H_{28}N_6P_2PtS_2$ (601.57): C 27.95, H 4.7, N 14.0, S 10.7. ^{1}H NMR (400 MHz, D_2O, 25 °C): δ 4.57 (6 H, s, N–C*H*$_2$–N), 4.29 (6 H, s, N–C*H*$_2$–P), 2.50 (4 H, t, $^3J_{HH}$ 9.0 Hz, S–C*H*$_2$). ^{13}C{^{1}H} NMR (100 MHz, D_2O, 25 °C): δ 70.3 (m, N–*C*H$_2$–N), 50.3 (d, $^3J_{CP}$ 11.5 Hz, N–*C*H$_2$–P), 36.6 (m, S–*C*H$_2$). ^{31}P{^{1}H} NMR (81 MHz, D_2O, 25 °C): δ –55.3 (s w/Pt satellites, $^1J_{PPt}$ 2554 Hz). MS (DEI): *m*/*z* 601.0 (M$^+$).

***cis*-Bis(PTA)$_2$(3-hydroxypropane-1,2-dithiolato)platinum(II) 13.** Prepared as described for **12** from 2,3-dimercaptopropane-1-ol (27 mg, 0.22 mmol), **4** (114 mg, 0.20 mmol) and K_2CO_3 (55 mg, 0.40 mmol) in EtOH (50 mL). The purification was carried out *via* Soxhlet extractor in $CHCl_3$, the collected fraction was dried under vacuum to give **13** (57 mg, 45%) as a colourless substance (decomp. 220 °C). Found: C 28.8, H 5.1, N 12.9, S 10.5. Calcd for $C_{15}H_{30}N_6OP_2PtS_2$ (631.59): C 28.5, H 4.8, N 13.3, S 10.15. IR (KBr): ν_{max}/cm^{-1} 3435 (ν_{OH}), 2933 ($\nu_{as,s\,CH2}$), 1483 ($\nu_{C=C}$), 743 ($\nu_{C\text{-}S}$). ^{1}H NMR (400 MHz, D_2O, 25 °C): δ 4.49 (6 H, s, N–C*H*$_2$–N), 4.22 (6 H, s, N–C*H*$_2$–P), 3.44–3.54 (2 H, m, O–C*H*$_2$), 2.94–3.15 (1 H, m, C*H*), 2.66–2.80 (1 H, m, S–C*H*$_2$), 2.54–2.61 (1 H, m, S–C*H*$_2$). ^{13}C{^{1}H} NMR (100 MHz, D_2O, 25 °C): δ 70.6 (d, $^3J_{CP}$ 7.2 Hz, N–*C*H$_2$–N), 63.7 (s w/Pt satellites, $^3J_{CPt}$ 32.4 Hz, O–*C*H$_2$), 52.6 (d, $^3J_{CP}$ 11.4 Hz, *C*H), 50.6 (m, N–*C*H$_2$–P), 37.8 (d, $^3J_{CP}$ 11.8 Hz, S–*C*H$_2$). ^{31}P{^{1}H} NMR (81 MHz, D_2O, 25 °C): δ –55.6 (AB spin system, $^1J_{PPt}$ 2546 Hz, $^2J_{PP}$ 59 Hz), –56.3 (AB spin system, $^1J_{PPt}$ 2568 Hz, $^2J_{PP}$ 59 Hz). MS (ESI): *m*/*z* 632.0 (M$^+$).

***cis*-(PPh3)(PTA)(2-hydroxypropane-1,3-dithiolato)platinum(II) 14.** Prepared as described for **12** from 1,3-dimercaptopropane-2-ol (27 mg, 0.22 mmol), *cis*-[(PPh_3)(PTA)$PtCl_2$] (137 mg, 0.20 mmol) and K_2CO_3 (55 mg, 0.40 mmol) in EtOH (50 mL). The purification was carried out by washing of the crude product with a small portion of H_2O, EtOH and Et_2O. Subsequently, the product was dried under vacuum to give **14** (82 mg, 56%) as a pale yellow powder. Found: C 43.7, H 4.3, N 5.55, S 8.5. Calcd for $C_{27}H_{33}N_3OP_2PtS_2$ (736.73): C 44.0, H 4.5, N 5.7, S 8.7. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.69 (6 H, m, Ph), 7.63 (3 H, m, Ph), 7.47 (6 H, m, Ph), 4.51 (1 H, m, C*H*), 4.19 (6 H, m, N–C*H*$_2$–N), 3.77 (6 H, s, N–C*H*$_2$–P), 2.84–2.72 (2 H, m, S–C*H*$_2$), 2.57–2.45 (2 H, m, S–C*H*$_2$). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, 25 °C): δ 134.6 (m, Ph), 131.3 (m, Ph), 130.6 (m, Ph), 128.3 (m, Ph), 73.0 (s, *C*H), 72.9 (d, $^3J_{CP}$ 7.1 Hz, N–*C*H$_2$–N), 51.3 (m, N–*C*H$_2$–P), 39.1 (m, S–*C*H$_2$). ^{31}P{^{1}H} NMR (81 MHz, $CDCl_3$, 25 °C): δ 21.5 (AB spin system, $^1J_{PPt}$ 2872 Hz, $^2J_{PP}$ 27 Hz), –63.6 (AB spin system, $^1J_{PPt}$ 2442 Hz, $^2J_{PP}$ 27 Hz). MS (ESI): *m*/*z* 736.0 (M$^+$).

UV-vis spectrophotometry

UV-vis absorption spectra were recorded on a Varian Cary 50 UV-vis spectrophotometer. Stock solutions (10^{-2} M) of each compound were prepared by dissolving the complex under investigation in DMSO. Stock solutions of **12** and **13** were prepared in H_2O. UV-vis measurements were then carried out by diluting the compounds' stock solutions to 10^{-4} M in phosphate buffer (50 mM, pH 7.4). Spectra were collected between 800–200 nm for 24 h at r.t., operating in 10 min intervals during the first hour and in 1 h intervals afterwards.

NMR spectroscopy

Hydrolysis studies were carried out on compound **10**. *Method A*: **10** was dissolved in DMSO-d_6/phosphate buffer (50 mM, prepared in D_2O) (9 : 1 ratio, 5×10^{-3} M). ^{31}P{^{1}H} NMR spectra of this solution were recorded at 37 °C over 24 h (30 min intervals) and remained identical over the course of the measurement. *Method B*: **10** was dissolved in DMSO-d_6/phosphate buffer (50 mM, prepared in D_2O) (4 : 1 ratio, 10^{-2} M) and stirred at 90 °C for 5 d. ^{31}P{^{1}H} NMR spectra of the solution were recorded over the course of the reaction until decomposition in favour of specific hydrolysis was observed, the last after 124 h. *Method C*: **10** was dissolved in DMSO-d_6/phosphate buffer (50 mM, prepared in

D_2O) (4 : 1 ratio, 10^{-2} M) with an overall NaCl content of 0.9%. $^{31}P\{^1H\}$ NMR spectra of this solution were recorded at 37 °C over 72 h (2 h intervals) and remained identical over the course of the measurement.

Cell lines

For cytotoxicity studies the representative cisplatin-sensitive ovarian carcinoma A2780/S human cell line and the cisplatin-resistant A2780/R cell line (produced by repeated 1 h weekly exposure to 50 μM of the sensitive parental cell line[60]) were used. Cell lines were maintained in RPMI 1640 medium supplemented with fetal bovine serum (FBS) and antibiotics at 37 °C in a 5% CO_2 atmosphere and subcultured twice weekly.

Cell growth inhibition studies

The cytotoxic effects of these platinum compounds were evaluated on the growth of A2780/S and A2780/R cell lines. The compounds were diluted in DMSO as stock solutions. In order to establish the potential antiproliferative effects of DMSO, its activity was evaluated using the same experimental conditions as for the compounds under investigation (*vide infra*).

Exponentially growing cells were inoculated into 96-well microtitre plates at plating densities of 2×10^3 cells/well. After cell inoculation, the microtitre plates were incubated under standard culture conditions (37 °C, 5% CO_2, 95% air and 100% relative humidity) for 24 h prior to the addition of experimental drugs. After 24 h, medium was removed and replaced with appropriate medium-containing drug concentrations ranging from 0.3 to 100 μM for a continuous exposure of 72 h for all platinum compounds tested. For comparison purposes the cytotoxicity effects of cisplatin, measured at the same experimental conditions, were also determined.

According to the procedure described by Skehan *et al.*,[61] the assay was terminated by the addition of cold tricholoroacetic acid (TCA). Cells were fixed *in situ* by 10% TCA and stained by sulforhodamine B (SRB) solution at 0.4% (w/v) in 1% acetic acid. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM tris base, and the absorbance was read on an automated plate reader at a wavelength of 540 nm.

The IC_{50} drug concentration resulting in a 50% reduction in the net protein content (as measured by SRB staining) in drug treated cells as compared to untreated control cells was determined after 72 h of drug exposure. The IC_{50} data represent the mean of at least three independent experiments.

Percentages of cell growth inhibition by DMSO present in solution have been recorded at the IC_{50} values of PtP_2S_2 compounds in order to determine the influence of DMSO cytotoxicity. The cell growth inhibition due to the percentage of DMSO present in solution (ranging from 0.05% to 0.5%) was lower than 10% at the IC_{50} level. Thus the influence of DMSO on the overall antiproliferative effects could be considered as only marginal (less than 20%).

To evaluate presence or lack of cross-resistance of cisplatin-resistant cells towards the substances the resistance ratio (*r*) was calculated as the ratio of the IC_{50} values in the resistant cell line and the IC_{50} values in the sensitive one.

Cytochrome c interaction studies

Samples were prepared dissolving horse heart cyt c (Sigma C7752) (100 μM) in tetramethylammonium acetate buffer (TMAA, 25 mM, pH 7.4). Then each of the selected metal complexes was added to the solutions (3 : 1 or 1 : 1 metal:protein ratio) and incubated at 37 °C for 24 h or 96 h. After a 20-fold dilution with HCOOH (0.1%), the ESI-MS spectrum was recorded (direct introduction, flow rate 5 μL min^{-1}) in an LTQ-Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA, USA), equipped with a conventional ESI source. The spectrometer's working conditions were the following: spray voltage 3.1 kV, capillary voltage 45 V and capillary temperature 220 °C. The sheath and the auxiliary gases were set, respectively, at 17 (arbitrary units) and 1 (arbitrary units). For acquisition, Xcalibur 2.0. software (Thermo) was used and monoisotopic and average deconvoluted masses were obtained by using the integrated Xtract tool. For spectrum acquisition a nominal resolution (at m/z 400) of 100,000 was used.

Acknowledgements

The Authors thank Heraeus Holding GmbH for a generous gift of $K_2[PtCl_4]$ and COST Action D39 for financial support (COST-STSM-D39-3461 (CR) and COST-STSM-D39-5610 (CM)). CM gratefully acknowledges financial support provided by the Studienstiftung des deutschen Volkes. AC gratefully acknowledges the Swiss Confederation (SER project C09.0027 "Interactions of metal-based anticancer agents with proteins") and the Swiss National Science Foundation for financial support (AMBIZIONE project number PZ00P2_121933/1).

Notes and references

1 J. Reedijk, *Eur. J. Inorg. Chem.*, 2009, 1303–1312.
2 L. Kelland, *Nat. Rev. Cancer*, 2007, **7**(8), 573–584.
3 M. J. Cleare and J. D. Hoeschele, *Plat. Met. Rev.*, 1973, **17**, 2–13.
4 E. Wong and C. M. Giandomenico, *Chem. Rev.*, 1999, **99**, 2451–2466.
5 A. M. Fichtinger-Schepman, J. L. Van der Veer, J. H. J. Den Hartog, P. H. M. Lohman and J. Reedijk, *Biochemistry*, 1985, **24**(3), 707–713.
6 A. V. Klein and T. W. Hambley, *Chem. Rev.*, 2009, **109**, 4911–4920.
7 M. J. Cleare and J. D. Hoeschele, *Bioinorg. Chem.*, 1973, **2**, 187–210.
8 Á. M. Montaña and C. Batalla, *Curr. Med. Chem.*, 2009, **16**, 2235–2260.
9 G. Natile and M. Coluccia, *Coord. Chem. Rev.*, 2001, **216–217**, 383–410.
10 J. Bravo, S. Bolaño, L. Gonsalvi and M. Peruzzini, *Coord. Chem. Rev.*, 2010, **254**, 555–607.
11 C. G. Hartinger and P. J. Dyson, *Chem. Soc. Rev.*, 2009, **38**(2), 391–401.
12 A. K. Renfrew, A. D. Phillips, A. E. Egger, C. G. Hartinger, S. S. Bosquain, A. A. Nazarov, B. K. Keppler, L. Gonsalvi, M. Peruzzini and P. J. Dyson, *Organometallics*, 2009, **28**, 1165–1172.
13 F. Mohr, S. Sanz, E. Vergara, E. Cerrada and M. Laguna, *Gold Bulletin*, 2006, **39**, 212–215.
14 E. Vergara, S. Miranda, F. Mohr, E. Cerrada, E. R. T. Tiekink, P. Romero, A. Mendia and M. Laguna, *Eur. J. Inorg. Chem.*, 2007, 2926–2933.
15 A. Dorcier, W. H. Ang, S. Bolano, L. Gonsalvi, L. Juillerat-Jeannerat, G. Laurenczy, M. Peruzzini, A. D. Phillips, F. Zanobini and P. J. Dyson, *Organometallics*, 2006, **25**, 4090–4096.
16 T. Servidei, C. Ferlini, A. Riccardi, D. Meco, G. Scambia, G. Segni, C. Manzotti and R. Riccardi, *Eur. J. Cancer*, 2001, **37**(7), 930–938.
17 V. Scarcia, A. Furlani, B. Longato, B. Corain and G. Pilloni, *Inorg. Chim. Acta*, 1988, **153**, 67–70.

18 G. Trovo, G. Bandoli, U. Casellato, B. Corain, M. Nicolini and B. Longato, *Inorg. Chem.*, 1990, **29**(23), 4616–4621.
19 G. Trovo, G. Valle and B. Longato, *J. Chem. Soc., Dalton Trans.*, 1993, 669–673.
20 G. Trovo, B. Longato, B. Corain, A. Tapparo, A. Furlani, V. Scarcia, F. Baccichetti, F. Bordind and M. Palumboe, *J. Chem. Soc., Dalton Trans.*, 1993, 1547–1550.
21 D. Montagner, E. Zangrando and B. Longato, *Inorg. Chem.*, 2008, **47**, 2688–2695.
22 A. R. Khokhar, Q. Xu and Z. H. Siddik, *J. Inorg. Biochem.*, 1990, **39**, 117–123.
23 F. Sampedro, J. I. Ruiz, A. M. Molins-Pujol, P. Santaló, C. Moranta, M. Pueyo, M. Llagostera and J. Bonal, *J. Inorg. Biochem.*, 1991, **43**, 599–599.
24 J. Kozelka, E. Segal and C. Bois, *J. Inorg. Biochem.*, 1992, **47**, 67–80.
25 N. Margiotta, A. Habtemariam and P. J. Sadler, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1185–1187.
26 H. Yuge and T. K. Miyamoto, *Inorg. Chim. Acta*, 1998, **279**, 105–110.
27 G. Puxty, H. Bjelosevic, T. Persson and S. K. C. Elmroth, *Dalton Trans.*, 2005, 3032–3038.
28 H. Bjelosevic, C. Spégel, A. Sykfont Snygg, L. Gorton, S. K. C. Elmroth and T. Persson, *Tetrahedron*, 2006, **62**, 4519–4527.
29 A. Messere, E. Fabbri, M. Borgatti, R. Gambari, B. D. Blasio, C. Pedone and A. Romanelli, *J. Inorg. Biochem.*, 2007, **101**, 254–260.
30 F. J. Ramos-Lima, A. G. Quiroga, B. García-Serrelde, F. Blanco, A. Carnero and C. Navarro-Ranninger, *J. Med. Chem.*, 2007, **50**(9), 2194–2199.
31 S. J. Berners-Price, P. J. Sadler, in *Structure and Bonding*, 70 (Bioinorg. Chem.), Springer, Berlin, Germany, 1988, pp. 27-102.
32 A. Romerosa, P. Bergamini, V. Bertolasi, A. Canella, M. Cattabriga, R. Gavioli, S. Mañas, N. Mantovani and L. Pellacani, *Inorg. Chem.*, 2004, **43**, 905–913.
33 P. Bergamini, V. Bertolasi, L. Marvelli, A. Canella, R. Gavioli, N. Mantovani, S. Mañas and A. Romerosa, *Inorg. Chem.*, 2007, **46**, 4267–4276.
34 S. Bombard, M. B. Gariboldi, E. Monti, E. Gabano, L. Gaviglio, M. Ravera and D. Osella, *JBIC, J. Biol. Inorg. Chem.*, 2010, **15**, 841–850.
35 E. Parkinson and F. Minty, *BioDrugs*, 2007, **21**(6), 375–385.
36 E. Amtmann, G. Schilling, Pat. WO 2000010543 A2 20000302, 2000.
37 E. Amtmann, M. Zöller, H. Wesch and G. Schilling, *Cancer Chemother. Pharmacol.*, 2001, **47**, 461–466.
38 S. Miranda, E. Vergara, F. Mohr, D. de Vos, E. Cerrada, A. Mendía and M. Laguna, *Inorg. Chem.*, 2008, **47**, 5641–5648.
39 D. Dolfen, K. Schottler, S. Valiahdi, M. A. Jakupec, B. K. Keppler, E. R. Tiekink and F. Mohr, *J. Inorg. Biochem.*, 2008, **102**, 2067–2071.
40 R. G. Pearson, *J. Am. Chem. Soc.*, 1963, **85**(22), 3533–3539.
41 C. K. Jørgensen, *Inorg. Chem.*, 1964, **3**, 1201–1202.
42 R. G. Pearson, *Inorg. Chem.*, 1973, **12**, 712–713.
43 T. B. Rauchfuss and D. M. Roundhill, *J. Am. Chem. Soc.*, 1975, **97**, 3386–3392.
44 A. K. Fazlur-Rahman and J. G. Verkade, *Inorg. Chem.*, 1992, **32**, 5331–5335.
45 G. J. Grant, D. F. Galas, I. M. Poullaos, S. M. Carter and D. G. VanDerveer, *J. Chem. Soc., Dalton Trans.*, 2002, 2973–2980.
46 S. E. Miller and D. A. House, *Inorg. Chim. Acta*, 1990, **173**, 53–60.
47 A. Casini, C. Gabbiani, G. Mastrobuoni, L. Messori, G. Moneti and G. Pieraccini, *ChemMedChem*, 2006, **1**(4), 413–417.
48 A. Casini, G. Mastrobuoni, W. H. Ang, C. Gabbiani, G. Pieraccini, G. Moneti, P. J. Dyson and L. Messori, *ChemMedChem*, 2007, **2**(5), 631–635.
49 C. Gabbiani, A. Casini, G. Mastrobuoni, N. Kirshenbaum, O. Moshel, G. Pieraccini, G. Moneti, L. Messori and D. Gibson, *JBIC, J. Biol. Inorg. Chem.*, 2008, **13**, 755–764.
50 C. G. Hartinger, A. Casini, C. Duhot, Y. O. Tsybin, L. Messori and P. J. Dyson, *J. Inorg. Biochem.*, 2008, **102**, 2136–2141.
51 T. G. Appleton, M. A. Bennett and I. B. Tomkins, *J. Chem. Soc., Dalton Trans.*, 1976, 439–446.
52 M. A. Andrews, G. L. Gould, W. T. Klooster, K. S. Koenig and E. J. Voss, *Inorg. Chem.*, 1996, **35**, 5478–5483.
53 M. P. Brown, R. J. Puddephatt, M. Rashidi and R. Seddon, *J. Chem. Soc., Dalton Trans.*, 1977, 951–955.
54 D. A. Krogstad, S. B. Owens, J. A. Halfen and V. G. Young, Jr., *Inorg. Chem. Commun.*, 2005, **8**, 65–69.
55 COLLECT, Data Collection Software; Nonius B.V., Netherlands, 1998.
56 Z. Otwinowski, W. Minor, in *Methods in Enzymology*, Vol. 276, Macromolecular Crystallography, Part A; ed. C. W. Carter, R. M Sweet, Academic Press 1997; pp 307-326.
57 G. M Sheldrick, *Acta Crystallogr., Sect. A: Found. Crystallogr.*, 2008, **64**, 112–122.
58 T. W. Greene, P. G. M. Wuts, *Protective Groups in Organic Synthesis*, Third Edition, John & Sons, Inc. New York 1999, pp. 273.
59 A. Yoshida, T. Hayashi, N. Takeda, S. Oida and E. Ohki, *Chem. Pharm. Bull.*, 1981, **29**(7), 1854–1861.
60 Y. Lu, J. Han and K. J. Scanlon, *J. Biol. Chem.*, 1988, **263**, 4891–4894.
61 P. Skekan, R. Stroreng, D. Scudiero, A. Monks, J. Mcmahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1107–1112.

