Coordination compounds in cancer: Past, present and perspectives

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Keywords:
Cancer
Coordination metal complexes Cisplatin
Omics Perspectives

Abstract

Metal-based coordination compounds have been used throughout the history of human medicine to treat various diseases, including cancer. Since the discovery of cisplatin in 1965, a great number of metal coordination complexes, such as platinum, ruthenium, gold or copper have been designed, synthesized and tested in order to develop clinically effective and safe drugs. Currently, many reviews cover applications of cytostatic metal complexes pointing out the most promising examples of platinum- and non-platinum-based compounds in preclinical and clinical trials. However, recent comprehensive reviews covering

chemical and biological aspects of metal-based coordination compounds in cancer therapy are still rare. In this review we wish to provide an overview of the coordination chemistry of current and novel cytostatic compounds, including an outline of their design and rationale of synthesis, and summarize bio-chemical reactivity and physicochemical properties of candidate metal complexes.

Introduction

Cancer is nowadays one of the leading causes of death in the developed world. Biologically, cancer represents a vastly heterogenic group of diseases sharing several common traits. One of these hallmarks is sustained proliferation, resulting in uncontrolled tumour growth (Hanahan and Weinberg, 2011). An extensive research has been done to characterize antiproliferative effect of various classes of compounds, ranging from naturally occurring molecules and their deriva- tives, to organometallic and inorganic compounds and their application in cancer therapy. The fortuitous discovery of the cytotoxic properties of cisplatin (diamminedichloroplatinum (II)) in 1965, opened new avenue for the application of metal complexes in cancer therapy (Arnesano et al., 2011) (Fig. 1). The antiproliferative effect of cisplatin and other compounds, however, induces adverse effects on normal tissues, decreasing therapeutic effectivity. Moreover, in many cancers, tumour cells may acquire resistance to the metal-based cytotoxic therapy resulting in virtually incurable relapsing disease (Desoize, 2004).

In the last 15 years, a great effort has been dedicated to the development of more effective and lesstoxic drugs. Variousnew trans-platinum(II) and platinum(IV) complexes have been synthesized, and some of them have been selected for clinical trials (Kelland et al., 1999), but with varying effectivity and safety. Therefore, less toxic metals, such as ruthenium, gold or copper were introduced as promising candidates for effective and safe therapy (Tiekink, 2002; Clarke, 2002; Marzano et al., 2009; Nobili etal., 2010). Various reviews have been published on the use of metalcomplexes asanticancer agents, with the intent to give an overview of the proposed approaches concerning the application of these systems in clinical practice (Tiekink, 2002; Boulikas et al., 2007; Milacic et al., 2008; Bruijnincx and Sadler, 2008; Todd and Lippard, 2009; Vilmar and Sørensen, 2009; Esteban-Fernández et al., 2010; Tisato et al., 2010; Wang and von Recum, 2011; Beija et al., 2012; Babu et al., 2013; Maldonado et al., 2013; Sukumar et al., 2013; Cao-Milán and Liz-Marzán, 2014; Mjos and Orvig, 2014; Muhammad and Guo, 2014; Petrelli et al., 2014). However, the majority of the available reviews point out the most relevant examples of platinum- or non-platinum- based compounds, eventually focusing on one particular metal ion or making a compendium on two or more metal ions. The aimof thisreview istobridge agap by summarizing onhistorical background, novel trends in synthesis of new metal complexes with antiproliferative effects and to describe their chemical reactivity, pharmacokinetic properties and interactions in the biological and biomedical context.

Platinum-based complexes

Platinum(II) complexes

Cisplatin and transplatin

Diamminedichloroplatinum(II) is a complex with square planar geometry and two possible *cis* and *trans* geometrical isomers, cisplatin and transplatin (Fig. 2).

Cisplatin has been a first-line therapy in many cancers and nowadays is used either alone or in combination with other compounds in many cancers, *e.g.* testicular, ovarian or bladder cancers or leukaemias. Due to low chemical stability of cisplatin, the direct intravenous administration is preferred over the other forms. In the blood stream, cisplatin rapidly interacts with plasma proteins such as human serum albumin (HSA), haemoglobin (Hb) or transferrin (Tf) (Rudnev et al., 2005) and 24 h after administration, 95% of cisplatin is bound to plasma proteins (Sooriyaarachchi et al., 2011). Cisplatin is widely distributed into body fluids and tissues, reaching the highest concentrations in kidneys (0.4–2.9 *m*g/g), liver (0.5–3.7 *m*g/g wet weight), and prostate (1.6–3.6 *m*g/g). Minor concentration levels can be found in muscles, bladder, testes, pancreas, and spleen (Stewart et al., 1982). Penetrance of cisplatin into tumour tissue differs in different cancers. However, the concentration of cisplatin and its analogues positively correlates with reduction of tumour mass and clinical parameters, such as recurrence free and overall survival, *e.g.* in non-small-cell lung cancer (Kim et al., 2012).

Cisplatin enters the cells either passively by a simple diffusion or by active protein-mediated transport systems, *e.g.* human organic cation transporter (hOCT2) and the copper transport protein (Ctr1) (Ishida et al., 2002; Song et al., 2004; Burger et al., 2010). In cytoplasm, cisplatin is hydrolysed and one of the two chloride ligands is displaced by a water molecule to form the $[PtCl(H_2O)(NH_3)_2]^+$ species, allowing for the binding of the platinum ion to DNA bases, especially in the N7 position of guanine and adenine and the N3 of cytosine, forming the monofunctional adduct $[PtCl(DNA)(NH_3)_2]^+$. The second chloride ligand can be displaced by a water molecule to form the adduct $[Pt(H_2O)(DNA)(NH_3)_2]^{2+}$. These species may re-interact with DNA by crosslinking forming a bifunctional adduct (Alderden et al., 2006) (Fig. 3) and trigger programmed cell death.

Several modes of cisplatin crosslinking with DNA have been proposed (Trzaska, 2005). The main adducts with DNA form 1,2-intrastrand cross-links with two adjacent guanines (1,2-d(GpG)) and 1,2-intrastrand cross-links with an adenine and an adjacent guanine (1,2-d(ApG)) (Jamieson and Lippard, 1999). The adducts 1,2-d(GpG) are supposed to be responsible for the cytotoxic activity of the drug (Todd and Lippard, 2009). Minor adducts are 1,3-intrastrand cross-links formed with nonadjacent guanines and interstrand adducts.

In contrast to cisplatin, the *trans* isomer of the diammine- dichloroplatinum(II), transplatin, shows only mild cytotoxic activity. This could be explained by different way of formation of DNA adducts. The interstrand cross-links between guanine and cytosine are formed by both isomers, while the 1,2- intrastrand cross-links are prevented by the geometry in the case of transplatin (Bernal-Méndez et al., 1997). In addition, the conversion of the monofunctional adducts into the bifunc- tional ones occurs particularly slowly for transplatin. In fact, after 24 h, the majority of DNA adducts

formed by transplatin are still monofunctional (Bernal-Méndez et al., 1997). The difference between cytotoxic effects of cisplatin and transpla- tin is therefore in the formation of the 1,2-intrastrand bifunctional adduct and in the fast conversion rate of the monofunctional adduct into the bifunctional one.

Other cis-Pt complexes

Because of the cisplatin success in clinical therapy, various new cis-Pt(II) complexes have been synthesized by substitu- tion of either chlorine or ammonia ligands with different structures. Up to now, only carboplatin and oxaliplatin, have shown better performance than cisplatin in some types of cancers, and their use has been approved worldwide.

The promising compound named *picoplatin* (*cis*-amine- dichloro-(2-methylpyridine)-Pt(II)) (Fig. 4) has been introduced for the treatment of patients with solid tumours, and its clinical trials started in 1997 (Kelland, 2007a; Wheate et al., 2010). Picoplatin has a marked steric bulk around the platinum ion that reduces its inactivation by thiol-containing species. Its cytotoxic activity is due to the interaction with DNA that leads mainly to the formation of intrastrand adducts. Picoplatin was found to be active against cisplatin- and oxaliplatin-resistant cell lines (Kelland, 2007b). When used as single agent its main dose-limiting side effect is myelosuppression. However, phase III trials did not confirm the previous promising results in the treatment of small-cell lung cancer and new trials are not currently planned (Lopez-Chavez and Sandler, 2012; Hamilton and Olszewski, 2013).

Recently, *cis*-Pt(II) complexes has been reconsidered and used as a scaffold for biologically active ligands. One example is the complex *cis*-[Pt(NH3)2(L)Cl] (L = 3-aza-5H-phenanthridin- 6-one) which contains a poly(ADP-ribose) polymerase (PARP-1) inhibitor as (B. Wang et al., 2014a). PARP-1 is a poly (ADP- ribose) polymerase involved in DNA replication, damage repair, and transcriptional regulation. After DNA damage, the activity of PARP-1 increases, stimulating the response of DNA-damage repairing proteins. This platinum complex exhibits increased activity and enhanced solubility with respect to those of the free inhibitor.

Other *cis*-Pt(II) compounds conjugated with side-directing molecules have been introduced with the aim to increase their selectivity. Analogues of cisplatin, carboplatin and oxaliplatin have been prepared with estrogens-like compounds in order to increase selectivity towards hormone-dependent breast, ovarian and uterine tumours (Descôteaux et al., 2003, 2008; Saha et al., 2012). The estrogens-derived cisplatin and carboplatin ligands possess a high affinity for the oestrogen receptor *a* (ER*a*) and improved cytotoxic activity. Interestingly, the cisplatin analogues show a better tumour regression than cisplatin alone in human breast cancer estrogens-receptor positive mouse xenograft model (Themsche et al., 2009). However, the substitution of cisplatin ligands has not extended significantly the range of applicability of plati- num-based drugs in cisplatin-resistant cancers probably because of the use of only one structural motif (Quiroga, 2012). Therefore, the research focused on platinum complexes with different geometry (*e.g.*, *trans*-Pt(II) complexes) and oxidation state (*e.g.*, Pt(IV) complexes).

Other trans-Pt complexes

Beside the low cytotoxic efficacy of transplatin, other *trans*-Pt (II) compounds with substituents different from ammonia have shown cytotoxic activity equal or higher than that of cisplatin. The cytotoxic effect induced by such complexes might be due to the formation of monofunctional adducts and interstrand crosslinking with DNA as well as to the interac- tions with proteins. The biological activity of these complexes appears related to the steric hindrance of the substituents that slow down the displacement of the chlorides (Marzano et al., 2010). Planar amines such as pyridine, quinoline, isoquinoline and thiazole were firstly used as substituents (Aris and Farrell, 2009). The first *trans*-platinum complex with marked antitumor efficacy *in vivo* was the *trans*-ammine- (cyclohexylamine)-dichloro-dihydroxo-Pt(IV) (known as JM335) (Fig. 5a) which combines the *trans* geometry with the higher oxidation state of the metal ion (Kelland et al., 1994). In *trans* platinum complexes, the substituents are chosen in order to modulate the solubility and stability in aqueous media. For example, the use of ligands containing carboxylic groups leads to *trans*-platinum complexes stable towards hydrolysis but water soluble and able to accumulate in cisplatin-resistant cell lines (Quiroga et al., 2005). Com- plexes containing iminoethers or aliphatic amines in place of planar ones were found active in cisplatin-resistant cell lines (Aris and Farrell, 2009). Various *trans*-[Pt(amine)$_2$(amidine)$_2$] Cl$_2$ compounds with four N-ligands bound to the platinum core have been synthesized (Fig. 5b) and tested with relevant results against a large panel of human cancer cell lines (Marzano et al., 2010). In addition, it has been observed that the size of the substituents and the cytotoxicity of the resulting molecule are directly correlated. The cyclohexyl derivative appears, in fact, as the most active. *In vivo* tests against Lewis lung carcinoma show a reduction in tumour size similar to that achieved using cisplatin (68 and 72%, respectively) but without the side effects typical of this last one (Marzano et al., 2010). Recently, the class of *trans*-Pt(II) compounds has been extended to complexes containing iminothioether ligands with general formula *trans*-[Pt{N(H) = C(SEt)R}$_2$Cl$_2$] (R = Me, Et, Ph, CH$_2$Ph) (Fig. 5c). The most active compound of such series is the *trans*-[Pt{N(H) = C(SEt) CH$_2$Ph}$_2$Cl$_2$] that shows higher cytotoxic activity than cisplat- in against a broad panel of human solid tumour cell lines (Sgarbossa et al., 2013). The aforementioned complexes are active against cisplatin-resistant and Multi Drug Resistance (MDR) cell lines. The MDR phenomenon occurs when the acquired resistance to a specific drug causes resistance to other drugs, even not chemically related.

Another interesting example is the family of *trans*-Pt(II) complexes with sulphonamide ligands. This class of com- pounds shows antitumor activity against cisplatin-resistant cell lines of cervix adenocarcinoma, ovarian carcinoma and ductal breast epithelial tumour (Pérez et al., 2014).

Platinum(IV) complexes

Complexes of Pt(IV) are thermodynamically stable, kinetically inert and diamagnetic. The metal ion is hexa-coordinated and its complexes have octahedral geometry. The biological properties of such complexes can be finely tuned thanks to the six coordination sites available.

Despite some contradictory results (Khokhar et al., 1993; Talman et al., 1998), it is now widely accepted that the antitumor activity of Pt(IV) complexes is due to their reduction to Pt(II) analogues. It was experimentally observed that DNA- binding activity of Pt(IV) complexes is increased in presence of some intracellular reductants (Choi et al., 1998), and Pt(IV) complexes are reduced by various biomolecules present in blood and cells. Pt(IV) complexes also show lower chemical reactivity in comparison to their Pt(II) counterparts. These findings suggest that Pt(IV) compounds undergo reduction prior getting a cytotoxic activity. Physiologically, these Pt(IV) complexes are reduced by biomolecules such as glutathione (GSH), methionine, cysteine, metallothioneins, serum albu- min, ascorbate, DNA nucleobases, nucleotides and their analogues. Depending on the reduction potential of the Pt(IV) complex, the reduction may occur into the bloodstream instead of within the cells, giving rise to side-reactions that lead to systemic toxicity (Hall and Hambley, 2002). The reduction rate of the Pt(IV) complexes increases by using bulky equatorial and axial electron-withdrawing ligands (Choi et al., 1998). Besides, the reduction rate may be also influenced from the low kinetics of axial ligand dissociation in Pt(IV) complexes (Wong and Giandomenico, 1999).

The cellular uptake of Pt(IV) complexes is higher than that of Pt(II) ones. This has been correlated with their higher lipophilicity which facilitates their passive diffusion into the cells. This could be also an explanation for their efficacy against some cisplatin resistant cancer cell lines (Hall and Hambley, 2002). However, the *in vivo* reduction of the Pt(IV) complex is accompanied by the loss of the axial ligands, and the resulting compound may show a lower activity due to decreased lipophilicity (Hall and Hambley, 2002).

Satraplatin

Satraplatin (bis-acetate-amine-dichloro-cyclohexylamine-Pt (IV)) (Fig. 6a), also known as JM216, is a drug for oral administration containing two acetate ligands that increase the lipophilicity of the compound. In the bloodstream it is reduced with consequent loss of the two acetate ligands, forming its main metabolite labelled JM118. This compound unwinds DNA and induces apoptosis. Unlike cisplatin and carboplatin, the DNA adducts formed by satraplatin are not detected by DNA mismatch repair proteins (Fink et al., 1996). Satraplatin is active against some cancers that show acquired resistance to cisplatin, thanks to its higher influx and reduced DNA-repair (Fokkema et al., 2002; Kelland, 2007b). Clinical trials using satraplatin for the treatment of advanced prostate cancer documented, however, only mild recurrence-free survival, but without overall survival benefits (Bhargava and Vaishampayan, 2009). In addition, it has been proposed for the treatment of non-small cell lung, and squamous head and neck cancers (Wong and Ang, 2012).

Besides satraplatin, other two Pt(IV) complexes have been tested in clinical trials: iproplatin and tetraplatin. The iproplatin, [Pt(isopropylamine)$_2$(OH)$_2$(Cl)$_2$] (Fig. 6b), has been extensively studied in phases I, II, and III trials for the treatment of a wide range of cancers, with lower efficacy than cisplatin and some cases of toxic death were reported (Clavel et al., 1988). The tetraplatin, [Pt(*trans*-1,2-diaminocyclohexane) Cl4], also known as ormaplatin (Fig. 6c), shows a good cytotoxic activity both *in vitro* and *in vivo*, but severe neurotoxicity observed in phase I trials prevents further clinical

applications (Boulikas et al., 2007).

A different approach is to combine a cytotoxic metal compound with biologically active molecules. It is possible to prepare Pt(IV) platinum complex with small molecular inhibitors of enzymes that inactivate platinum complexes in resistant cells. Ethacrynic acid is an inhibitor of glutathione-S- transferase (GST) which catalyses the reaction of cisplatin with glutathione (Ang et al., 2011). Overexpression of GST enzyme has been reported in cisplatin-resistant cell lines and the GST inhibitors have already been used in combination with other drugs in anticancer therapy. The GST inhibition induced by the Pt(IV) complex containing ethacrynic acid (Fig. 6d) is more effective than that induced by the ethacrynic acid alone. In addition, it shows cytotoxic activity higher than that of cisplatin in the first 24 h and equal to that of cisplatin after 72 h. The high GST inhibition shown by the complex has been attributed to its covalent binding to the enzyme and subse- quent binding of ethacrynate ligands at the active sites (Parker et al., 2011).

Polynuclear platinum complexes

Polynuclear platinum complexes containing aliphatic amines as bridging linkers have been designed with the intent to overcome drug resistance. They react rapidly with DNA forming long-range interstrand and intrastrand cross-links (Wong and Giandomenico, 1999; Wang and Guo, 2008). Some of them have been found to be active even against cisplatin- resistant cells.

The triplatin tetranitrate or dichloro-hexamine-bis(*m*-1,6- hexane-1,6-diamine)-tri-Pt(II), known as BBR3464 (Fig. 7a), is a multiply charged (+4) trinuclear complex that interacts with DNA by forming 1,4-intrastrand and 1,4-interstrand cross- links that cannot be repaired by the excision repair mecha- nism. The cytotoxic activity of the drug is due to the long life of the intra- and interstrand cross-links (Kasparkova et al., 2002). The BBR3464 shows no cross-resistance in cisplatin-resistant cell lines (Perego et al., 1999), but phase II trials were not convincing (Wheate et al., 2010). Its lack of activity is probably due to the binding with plasma proteins that results in drug deactivation. Among trinuclear Pt(II) complexes, the [{trans-Pt (NH3)2(NH2(CH2)6(NH3))}2-m-{trans-Pt(NH3)2(NH2(CH2)6NH2)2}]+ (Fig. 7b) shows a peculiar interaction with DNA. In fact, it binds via hydrogen bond to the oxygen atoms of the phosphate groups on the DNA backbone. This complex has been presented as the first example of a non-covalent platinum compound with cytotoxicity equivalent to that of cisplatin (Komeda et al., 2006). Polynuclear complexes with rigid bridging ligands such as aromatic compounds and with more flexible linkers like 4,4-methylenedianiline have been devel- oped (Mlcouskova et al., 2012; Zerzankova et al., 2010; Olivova et al., 2012). The substitution of chloride ligands with alkylcarboxylates leads to a class of polynuclear complexes with increased stability and reduced ability to bind plasma proteins with respect to BBR3464. Among these compounds, dinuclear platinum complex CT-47463 (Fig. 7c) possesses a cytotoxic activity against cisplatin-resistant ovarian and squamous cell carcinoma, and osteosarcoma human cell lines with IC50 (i.e., drug concentration required for 50% inhibition of cell growth) of 0.003, 0.77 and 0.041 mM (Gatti et al., 2009). The CT-47463 inhibits tumour growth in platinum-resistant human ovarian carcinoma xenograft by 80% (Barry and Sadler, 2013).

Platinum drugs approved for clinical practice

Despite the huge number of platinum complexes synthe- sized up to now, less than 30 have reached the human dicarboxylic ligand. Because the carboxylate group is more stable than the chloride, carboplatin exhibits lower reactivity and slower DNA binding kinetics. Within the cellular envi- ronment it forms the same DNA adducts formed by cisplatin, but with a product profile noticeably different (Blommaert et al., 1995). The major carboplatin adduct identified is the *cis*- [Pt(NH3)2(dG)2] (40%). Minor products are 1,2-d(GpG) (30%), 1,2- d(ApG) (16%), and a small number of interstrand cross-links (3– 4%), together with monofunctional adducts. The lower reactivity of carboplatin results also in less toxicity for urinary and gastrointestinal tract, when compared to cisplatin (Kel- land, 2007a). The dose-limiting toxicity (*e.g.*, adverse side-effects) is myelosuppression (Calvert et al., 1982). Despite many studies have shown that carboplatin and cisplatin present the same cytotoxic activity, carboplatin has success- fully replaced cisplatin in the treatment of some kinds of cancers, such as advanced, metastasized or recurrent non- small cell lung, and advanced or recurrent ovarian cancer (see www.cancer.gov). Moreover, the lower toxicity allows the use of higher doses and the prolongation of treatment time (Lebwohl and Canetta, 1998). However, carboplatin does not overcome the problem of drug resistance due to the cross- resistance with cisplatin (Desoize, 2004).experimentation. Among them, only cisplatin, carboplatin, and oxaliplatin have been approved worldwide for clinical use. Nedaplatin, heptaplatin, and lobaplatin have been approved only in Japan, China, and South Korea, respective- ly. Other drugs previously tested have evidenced severe side-effects or lack of activity in phase I or II trials, and their development has been abandoned (Wheate et al., 2010). Recently, satraplatin and picoplatin development has been stopped as well (Hamilton and Olszewski, 2013).

Carboplatin

Carboplatin (*cis*-diamine(1,1-cyclobutanedicarboxylato)-Pt(II)) (Fig. 8a) is the first cisplatin derivative used in clinical therapy. In this complex, the metal ion is coordinated by a bidentate

Oxaliplatin

Oxaliplatin (1R,2R-diaminocyclohexane-oxalate Pt(II)) (Fig. 8b) is now used for the treatment of cancers resistant to other platinum drugs, particularly in colorectal cancer. It is typically administered with fluorouracil and leucovorin in a combina- tion known as FOLFOX (Maindrault-Goebel et al., 1999; Desoize, 2004). In oxaliplatin, the metal ion is coordinated by 1,2-diaminocyclohexane and oxalate ligands. The cytotoxic activity of this drug arises from the inhibition of DNA synthesis in cancer cells. In fact, it forms both inter- and intrastrand cross links in DNA, preventing DNA replication and transcrip- tion, and triggering cell death (Graham et al., 2004). Oxaliplatin does not show cross-resistance with other platinum drugs because it binds DNA differently than cisplatin, and the resulting adducts are not recognized by the DNA mismatch repair proteins (Fink et al., 1996). Also, it seems that no interaction with the copper

transporter CTR1 occurs, and this prevents its efflux outside the cell in some kinds of cisplatin- resistant cancers (Holzer et al., 2006). Oxaliplatin is less toxic than cisplatin but its dose-limiting toxicity is associated with a not predictable occurrence of sensory neuropathy.

Nedaplatin

Nedaplatin(diamine(1,2-(O,O')-2-hydroxyacetato)-Pt(II)) (Fig. 8c) is a second-generation cisplatin analogue. This drug was developed with the aim to avoid the nephrotoxicity and gastrotoxicity of cisplatin while maintaining the same efficacy (Mabuchi and Kimura, 2010). It presents two ammo- nia and a glycolate ligand which forms a five-membered ring with the platinum ion. The water solubility of nedaplatin is ten times higher than that of cisplatin. It is actually less nephrotoxic than cisplatin and carboplatin (Alberto et al., 2009; Kuwahara, 2009), presenting anticancer activity comparable to that of cisplatin (Kawai et al., 2005; Alberto et al., 2009). Nedaplatin interacts with DNA forming mainly inter- strand cross-links. It reacts with GSH and metallothioneins in

minor extent because of the presence of the five-membered ring which prevents the binding to the platinum core. Nedaplatin can cause thrombocytopenia and also nephrotoxicity in absence of a pre- and post-treatment hydration. Its efficacy is not higher than that of cisplatin, but it has been proved to be less toxic for kidneys, gastrointestinal tract, and nervous system. It is currently registered in Japan for the treatment of head and neck, testicular, lung, ovarian, cervical, and non-small-cell lung cancer (Wheate et al., 2010). Clinical trials are ongoing for the use of nedaplatin in different schedules, in particular in combination with other drugs (Oshita et al., 2004; Gong et al., 2009; Kurita et al., 2010), against non-small-cell lung, cervical, oesophageal, testicular, and head and neck cancers.

Lobaplatin

In lobaplatin (1,2-diaminomethyl-cyclobutane-lactate-Pt(II)) (Fig. 8d), the Pt(II) ion is coordinated by the nitrogen atoms of a 1,2-diaminomethyl-cyclobutane and by one molecule of lactic acid. Lobaplatin binds DNA preferentially at guanine residues, forming mainly intrastrand cross-links, and in parallel probably inhibits the DNA and RNA polymerases. Lobaplatin shows *in vitro* cytotoxic activity against a wide range of cancer cell lines, including some cisplatin and carboplatin resistant ones (McKeage, 2001). In clinical trials the dose-limiting side-effect was found to be thrombocytope- nia (Gietema et al., 1993). Lobaplatin has been approved in China for the treatment of chronic myeloid leukaemia, and inoperable metastatic small-cell lung and breast cancers. Phase II clinical trials have been also completed in other countries such as USA, Australia, Europe, Brazil, and South Africa for the treatment of breast, oesophageal, lung, and ovarian cancers, and chronic myelogenous leukaemia (Bouli- kas et al., 2007). Despite the lack of cross-resistance estab- lished *in vitro*, in a clinical trial lobaplatin has shown no activity against a cisplatin-resistant form of ovarian cancer (Kavanagh et al., 1995).

Heptaplatin

Heptaplatin (2-(1-methylethyl)-1,3-dioxolane-4,5-dimetha- namine-[N,N^{0}][propanedioato-O,O']-Pt(II)) (Fig. 8e) has been proposed for the treatment of gastric cancers and was approved for clinical therapy in South Korea. It is a Pt(II) square planar complex with a malonato ligand as leaving group and a dimethanamine-1,3-dioxolane derivative. It has been designed to have higher antitumor activity and lower toxicity with respect to cisplatin. It shows activity *in vitro* and in human tumour xenografts against different types of cisplatin-resistant tumours (Kim et al., 1995). The activity of heptaplatin on cisplatin-resistant cell lines is partially due to a major resistance to deactivation by metallothioneins (Choi et al., 2004). Nephrotoxicity, hepatotoxicity, and myelosuppression are its dose-limiting side-effects. The toxicity of heptaplatin has been confirmed to be lower than that of cisplatin as supposed by its developers but it does not present a higher cytotoxic activity, at least in advanced gastric adenocarcinoma and small-cell lung cancer (Kim et al., 1999; Zang et al., 1999). Currently, heptaplatin is used for the treatment of advanced gastric and lung cancers (Graf et al., 2012).

Overcoming of platinum resistance – lessons from the ''omics''

Any cytostatic treatment reducing a tumour mass induces a selection pressure on proliferating cancer cells. Cells that survive are either slow-cycling, quiescent or those that acquired resistance to the cytostatic drug. Generally, metal- based compounds, such as cisplatin, induce programmed cell death by DNA damage or interactions with other cytoplasmic targets. However, the cell response triggered by cytotoxic compounds is highly dependent on cancer type and its particular genetic and epigenetic context. Genes affecting the therapeutic effectivity are remarkably diverse, coding for membrane transporters, DNA damage response machinery, detoxification enzymes or programmed cell death effectors, affecting greatly the results of the therapy (Scanlon et al., 1989; Andrews and Howell, 1990) (Fig. 9). However, plethora of other genes, involved in many aspects of cell life, increase the enormous complexity of mechanism of action of individual coordination compounds in cancer cells.

Progress in development of global gene expression tech- niques enabled deeper and complex understanding of devel- opment of resistance or even cross-resistance. The classical proteomic approaches identified tens or hundreds of proteins differentially expressed in cisplatin-resistant and sensitive cancers. Interestingly, these proteins fall into virtually all functional families in human proteome, ranging from tran- scription factors, splicing and translation machinery, cyto- skeleton, junction proteins to signalling pathways to microsomal metabolic and detoxication enzymes, mitochon- drial respiration proteins or cell cycle (Stewart et al., 2006). Next generation sequencing (NGS) is sufficiently robust to address the expression complexity in various cancer types and models. NGS-based whole exon sequencing, mRNA sequenc- ing, miRNA quantification or global analysis of single nucleo- tide polymorphisms (SNPs) can provide a multidimensional alteration landscape that can determine the effect of cytotoxic drugs (Roukos et al., 2012). NGS was used to identify and quantify individual mRNAs in cell line models of ovarian cancer chemoresistance, indicating

that expression of more 2500 genes is altered in cisplatin-sensitive and resistant cancer cells (Li et al., 2013). Further integration of global gene expression data, miRNA and SNPs into quantitative trait loci (QTLs) allowed for identification of functional links, *e.g.* between particular SNPs (rs11138019), expression of miRNA (miR30a), activity of membrane efflux transporters (ABCD2) and cisplatin sensitivity in ovarian cancer (LaCroix et al., 2014). Recent global studies focused on other metal-based cytotoxic drugs, *e.g.* copper and gold that specifically targets proteasome and metabolism of reactive oxygen species (ROS) (Guidi et al., 2012; Ng et al., 2014) or ruthenium, altering the general cell thioredoxin system (Guidi et al., 2013). Particularly relevant are the recent metallomic studies that documented a critical impact of distribution of metal-based drugs in tissues *in vivo* and three dimensional (3D) tissue-like structures *in vitro*. Encapsulation of cisplatin in nanocarriers (Oberoi et al., 2013) or modification of tumour-specific antigens (Dhar et al., 2008) improves homing to the tumour lesion and stabilizes concentration of the cisplatin in the vicinity of the tumour.

Zhang et al. analysed distribution of platinum in cisplatin- and platinum-anthraquinone conjugate-treated colon cancer 3D spheroids, revealing hotspots of platinum accumulation in necrotic foci, often responsible for tumour relapse. Exposition of such foci to cytotoxic concentrations of cytostatics can eliminate quiescent or stem cell populations (Zhang et al., 2012). Moreover, information provided by integrative global approaches, including NGS, pharmacokinetic analysis and metallomics, can even serve as a biomarker tool predicting a cancer relapse (Araujo et al., 2014). In summary, the integrative approach is important for understanding the cell responses to metal-based compounds and overriding the cancer chemore- sistance.

Non-platinum complexes

Despite the extensive research done in last decades, only platinum-based drugs has been approved for clinical practice. Therefore, the possibility to replace platinum with other metal ions has been studied in order to obtain new complexes with low systemic toxicity and active against cancers unresponsive to cisplatin. The rationale behind this approach is that complexes based on other metal ions may act on different targets in cells, including DNA, enzymatic pathways, ROS or mitochondria. Moreover, therapeutic metal-based complexes may mimic endogenous compounds and specifically use physiological endocytic pathways to increase cytotoxic effect and possibly reduce the systemic adverse effects.

Ruthenium

Ruthenium(II) forms either penta- or hexa-coordinated com- plexes, while ruthenium(III) only hexa-coordinated ones. Both

of them show antitumor activity, but Ru(III) is probably *in vivo* first reduced to Ru(II) acting rather as a prodrug. This reduction process seems to be more effective in hypoxic tumour tissues, lacking normal oxygen partial pressure (Clarke et al., 1999; Clarke,

2002; Spreckelmeyer et al., 2014). The reduction process, Ru(III) ⬇ Ru(II), can be reverted in presence of molecular oxygen. Generally, Ru(II) compounds show lower systemic toxicity than that of platinum ones. Interestingly, Ru(II) is bound by iron-binding proteins such as transferrin and albumin, leading to accumulation of Ru(II) in cancer cells by highly selective iron transport mechanisms and reducing the concentration of the free complex in the bloodstream (Allardyce et al., 2005).

Complexes of Ru(II) with arene ligands represent the most studied anticancer ruthenium compounds because of their amphiphilicity and a relatively easy tunability of their properties. In these complexes the metal ion represents the hydrophilic part while the arene ligand the hydrophobic one. Cationic arene-ruthenium compounds can act as cage for molecules containing other drugs representing an interesting strategy for the development of synergic anticancer drugs (Süss-Fink, 2010). Cytotoxic activity of ruthenium complexes is due to their interaction with DNA and with other biological targets influencing different cellular mechanisms (Fig. 10). The DNA is the target of a family of ruthenium compounds with general formula $[Ru(\eta^6\text{-arene})(N,N')X]^+$ (X = Cl or I , N, N^0 = ethylenediamine or N-ethylethylenediamine). Monofunc- tional and bifunctional adducts with DNA are formed by reaction with guanine nucleobases. These Ru(II) compounds show cytotoxic activity similar to that of carboplatin, but lower than that exhibited by cisplatin against human ovarian cancer cell line A2780 (Morris et al., 2001). In addition, these Ru(II)- arene compounds do not present cross-resistance with cisplatin neither *in vitro* nor *in vivo* (Aird et al., 2002). Among Ru(II)-arene complex family, the most interesting compound is $[Ru(biphenyl)(en)Cl]PF_6$, named as RM175 (Fig. 11a). RM175 binds DNA either by intercalation through the aromatic ligand or by covalent binding with the metal ion (Hayward et al., 2005). Anionic acetylacetonate (acac) derivatives of Ru(II)- arene may be used instead of

reacts with DNA and induces apoptosis *via* the intrinsic mitochondrial pathway, being accumulated mainly in cell nucleus (Pongratz et al., 2004; Hartinger et al., 2006). Although the target of this compound is the same of platinum-based drugs, the induced DNA lesions are different. The complex $[Ru(HIn)_2Cl_4](H_2In)$ shows biological activity against tumour cell lines overexpressing several multidrug-resistance-associated proteins (multidrug resistance protein 1 MRP1, breast cancer resistance protein BCRP, and lung resistance protein LRP) (Hartinger et al., 2006). In phase I trial no dose-limiting toxicity has been observed (Dittrich et al., 2005). The lower occurrence of adverse side effects is probably due to the ability of $[Ru(HIn)_2Cl_4](H_2In)$ to rapidly bind transferrin in blood, maintain- ing its cytotoxic activity preferentially against tumour cells (Kratz et al., 1994). Recently, a conjugation of a Ru(III)-indazole complexes with PARP-1 was reported, suggesting its role in disrupting DNA repair machinery (Z. Wang et al., 2014b). Interestingly, some compounds derived from Ru(II) complexed with arene ligands and water-soluble phosphines, in particular 1,3,5-triaza-7-phospha-adamantane (PTA), show promising activity against dispersed cancer. $[Ru(\eta^6\text{-}p\text{-cym})(PTA)Cl_2]$ complex (RAPTA-C) (Fig. 12a) is capable of interaction with

ethylenediamine with enhanced DNA binding capability and increased extent of hydrolysis. The hydrolysis appears to be a fundamental step for the activation of the Ru(II) complex. The [Ru(h^6-*p*-cym)(acac)Cl] (*p*- cym = *para*-cymene) compound (Fig. 11b) can undergo the rapid hydrolysis leading to the loss of the chloride ligand. The resulting species is then able to bind aminoacids, guanine, and adenine (Fernández et al., 2004). In addition to Ru(II), Ru(III) complexes with indazole motifs as ligands also show interesting antitumor properties due to DNA interaction. One of the most studied Ru(III) complexes is the [Ru (HIn)$_2$Cl$_4$](H$_2$In) (HIn = indazole) (KP1019) (Fig. 11c), which proteins (Dorcier et al., 2005; Ang et al., 2011) *e.g.* cathepsin B, a cysteine peptidase responsible for degradation of extracellular matrix (ECM) and promoting metastasis (Casini et al., 2008). RAPTA-C complex also shows a pH-dependent selectivity between cancer and normal cells (Vock et al., 2008). The use of N-methyl-PTA (mPTA) as ligand in water-soluble ruthenium complexes containing chloride and cyclopentadiene (Cp) motifs was found to improve the interaction with super- coiled DNA (Romerosa et al., 2006). The [RuCpCl(mPTA)$_2$] (OSO$_2$CF$_3$)$_2$ (Fig. 12b), shows interesting behaviour during hydrolysis as documented by mass spectrometry (MS) and UV-Vis spectrophotometry (Peña-Méndez et al., 2009). It was found that this complex forms hydrated species and exchanges chloride ions or mPTA and Cp ligands with water, hydroxyl or counter ions following the hydrolysis scheme (Fig. 13), increasing the number of potential targetable molecules. To prevent uncontrolled hydrolysis of [RuCpCl (mPTA)$_2$]$^{2+}$, it has been suggested either to dissolve the complex in isotonic 0.15 M NaCl solution or to replace the OSO$_2$CF$_3$ ligand. Following this strategy, [RuCpCl(mPTA)$_2$](BF$_4$) compound has been synthesized but its biological activity has not been evaluated (González et al., 2009). The [Ru(2-phenyl-pyridine)(NCMe)$_2$phen]PF$_6$ (phen is 1,10-phenthroline) (RDC-11) (Fig. 14a) belongs to RDC family of organometallic Ru(II)
compounds, and presents an atypical mechanism of cytotoxic rheumatoid arthritis showed unexpectedly lower malignancy rates (Fries et al., 1985). Auranofin is a complex of Au(I) with a phosphine ligand that acts as thioredoxin reductase (TR) inhibitor. The TR is a class of seleno-cysteine enzymes catalyzing the reduction of thioredoxins, which are ubiquitous redox proteins containing a redox-active disulphide bond in the active site (Holmgren, 1989). Thioredoxins are involved in several biological processes, including ROS reduction (Nordberg and Arnér, 2001). Elevated concentrations of TR have beenfound in human tumour cells and they have been associated with tumour proliferation (Bruijnincx and Sadler, 2008). The inhibi- tion of TR induces mitochondria-dependent apoptosis (Gromer et al., 1998). In addition, TR inhibition is also probably responsible for the side-effects observed during the treatment with auranofin (Ott and Gust, 2007).activity (Fernandez et al., 1999). In fact, RDC-11 induces the apoptosis of tumour cells by activating the pro-apoptotic protein CHOP (CCAAT/Enhancer-Binding Protein Homologous Protein), which is a transcription factor involved in unfolded protein response, induced upon endoplasmic reticulum stress (Meng et al., 2009). Moreover, RDC-11 is able to arrest lymphoblastoma (RDM4, TK6) and glioblastoma (A172) cells in G1 phase of cell cycle. RDC-11 also induces the tumour suppressor p53 and p53-regulated effectors, the inhibitor of cell cycle p21 and pro-apoptotic Bax (Gaiddon et al., 2005).

An interesting example of ruthenium complex that shows cytotoxic activity due to interaction with non-classical targets is *trans*-[Ru(HIm)(DMSO)Cl$_4$](H$_2$Im) (Him =

imidazole; DMSO = - dimethylsulfoxide) labelled as NAMI-A (Fig. 14b). Surprisingly, despite the poor activity *in vitro* and no effect on tumour growth *in vivo*, it displays a significant anti-metastasis activity, *e.g.* in lung cancer. NAMI-A accumulates in kidneys, liver, and collagen-rich tissues (Sava et al., 2003). It interferes with the cell cycle by decreasing the percentage of cells in synthesis phase (S phase) (Gava et al., 2006). NAMI-A also decreases frequency of cells overexpressing receptors for ECM, such as CD44, CD54 and integrin beta 3. Moreover, it presents anti- angiogenic activity due to the inhibition of angiogenesis induced by the vascular endothelial growth factor (VEGF) (Ott and Gust, 2007). The mechanism of action of NAMI-A is therefore supposed to be based on the inhibition of some tumour invasion by the reduction of ECM degradation and prevention of migratory phenotype (Gava et al., 2006).

Promising pilot data therefore led to the introduction of both KP1019 and NAMI-A in clinical evaluation (Rademaker- Lakhai et al., 2004; Hartinger et al., 2006).

In summary, ruthenium compounds act in different way compared to cisplatin. In the majority of ruthenium com- plexes, ligand affinities, electron transfer, substitution rates, and reduction potentials may be easily tuned. Currently, novel ruthenium compounds are also designed and characterized, as ruthenium half-sandwich complexes comprising combined metal centrochirality and planar chirality and having promis- ing anticancer activity (Meggers et al., 2009; Streu et al., 2011; Martin et al., 2014).

Gold

The attention to gold complexes as antitumor drugs has been attracted when patients treated with auranofin (Fig. 15a) for rheumatoid arthritis showed unexpectedly lower malignancy rates (Fries et al., 1985). Auranofin is a complex of Au(I) with a phosphine ligand that acts as thioredoxin reductase (TR) inhibitor. The TR is a class of seleno-cysteine enzymes catalyzing the reduction of thioredoxins, which are ubiquitous redox proteins containing a redox-active disulphide bond in the active site (Holmgren, 1989). Thioredoxins are involved in several biological processes, including ROS reduction (Nordberg and Arnér, 2001). ElevatedconcentrationsofTRhavebeenfound in human tumour cells and they have been associated with tumour proliferation (Bruijnincx and Sadler, 2008). The inhibi tion of TR induces mitochondria-dependent apoptosis (Gromer et al., 1998). In addition, TR inhibition is also probably responsible for the side-effects observed during the treatment with auranofin (Ott and Gust, 2007).

Complexes of Au(III) have also been extensively studied because its chemistry is similar to that of Pt(II). In fact, Au(III) is isoelectronic with Pt(II) and forms complexes with square planar geometry. However, under physiological conditions Auis rapidly hydrolysed and reduced to Au(I) (Wang and Guo, 2008). Therefore, the stabilization of Au(III) complexes by using suitable ligands is needed. Chelating nitrogen donors such as phen, 2,2^{0}-bipyridine (bipy), 2,6-bis(2-pyridyl)-pyridine (terpy) and ethylenediamine (en) have been proposed for this purpose (Marcon et al., 2002). The Au(III) complexes with phen and bipy derivatives show cytotoxic activity comparable to that of their ligands. On the contrary, the cytotoxicity of $[Au(en)_2]Cl_3$ (Fig. 15b) was found to be due to the presence of the gold centre (Tiekink, 2002). The cytotoxicity of Au(III) complexes containing terpyridine derivatives as ligands (Fig. 15c) is mainly due to DNA

intercalation. Such complexes are also stable towards reduction by GSH and represent the first example of Au(III) complexes interacting with DNA (Wang and Guo, 2008).

The cytotoxic activity of Au(III) complexes with porphyrin ligands might be due to their action on mitochondria. As a consequence, apoptosis is induced by caspase-dependent and caspase-independent pathways (Wang et al., 2005). The leader compound [Au(TPP)]Cl (H_2TPP = tetraphenyl-porphyrin) (Fig. 15d) shows IC_{50} values on the *m*M order towards several cell lines, including human cervix epitheloid (HeLa) and hepatocellular (HepG2) carcinoma. It presents a similar activity also against cisplatin-resistant and MDR cell lines (Che et al., 2003). This compound shows tumour inhibition of about 80% (Sun et al., 2007) in cisplatin-resistant nasopharyngeal carcinomas (NPC) cells implanted into mice.

Several Au(III)-thiocarbamates complexes with general formula [Au(dtc)X_2] (X = Cl, Br; dtc = N,N-dimethyl-dithio- carbamate, ethyl-sarcosine-dithiocarbamate) (Fig. 15e) in- hibit the proteasome-dependent protein degradation both *in vitro* and *in vivo* (Milacic et al., 2006). Proteasome inhibitors are effective against tumour cells because they induce apoptosis by perturbing the regulated degradation of pro-growth cell cycle proteins (Orlowski, 1999). The Au (III)-thiocarbamate compounds are equally or more active than cisplatin against various human tumour cell lines and show low cross-resistance with cisplatin. These complexes show 50% reduction of tumour growth in human breast cancer MDA-MB-231 and no toxic side-effects *in vivo* (Milacic et al., 2006).

Recently, Au(III)-peptide-dithiocarbamate complexes with general formula [AuX_2(pdtc)] (X = Cl, Br; pdtc = oligopeptide- dithiocarbamate) (Fig. 15f) have been synthesized using di-, tri-
, tetra- and pentapeptides (Kouodom et al., 2012). The incorporation of peptides makes the complex recognizable by intracellular peptide transporters (PEPTs). Therefore, cellu- lar uptake is enhanced, and side-effects reduced. The most promising results have been obtained with the tripeptide derivatives, such as H-Sar-Aib$_2$-O(t-Bu) and H-D,L-Pro-Aib$_2$-O(t- Bu) (Sar = sarcosine (N-methylglycine)); Aib = *a*-aminoisobu- tyrric acid (2-methylalanine), which show IC_{50} values lower

than that of cisplatin *in vitro*. They also show no cross- resistance with cisplatin, confirming a different cytotoxic mechanism. The study of *in vivo* activity and mechanism started recently (Kouodom et al., 2012).

The variety of cytotoxic mechanisms reported for gold complexes (Fig. 16) expands the possibility to find out new compounds able to overcome platinum-drug resistance. Nevertheless, the application of gold complexes in clinical practice still requires an extensive evaluation of their chemical and pharmaceutical properties such as hydrolysis equilibria, cellular uptake, biodistribution, and pharmacokinetics.

Silver

Silver complexes show therapeutic potential in treating bacterial infections but detailed reports on the antitumor activity of silver (I) are scarce. Recently, the anti-proliferative activity of silver (I) complexes containing various type of ligands such as carboxylic acids, amino acids, nitrogen, phosphorus or sulphur donor ligands, was reviewed (Banti

and Hadjikakou, 2013). In this review comparison with the corresponding activity of cisplatin is given, and it was found that under specific conditions silver(I) complexes are more active than cisplatin *in vitro*.

Interestingly, it was reported that complexes of copper, silver, and gold show enhanced selectivity for breast cancer while causing fewer and weaker adverse side-effects (Biersack et al., 2012) when compared to platinum based drugs, being promising new candidates (Tan et al., 2010, 2014; Kyros et al., 2014).

Copper

Copper is an essential metal ion present as cofactor in many enzymes. It is involved in haemoglobin formation, xenobiotics and carbohydrates metabolism, catecholamine biosynthesis, cross-linking of collagen, elastin, and hair keratin. Copper ion is also engaged in antioxidant defence mechanism. In fact, copper-dependent enzymes, such as cytochrome C oxidase, superoxide dismutase, ferroxidases, monoamine oxidase, and dopamine *b*-monooxygenase, are involved in neutralizing ROS or molecular oxygen. Importantly, efflux of cisplatin outside the cells employs specific copper-efflux transporters ATP7A and ATP7B, next to the multidrug efflux pumps belonging to the ABC superfamily (P-glycoprotein (Pgp, ABCB1) and multi- drug resistance protein 2 (MRP2, ABCC2)) (Samimi et al., 2004; Leslie et al., 2005).

Copper has been selected for the synthesis of antitumor drugs under the hypothesis that complexes with endogenous metal ions might give lower systemic toxicity. Several Cu(II) complexes with a variety of N-, S-, or O-containing ligands have been designed, synthesized and tested as antitumor drugs. The different biological actions of these complexes, in comparison to that of cisplatin, suggest that different mechanisms for their antitumor activity are involved (Fig. 17). However, such mechanisms are not completely clarified to date (Santini et al., 2013).

Copper(II) is also an angiogenesis promoter because it enhances the formation of new blood vessels from pre- existing ones. In cancer cells, this physiological process may eventually trigger the transition from a benign to malignant state of tumours. Further studies are required to completely clarify the role of copper in angiogenesis processes.

Copper(II) complexes are supposed to act by triggering cell apoptosis or inhibiting enzymes (Tripathi et al., 2007). In fact, the expression of tyrosine-protein kinase CSK is inhibited by complexes of Cu(II) with pyridine-2-carbohidrazide deriva- tives. Copper(II) chelate of salicylaldoxime induces cell cycle arrest and apoptosis, perhaps involving the inhibition of the topoisomerase II enzyme (Jayaraju and Kondapi, 2001). Topoisomerases (Topo) are ubiquitous enzymes able to break and reseal the DNA polyphosphate backbone, preventing the overwinding or underwinding of the double helix. The disruption of Topo activity leads to single and double stranded breaks in DNA, inducing apoptosis. Among Topo-I inhibitors, the [Cu(phen)L](NO3)2 (L = 2,4,6 tri-(1H-pyrazol-1-yl)-pyrimi- dine) is a Cu(II) complex containing a ligand derived from barbituric acid and pyrazole (Tabassum et al., 2014). This complex inhibits Topo-I at 10 *m*M concentration. Another family of Topo-I inhibitors with general formula [Cu(N)L]Cl (N = phen, bipy or 5,5′-dimethyl-2,2′-bipyridine; L = doubly

deprotonated 5-(triphenyl-phosphonium-methyl)-salicylalde- hyde-benzoyl-hydrazone) show a good cytotoxic activity against human lung carcinoma (A549) and prostate adenocar- cinoma (PC-3) cell lines (Chew et al., 2014). The most active compound of this family is the one containing phen motif. It shows an IC_{50} value of 3.2 *m*M against PC-3 cell line and starts to inhibit Topo I at 40 *m*M. A metalloprotease activity (Shrivastava et al., 2002) is evidenced by the complex 2,6-bis- (benzimidazo-2-yl)-pyridine copper(II) chloride. Proteasome inhibition resulting in apoptosis was reported when Cu(II) binary complexes containing neutral or anionic molecules such as phen, 8-hydroxyquinolinate, pyrrolidine dithiocarbamate, or (pyridine-2-ylmethylamino)-methyl phenolate are used. Evaluation of proteasome inhibition shows that both the complexes and the copper ion inhibit the enzyme in the same extent while the free ligands have no activity. Then, the complex behaves as carrier of the metal ion through the cell membrane. This is achieved by tuning the lipophilicity of the complexes by suitable ligands (Hindo et al., 2009).

Copper complexes with thiosemicarbazone ligands, which possess antitumor activity and are used in clinical practice, inhibit enzymatic activity and induce cell apoptosis (Tisato et al., 2010). The cytotoxic properties of copper complexes with phen ligand have been firstly reported by Sigman (Sigman et al., 1979). The complex with two phen ligands is able to cleave DNA by binding to the deoxyribose units and thus acting as a chemical nuclease. It has been tested against a great number of cancer cell lines, both solid and hematologic (Cai et al., 2007; Pivetta et al., 2012). Consequently, many other copper complexes with phen, phen-derivatives or structurally related compounds such as bipy have been studied. Modula- tion of the cytotoxic activity of $[Cu(phen)_2]^{2+}$ species with insertion of substituted imidazolidine-2-thione ligands (Fig. 18a) has been evaluated against acute T-lymphoblastic leukaemia (CCRF-CEM), acute B-lymphoblastic leukaemia (CCRF-SB), lung squamous carcinoma (K-MES-1), and prostate carcinoma (DU-145). Correlation between the dipole moment of the complexes and the cytotoxic activity has been found (Pivetta et al., 2011). Complexes with high dipole moment result more active against haematological tumour cell lines, while less polar complexes show higher activity against solid tumour ones. The use of serinol bridge (called Clip) in position 2 or 3 to link two phenanthroline (phen) units has led to the preparation of a new class of compounds (Pitie et al., 1998). The copper(II) complexes obtained by reaction with 2- and 3-Clip- phen show a 2- to 60-fold increased ability to cleave DNA in comparison to phen complexes (Pitie et al., 1998). Further studies have shown that the optimal length of the bridge to achieve optimal DNA cleavage activity corresponds to three methylene units (Pitié et al., 2003). Compounds with a functionalized serinol bridge have also been prepared by using a conjugate of 3-Clip-phen (Fig. 18b) with a cisplatin derivative (De Hoog et al., 2007). A class of Cu(II) complexes with general formula $[Cu(N\text{-}N)(A\text{-}A)]NO_3$, where N-N is phen or bipy and A-A is either a nitrogen–oxygen or oxygen–oxygen donor ligand is capable of inducing autophagy and pro- grammed cell death of glioma cells by ROS and JNK activation (Trejo-Solís et al., 2012) or caspase activity in colon cancer cells. They also exhibit high DNA binding and nuclease activity towards plasmid, genomic, and internucleosomal DNA (Marín Hernández et al., 2003). Other complexes of Cu(II) with Schiff bases and 2-

amino-2-thiazoline show interesting anti-inflam- matory, antibacterial, and anticancer activity against various cell lines (Chaviara et al., 2005). The development and use of Cu (I) complexes as antitumor agents is limited by their low stability and their tendency to be easily oxidized. Some complexes, such as [Cu(N,N′-disubstituted thioureas)Cl] and $[Cu(1,3,5\text{-triaza-7-phosphaadamantane})_4]^+$, exhibit moderate cytotoxicity against various human cell lines (Porchia et al., 2009). In analogy with gold, Cu(I)-phosphine complexes have been synthesized and evaluated as antitumor compounds (Marzano et al., 2006; Plotek et al., 2013; Porchia et al., 2013; Santini et al., 2013). Some mixed Cu(I) complexes of triazo- lylborate and alkyl- or aryl-phosphines have been found to be effective against A549 adenocarcinoma cisplatin resistant cells (Marzano et al., 2006).

Iron

Iron is involved especially in oxygen transport, DNA synthesis, oxidative phosphorylation, and cell cycle progression, and all of them have a role in carcinogenesis (Boult et al., 2008; Brookes et al., 2008; Coombs et al., 2012). The potential cytotoxic activity of iron complexes appears to be related to the redox reactions occurring between Fe(II) and Fe(III) in physiological conditions (Jungwirth et al., 2011). A promising compound is a complex between iron and bleomycin, a molecule already used for the treatment of testicular carcino- ma. The biological activity of this complex arises from the ROS production that leads to the apoptosis of the cancer cells (Chen and Stubbe, 2005). Also complexes with ferrocene (ferroci- fenes) show anticancer activity, with a selectivity that depends on the present substituents. Several hydroxy-substituted ferrocifens present high affinity with oestrogen receptor and are used for the treatment of breast cancer (Lange et al., 2008; Rafique et al., 2010). Interestingly, the ferrocene alone does not show anticancer activity.

Iron deprivation by selective chelators is a promising strategy in reduction of tumour growth. Treatment with iron chelator has been then proposed in cancer therapy by using iron selective chelators such as triapine and desferrioxamine (DFO) (Kalinowski and Richardson, 2005). It has been suggested that the antitumor activity exhibited by these molecules may be due to the inhibition of ribonucleotide reductase by bonding the iron(II,III) ion. In fact, the enzyme requires iron and oxygen for its biological activity that is elevated in tumour tissues (Richardson, 2002). The thiose- micarbazone triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) is able to overpass the blood-brain barrier showing a potent activity against leukaemia brain metastasis. It is also able to inhibit the growth of mouse M109 lung and human A2780 ovarian cancer cells *in vivo* (Finch et al., 2000). Desferrioxamine (DFO) is a hexadentate side-rophore currently in use for *b*-thalassemia treatment because of its great affinity for iron (Richardson, 2002). Clinical trials have confirmed that some cancers, as neuroblastoma and leukaemia, are sensitive to DFO therapy while normal tissues are affected only in little extent (Desoize, 2004). Nowadays, iron depletion has been proposed for the treatments of several kinds of cancers and metastatic progression (Kovacevic, 2012; Keeler and Brookes, 2013; Richardson et al., 2013; Torti and Torti,

2013). A triapine derivative, di-2-pyridylketone-4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC) is currently under preclinical evaluation (Lane et al., 2014). From several clinical evidences, it seems that iron deprivation could be an effective therapeutic approach. However, adverse effects, such as anaemia, induced by the iron-chelating agents, can develop (Beguin et al., 2014). However, more studies *in vitro* and *in vivo* should be done, to clarify the action mechanisms and in particular their extra- and intracellular effects. This kind of studies will help in the design and synthesis of more potent iron chelators.

Cobalt

The Co–ASS (Fig. 19) is a cobalt(II) complex containing an acetylsalicylic acid (ASA) derivative which inhibits the cyclooxygenase enzymes COX-1 and COX-2. Recently, it was found that the regular use of aspirin reduces cancer incidence (Algra and Rothwell, 2012). The COX inhibition induced by Co– ASS complex is more efficient than that induced by ASA alone and this probably determines the resulting higher cytotoxic properties, in particular against breast cancer cell lines (Ott et al., 2004; Bruijnincx and Sadler, 2008). The use of COX inhibitors is suggested in combinational therapies with other antitumor drugs (Ott and Gust, 2007). Cobalt(III) complexes have been developed as prodrugs which exploit the hypoxic environment in tumours to release highly cytotoxic ligands. In fact, in hypoxic environment Co(III) complexes can be reduced to Co(II) with subsequent release of one neutral ligand. This strategy has been widely exploited for the release of compounds such as DNA alkylators and a matrix metalloproteinase inhibitor (Ahn et al., 2006; Failes et al., 2007; Lu et al., 2011).

Gallium

Gallium is a metalloid that has been used in different medical applications, such as the treatment of accelerated bone resorption, autoimmune and infective diseases (Gârban et al., 2014). Using the isotope ^{67}Ga for imaging in rodents, it has been reported that gallium accumulates in tumour tissues.

This observation led to the introduction of gallium com- pounds in anticancer therapy. The biological activity of gallium derives from its chemical similarity with iron. In fact, Ga(III) and Fe(III) have similar ionic radius, ionization potential, and electron affinities (Chitambar, 2012). These characteristics allow gallium to bind transferrin. Because some cancers, as lymphoma (Gatter et al., 1983), bladder (Smith et al., 1990) and breast (Ding Cheng Yang et al., 2001) cancers, overexpress transferrin receptors, gallium is selec- tively delivered to tumour cells *via* iron transporters similarly to ruthenium complexes (Chitambar and Antholine, 2013). The gallium uptake perturbs iron homeostasis, reducing the intracellular iron concentration. In addition, the activity of the enzyme ribonucleotide reductase is blocked by iron- substitution in the active site by gallium and by gallium- induced iron deprivation. This results in the inhibition of DNA synthesis and cell proliferation. An interaction with mito- chondria has been also recently proposed as a possible cytotoxic mechanism (Chitambar et al.,

2007).

The first gallium compound that has been investigated in phases I and II trials as anticancer agent was the gallium nitrate. Some antitumor activity in advanced bladder cancer and non-Hodgkin's lymphoma was demonstrated (Chitam- bar, 2012). Since it does not show myelosuppressive activity, it can be safely administered in patient with pancytopenia. Despite the good activity shown by gallium nitrate, its administration is dependent on long intravenous infusions and pre- and post-hydratation required to prevent nephro- toxicity (Chitambar, 2012).

Gallium chloride has been also tested, showing an *in vitro* cytotoxicity similar to that of gallium nitrate. A modified formulation of gallium nitrate suitable for oral administration, G4544, is currently in phase I clinical trial. G4544 ensures an increased level of gallium in blood *in vivo* and a longer circulation time, when compared with gallium nitrate. In humans, the gallium levels in blood are similar to those obtained with the continuous intravenous infusion of gallium nitrate and no adverse side-effects are observed (Novick et al., 2008). Also gallium maltolate (tris(3-hydroxy-2-methyl-4H- pyran-4-onato)gallium(III)) (Fig. 20a) has been studied as anticancer agent. This complex is degraded after the adminis- tration and the free gallium can bind transferrin in blood (Bernstein et al., 2011). Gallium maltolate has been tested onphase I trials, and it is effective in advanced hepatocellular carcinoma (Bernstein et al., 2011). Gallium maltolate shows activity also in gallium nitrate-resistant lymphoma cell lines (Chitambar and Purpi, 2010). This lack of cross-resistance allows to expand the action spectrum range of gallium compounds. To enhance their antitumor activity and bio-availability, complexes of gallium(III) stable in physiological conditions were investigated (Tan et al., 2014). An interesting example of a gallium compound developed for oral adminis- tration is the tris(8-quinolinolato)gallium(III), also known as KP46 (Fig. 20b). Interestingly, this complex is not destroyed in the blood (Enyedy et al., 2012, 2015; Hummer et al., 2012). It shows *in vitro* a cytotoxic activity higher than that of gallium nitrate (Lessa et al., 2012). The cytotoxicity mechanism involves p53 activation mediated by Ca^{2+}-signalling and ROS production (Gogna et al., 2012; Madan et al., 2013). Recently, new insights onto the mechanism of action have been highlighted (Jungwirth et al., 2014). KP46 has been tested in various phase II clinical trials and no dose-limiting toxicity was found. In particular, the results in renal cancer treatment are promising, improving the remission rate (Timerbaev, 2009). Gallium complexes with thiosemicarbazone ligands are widely studied (Ismail et al., 2013; Lessa et al., 2013). In these compounds, the cytotoxic activity of the thiosemicar-bazone ligands is enhanced of several folds by the formation of the metal complex (Mendes et al., 2009). A family of gallium complexes with pyridine and derivatives of phenolate as ligands shows cytotoxic activity greater than that of cisplatin (Shakya et al., 2006). The biological action of these complexes is due to the inhibition of proteasome with activation of apoptosis (Chen et al., 2007). Recently, the antitumor activity of phosphinoarylbisthiolato gallium(III) complexes was reported (Fischer-Fodor et al., 2014). They bind DNA in 7-methylguanine and 8-oxoguanine positions, oxidizing the pyrimidine bases and inducing apoptosis. These complexes show *in vitro* antitumor activity against the cisplatin-resistant ovarian tumour cell line A2780cis

(Fischer-Fodor et al., 2014). Com- plexes with N,N,O donors have been also tested against ovarian, breast, and prostate adenocarcinoma cell lines. Against these tumour cell lines, the complex bis(2-(benzothia- zol-2-yl-hydrazonomethyl)-6-methoxyphenolate) gallium(III) nitrate shows a good cytotoxic activity, with CC_{50} on the micromolar range (Machado et al., 2014). In order to combine chemotherapy with photodynamic therapy, a gallium(III) complex with the photosensitizer hypocrellin A has been recently proposed (Xie et al., 2014). This complex induces i*n vitro* nuclear morphological changes in tumour cells after light irradiation. Up-to-now, the research of gallium complexes for anticancer treatment has led to the clinical evaluation of the two candidate compounds KP46 and gallium maltolate. They both seem to possess interesting activity in renal malignan- cies. The search for new compounds extending the activity of gallium complexes to other types of cancer is ongoing, especially in context of chemoresistance developed in some cancers. For example, lung cancers seem to be resistant to gallium compounds because of over-expression of tyrosine-protein kinase receptors (Oyewumi et al., 2014). Starting from the promising results of gallium compounds undergoing clinical trials, the development of new gallium compounds, as well as the study of their mechanism of activity, seems to be one of the key topics of anticancer research in the next years (Mikuš et al., 2014).

Rhodium

Many complexes of rhodium have been synthesized but most of them show severe nephrotoxicity and thus no further studies were done (Katsaros and Anagnostopoulou, 2002). Recently, it has been suggested that rhodium(III) complexes that are inert towards substitution may show low systemic toxicity (Geldmacher et al., 2012). Some interesting rhodium complexes are $[Rh(2\text{-}(2'\text{-hydroxy-}5'\text{-methylphenyl})\text{-benzotriazole})_2(H_2O)_2]Cl$ that shows promising activity against human breast cancer (MDA-MB231) and human ovarian cancer (OVCAR-8) cell lines (El-Asmy et al., 2014) and also a series of rhodium(I)-N-heterocyclic carbene complexes with CO as secondary ligand, which shows marked antiproliferative effects together with moderate inhibitory activity of thior- edoxin reductase and efficient binding to biomolecules (*e.g.*, DNA, albumin). With the use of these complexes, modifica- tions in the mitochondrial membrane potential and DNA fragmentation were observed in wild-type and daunorubicin- or vincristine-resistant Nalm-6 leukaemia cell lines (Oehnin- ger et al., 2013).

Despite the encouraging results, in order to define their activity spectrum, selectivity and systemic toxicity, *in vivo* studies need to be performed (Zhong et al., 2014).

Titanium

The $cis\text{-}[Ti(CH_3CH_2O)_2(bzac)_2]$ (bzac = 1-phenylbutane-1,3-dio- nato) complex was the first non-platinum compound tested in clinical trials but surprisingly, it has no antitumor activity. Afterwards, complexes with arene ligands have been tested. The titanocene dichloride (Fig. 21) binds DNA *via* the phosphate backbone, inducing apoptosis (Meléndez, 2002). It shows *in vitro* cytotoxic activity against a broad spectrum of cancers, in particular human stomach and colon adenocarci- nomas, and

has been tested in phases I and II trials. Its development has been abandoned because it shows, as the others Ti(IV) complexes, nephrotoxicity as dose-limiting toxicity and poor antitumor activity, probably due to low water solubility and high deactivation by plasma proteins (Ott and Gust, 2007).

Recently, coordination compounds of Ti(IV) with isoprop- oxide supported by pyrrolyl Schiff base ligands were synthe- sized and tested against human colon (HCT-116), prostate (PC3) and breast (MCF-7) cancer cell lines showing cytotoxic activity either lower or higher than cisplatin (Lin et al., 2014).

The reasons for the different behaviour observed are still not clear. Perhaps high hydrolytic resistant ligands strongly bound to the metal centre and are probably responsible for the high cytotoxicity observed for some complexes.

Titanium(IV) complexes with ortho-bromo-para-methyl- substituted diaminobis(phenolato) ligands were prepared with NH-, NMe-, and bipyrrolidine-based diamino bridges. The hydrolytic stability of such compounds as well as their cytotoxic activity in human colon cancer cells (HT-29) was investigated (Miller and Tshuva, 2014). The NMe-based complexes, although highly hydrolytically stable, were found inactive while, the NH- and bypirrolidine-based compounds were found active. Interestingly, the highest cytotoxic activity of such compounds was observed when pure enantiomers were used in place of racemic mixtures (Miller and Tshuva, 2014).

Arsenic

Arsenic trioxide (As_2O_3) represents an important metalloid compound for cancer treatment. It has been used in leukaemia treatment with increased rate of survival (Wang and von Recum, 2011). It has been approved by FDA under the name of trisenox and it is the most active single agent for acute promyelocytic leukaemia treatment (Barry and Sadler, 2013). However, the antitumor mechanism is still unclear and cardiotoxicity has been reported as side-effect (Desoize, 2004). The compound darinaparsin (S-dimethylarsino-gluta- thione) (Fig. 22) has been approved for clinical therapy in the treatment of peripheral T-cell lymphoma and other arsenic compounds are currently under clinical trials (Barry and Sadler, 2013).

Antimony

Antimony in form of potassium antimonyl tartrate (PAT) shows antibacterial activity and antiangiogenic effect in non- small cell lung cancer *in vitro*, in an extent comparable to doxorubicin or cisplatin (Duffin and Campling, 2002). Sodium stibogluconate (SSG) is used as drug to treat leishmaniasis but recently, its synergistic effect with IFN-*a* to overcome IFN-*a* resistance in various human cancer cell lines was demon- strated (Yi et al., 2002). Complexes of antimony(V) shows 10- fold lower toxicity than the corresponding antimony(III) ones (Asghar et al., 2012). Furthermore, ferrocenyl benzoate- antimony complexes have been shown to be promising chemotherapeutic compounds (Liu et al., 2003; Li et al., 2004; Yu et al., 2004) but their mechanism of action is not yet known. Studies attempting to investigate

interaction of ferrocenyl benzoate-antimony complexes with DNA and other biomolecules are currently under investigation.

Final remarks

From all the results presented in literature in the field of various anticancer metal complexes so far, it appears neces- sary to choose a more constructive and rational approach. The continued study to optimize the design and potency of a particular metal complex used as anticancer agent, sometimes has led to undesirable results. Therefore, to overcome these obstacles, the effective design of metal complexes with potential antitumor properties should now focus not sepa- rately on achieving the high cytotoxic activity or interactions with the biomolecules, but rather on the simultaneous evaluation of all the involved parameters. In particular, it must be clearly understood that the proper strategy for the possible use of the metal complex, the selection of the target and interactions of the drug with biomolecules should involve highly interdisciplinary approach.

The proper strategy

Two different strategies can be exploited for the use of a metal complex, and it is fundamental to choose in advance the strategy most suitable to the needs. A metal complex, in fact, might be used as active agent if it presents cytotoxic activity, or as a carrier of organic ligands that present independent biological activity, or as a carrier for metal ions that present some other biological activity.

The selection of the target

The optimal design of a metal complex and its potential target should be considered and estimated before its synthesis. In this way, a molecule with features suitable to specifically interact with the chosen target may be prepared, and the antitumor activity might be exerted according to the expected mechanism. The traditional approaches in this field are still founded mainly on the interaction of the metal complex with DNA. Unfortunately, the continuous research for compounds able to form more stable DNA-adducts, by intercalation, groove binding or electrostatic interactions, does not always lead to a better drug. Instead, other types of targets may play a key role and should be seriously considered. In fact, the antitumor activity can be obtained also through reactions with proteome, disruption of mitochondrial processes or through inhibition of angiogenesis or metastatic routes.

Drug and biomolecules

In parallel with the choice of the proper strategy and the required target, the interaction of the drug with the biomo- lecules naturally present in the body should be estimated or at least hypothesized. In fact, the resulting biochemical reactions may eventually trigger the onset of side effects. Moreover, the metal complex, due to its structural

characteristics, might be susceptible to mechanisms that can cause the drug resistance, deactivation for transformation or loss of the functional groups, for hydrolysis or redox reactions.

In the last three years (2012–2014) the drugs that have obtained FDA approval for oncology are primarily monoclonal antibody and kinase inhibitors. This fact shows that the development of new metal complexes has come to a break since the approval of oxaliplatin in 2002. However, rational development of coordination compounds acting in specific cellular and tissue contexts in patient-tailored therapy has an immense potential in oncology.

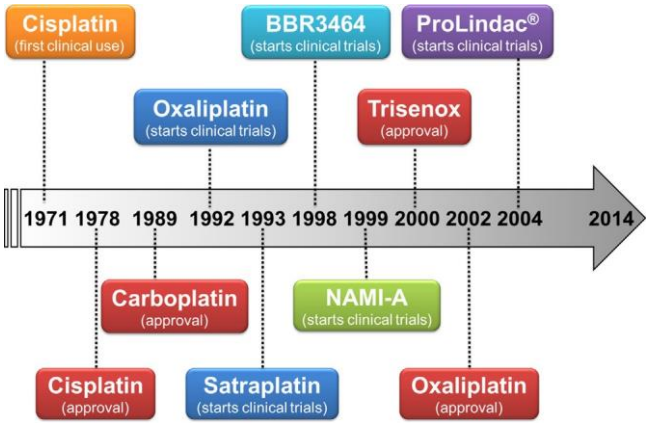

Fig. 1 – Historical overview of the cytotoxic metal and metalloid complexes that have been approved or entered the clinical practice.

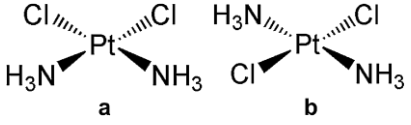

Fig. 2 – Structures of the *cis* (a) and *trans* (b) isomers of diamminedichloroplatinum(II), the cisplatin and transplatin, respectively.

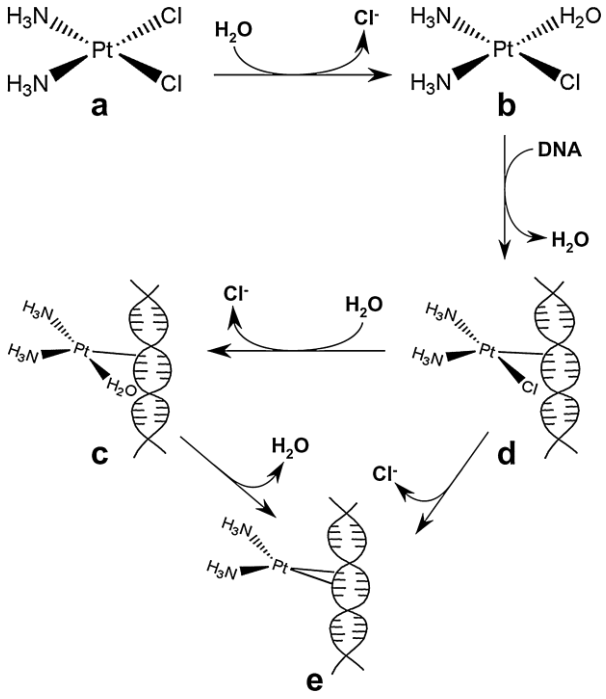

Fig. 3 – Scheme of the reaction pathway leading to the formation of adducts between cisplatin (a) and DNA. One chloride ligand is displaced by water to form the aqua- complex $[PtCl(H_2O)(NH_3)_2]^+$ (b) which interacts with DNA forming the monofunctional adduct $[PtCl(DNA)(NH_3)_2]^+$ (c). This last might exchange the chloride ligand with one molecule of water forming the hydrated monofunctional adduct $[Pt(H_2O)(DNA)(NH_3)_2]^{2+}$ (d). Both the monofunctional adduct (c) and its hydrated form (d) lead to the formation of the bifunctional adduct $[Pt(H_2O)(DNA)(NH_3)_2]^{2+}$ (e).

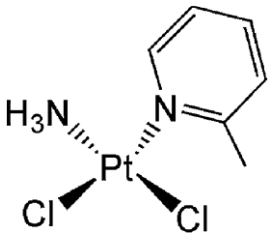

Fig. 4 – Structure of picoplatin (*cis*-amine-dichloro-(2- methylpyridine)-Pt(II)).

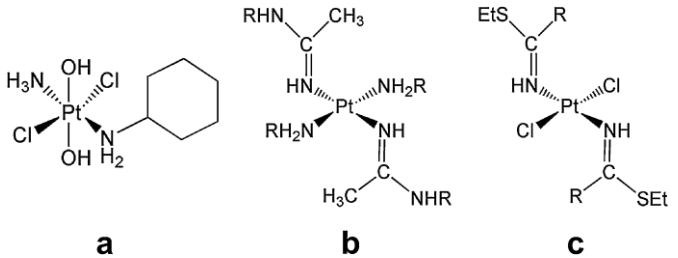

Fig. 5 – Structures of *trans*-ammine-(cyclohexylamine)- dichloro-dihydroxo-Pt(IV) (JM335) (a), *trans*-[Pt (amine)$_2$(amidine)$_2$]Cl_2 complex (b) and *trans*-[Pt{N(H) = C (SEt)R}$_2$$Cl_2$] (R = Me, Et, Ph, CH_2Ph) complexes (c).

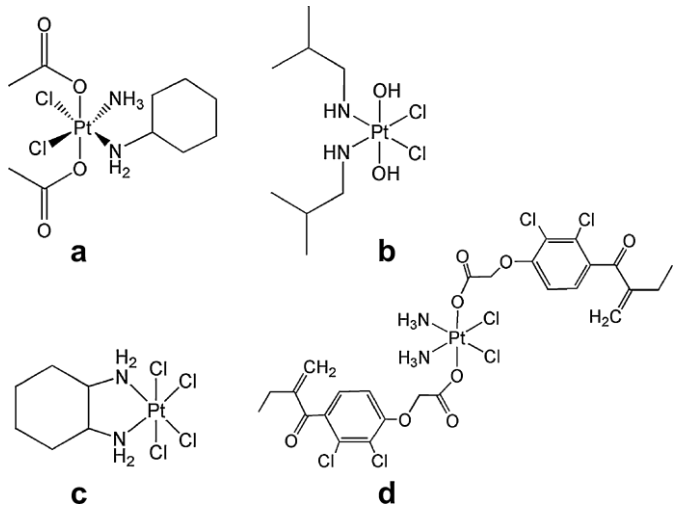

Fig. 6 – Structures of satraplatin (a), tetraplatin (or ormaplatin) (b), iproplatin (c) and Pt(IV) complex containing ethacrynic acid as ligand (d).

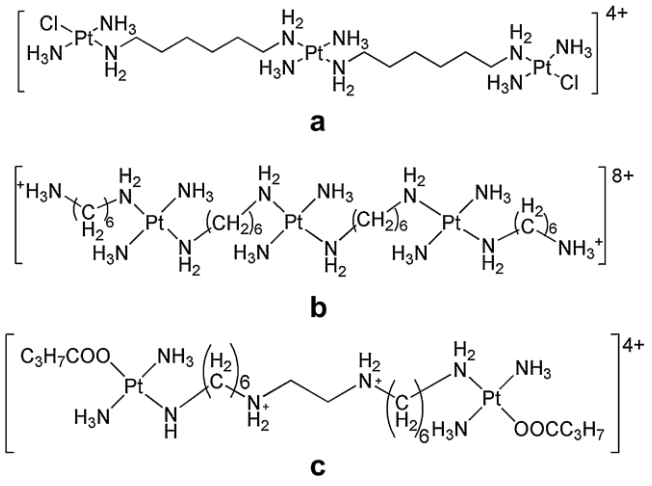

Fig. 7 – Structures of BBR3464 (a), [{trans-Pt $(NH_3)_2(NH_2(CH_2)_6(NH_3$))}$_2$-m-{trans-Pt $(NH_3)_2(NH_2(CH_2)_6NH_2)_2$}]$^{8+}$ (b) and CT-47463 (c).

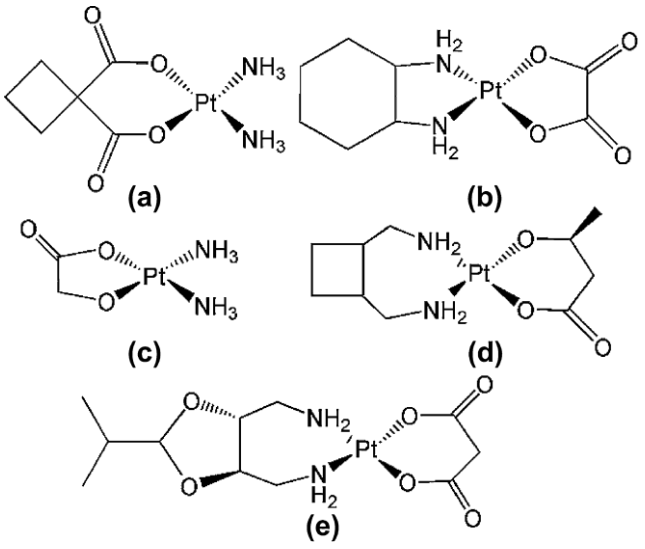

Fig. 8 – Overview of platinum-based drugs used in clinical practice: carboplatin (a), oxaliplatin (b), nedaplatin (c), lobaplatin (d) and heptaplatin (e).

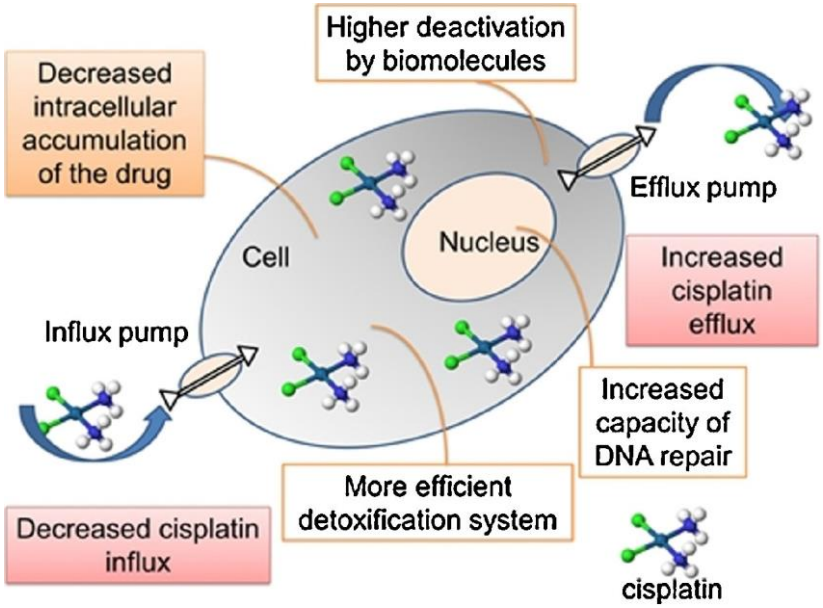

Fig. 9 – Mechanism of cisplatin resistance in cancer cells involves the reduced cisplatin influx and therefore a decreased intracellular accumulation of the drug, a higher enzymatic deactivation, an increased capacity of DNA repair, and an increased cisplatin efflux.

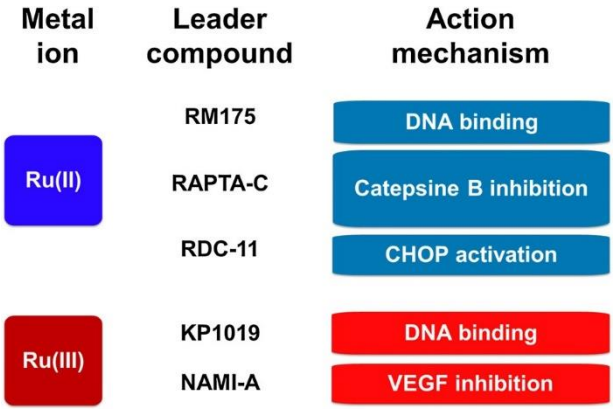

Fig. 10 – Overview of Ru(II) and Ru(III) leading compounds and their main action mechanisms. The acronyms CHOP and VEGF refer to CCAAT/enhancer-binding protein homologous protein and vascular endothelial growth factor, respectively.

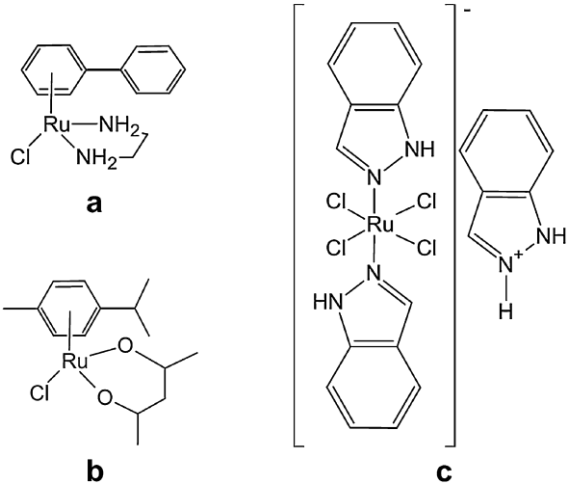

Fig. 11 – Structures of main Ru(II) and Ru(III) complexes: RM175 (a), [Ru(h^6-*p*-cym)(acac)Cl] (*p*-cym = *para*-cymene) (b) and KP1019 (c).

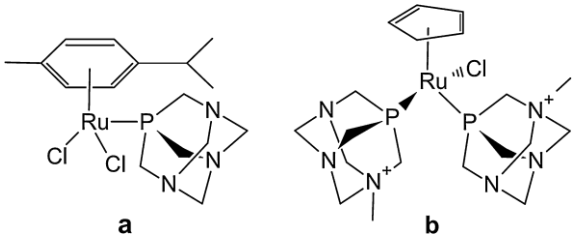

Fig. 12 – Structures of the Ru(II) complexes active against tumour RAPTA-C (a) and $[RuCpCl(mPTA)_2](OSO_2CF_3)_2$ (b).

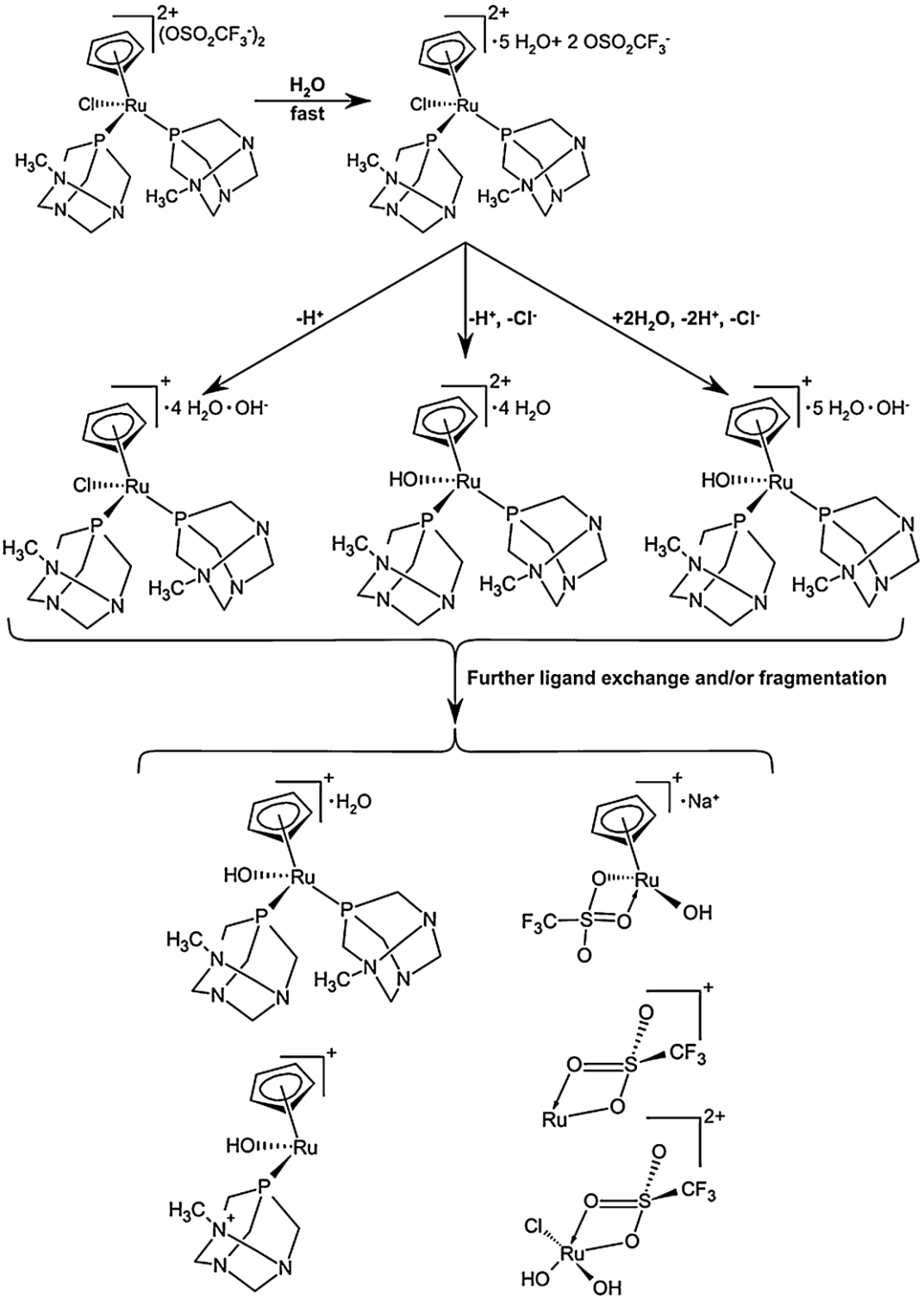

Fig. 13 – Scheme of $[RuCpCl(mPTA)_2](OSO_2CF_3)_2$ hydrolysis as determined by mass spectrometric and spectrophotometric investigation.

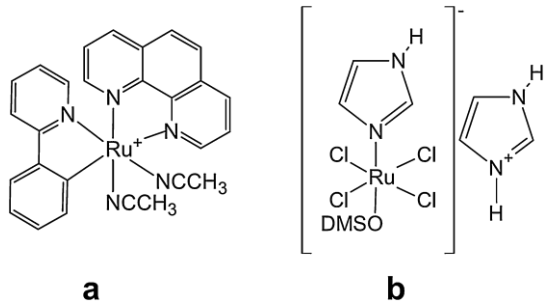

Fig. 14 – Structures of the Ru(II) complexes RDC-11 (a) and NAMI-A (b).

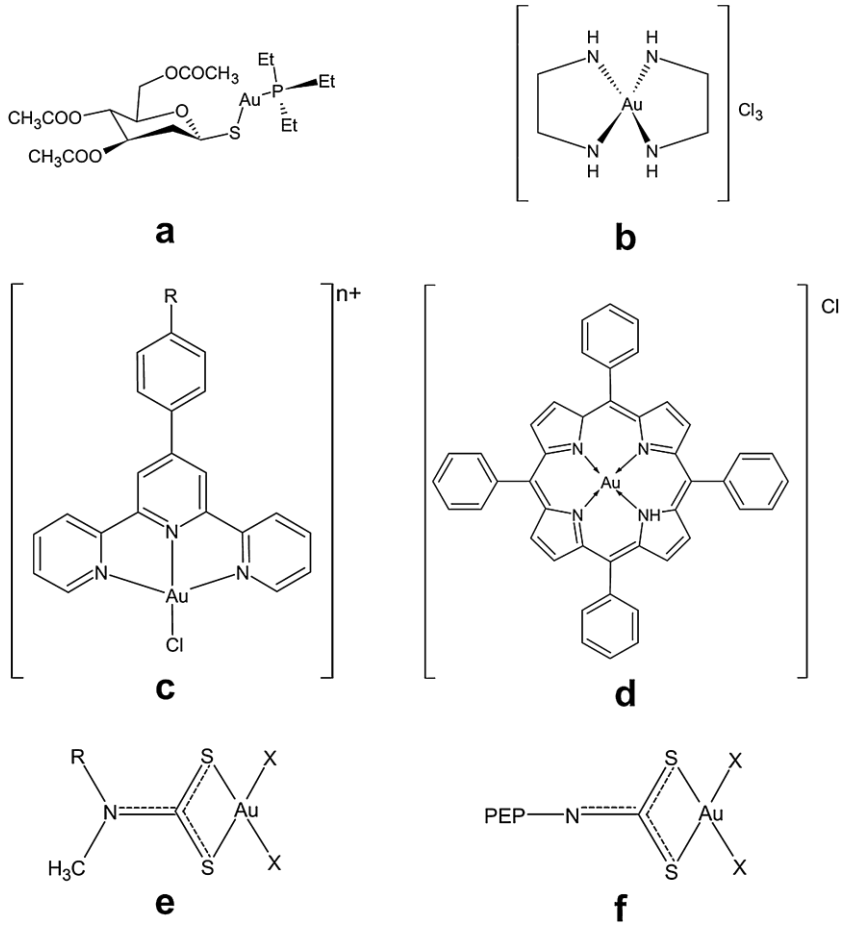

Fig. 15 – Structures of selected antitumor gold complexes: auranofin (a), $[Au(en)_2]Cl_3$ (b), Au(III) complex with substituted terpyridine ligand (c), [Au(TPP)]Cl (H_2TPP = tetraphenyl-porphyrin) (d), $[Au(dtc)X_2]$ (X = Cl, Br; dtc = N,N-dimethyl- dithiocarbamate, ethyl-sarcosine-dithiocarbamate) (e) and $[AuX_2(pdtc)]$ (X = Cl, Br; pdtc = oligopeptide-dithiocarbamate, PEP- N is a di-, tri-, tetra- or penta-peptide) (f).

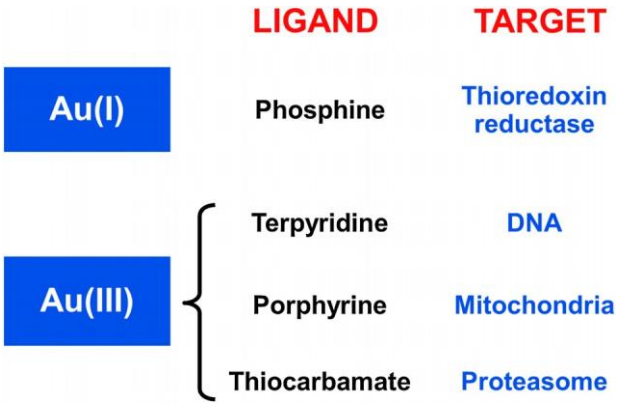

Fig. 16 –Overview of main ligand families used in Au(I) and Au(III) antitumor complexes and principal biological targets.

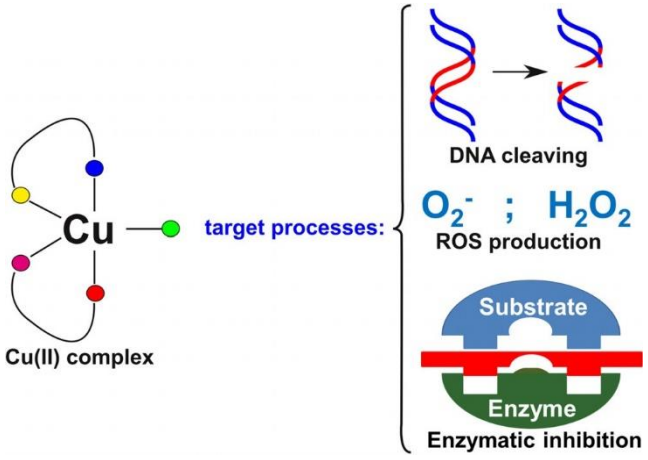

Fig. 17 – Biological target processes of Cu(II) complexes with antitumor activity.

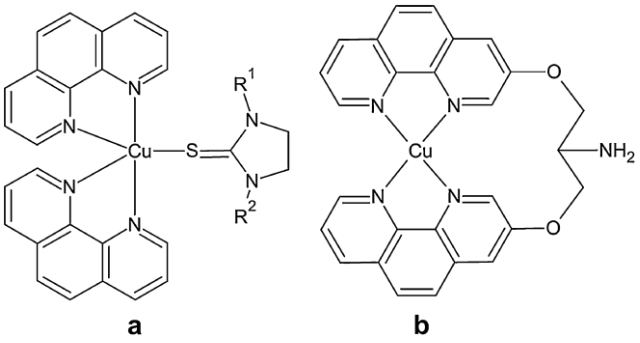

Fig. 18 – Structures of some Cu(II)-phen complexes: $[Cu\ (phen)_2(imidazolidine\text{-}2\text{-}thione)]^{2+}$ (R^1, R^2 = H, Me or Et) (a) and $[Cu(phen)_2]^{2+}$ with serinol bridge. Charges are omitted.

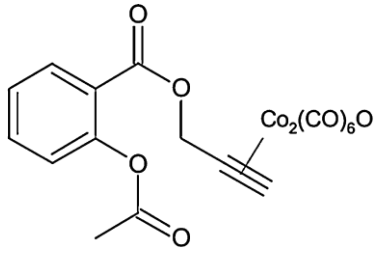

Fig. 19 – The Co–ASS cobalt(II) complex containing an acetylsalicylic acid (ASA) derivative.

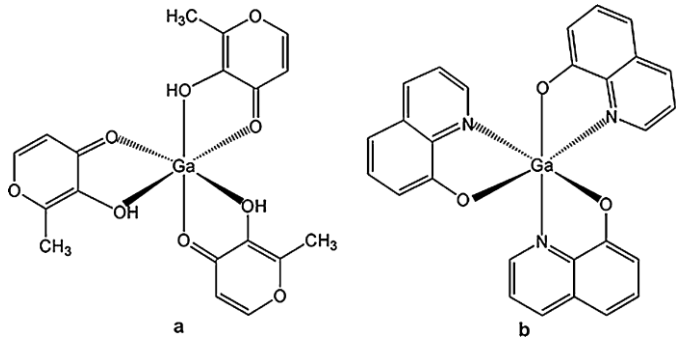

Fig. 20 – Structure of gallium maltolate (tris(3-hydroxy-2- methyl-4H-pyran-4-onato)gallium(III)) (a) and KP46 (tris(8-quinolonato)gallium(III)) (b).

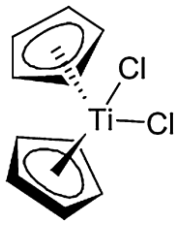

Fig. 21 – Structure of the titanocene dichloride.

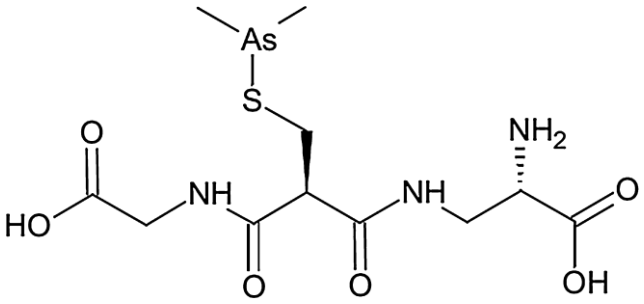

Fig. 22 – Structure of darinaparsin (S-dimethylarsino- glutathione).

Acknowledgements

Roukos, D., Batsis, C., Baltogiannis, G., 2012. Assessing tumor heterogeneity and emergence mutations using next- generation sequencing for overcoming cancer drugs resistance. Expert Rev. Anticancer Ther. 12, 1245–1248.

Rudnev, A.V., Aleksenko, S.S., Semenova, O., Hartinger, C.G., Timerbaev, A.R., Keppler, B.K., 2005. Determination of binding constants and stoichiometries for platinum anticancer drugs and serum transport proteins by capillary electrophoresis using the Hummel–Dreyer method. J. Sep. Sci. 28, 121–127.

Saha, P., Descôteaux, C., Brasseur, K., Fortin, S., Leblanc, V., Parent, S., Asselin, É., Bérubé, G., 2012. Synthesis, antiproliferative activity and estrogen receptor *a* affinity of novel estradiol-linked platinum(II) complex analogs to carboplatin and oxaliplatin: potential vector complexes to target estrogen-dependent tissues. Eur. J. Med. Chem. 48, 385–390.

Samimi, G., Katano, K., Holzer, A.K., Safaei, R., Howell, S.B., 2004. Modulation of the cellular pharmacology of cisplatin and its analogs by the copper exporters ATP7A and ATP7B. Mol.
Pharmacol. 66, 25–32.

Santini, C., Pellei, M., Gandin, V., Porchia, M., Tisato, F., Marzano, C., 2013. Advances in copper complexes as anticancer agents. Chem. Rev. 114, 815–862.

Sava, G., Zorzet, S., Turrin, C., Vita, F., Soranzo, M., Zabucchi, G., Cocchietto, M., Bergamo, A., DiGiovine, S., Pezzoni, G., Sartor, L., Garbisa, S., 2003. Dual action of NAMI-A in inhibition of solid tumor metastasis: selective targeting of metastatic cells and binding to collagen. Clin. Cancer Res.: Off. J. Am. Assoc. Cancer Res. 9, 1898–1905.

Scanlon, K.J., Kashani-Sabet, M., Miyachi, H., Sowers, L.C., Rossi, J., 1989. Molecular basis of cisplatin resistance in human carcinomas: model systems and patients. Anticancer Res. 9, 1301–1312.

Sgarbossa, P., Sbovata, S.M., Bertani, R., Mozzon, M., Benetollo, F., Marzano, C., Gandin, V., Michelin, R.A., 2013. Novel imino thioether complexes of platinum(II): synthesis, structural investigation, and biological activity. Inorg. Chem. 52, 5729– 5741.

Shakya, R., Peng, F., Liu, J., Heeg, M.J., Verani, C.N., 2006.
Synthesis, structure, and anticancer activity of gallium(III) complexes with asymmetric tridentate ligands: growth inhibition and apoptosis induction of cisplatin-resistant

neuroblastoma cells. Inorg. Chem. 45, 6263–6268.
Shrivastava, H.Y., Kanthimathi, M., Nair, B.U., 2002. Copper(II) complex of a tridentate ligand: an artificial metalloprotease for bovine serum albumin. Biochim. Biophys. Acta BBA – Gen. Subj. 1573, 149–155.
Sigman, D.S., Graham, D.R., D'Aurora, V., Stern, A.M., 1979. Oxygen-dependent cleavage of DNA by the 1,10- phenanthroline. cuprous complex. Inhibition of *Escherichia coli* DNA polymerase I. J. Biol. Chem. 254, 12269– 12272.
Smith, N.W., Strutton, G.M., Walsh, M.D., Wright, G.R., Seymour, G.J., Lavin, M.F., Gardiner, R.A., 1990. Transferrin receptor expression in primary superficial human bladder tumours identifies patients who develop recurrences. Br. J. Urol. 65, 339–344.
Song, I.-S., Savaraj, N., Siddik, Z.H., Liu, P., Wei, Y., Wu, C.J., Kuo, M.T., 2004. Role of human copper transporter Ctr1 in the transport of platinum-based antitumor agents in cisplatin- sensitive and cisplatin-resistant cells. Mol. Cancer Ther. 3, 1543–1549.
Sooriyaarachchi, M., Narendran, A., Gailer, J., 2011. Comparative hydrolysis and plasma protein binding of cis-platin and carboplatin in human plasma *in vitro*. Metallomics 3, 49–55.
Spreckelmeyer, S., Orvig, C., Casini, A., 2014. Cellular transport mechanisms of cytotoxic metallodrugs: an overview beyond cisplatin. Molecules 19, 15584–15610.
Stewart, D.J., Benjamin, R.S., Luna, M., Feun, L., Caprioli, R., Seifert, W., Loo, T.L., 1982. Human tissue distribution of platinum after cis-diamminedichloroplatinum. Cancer Chemother. Pharmacol. 10, 51–54.
Stewart, J.J., White, J.T., Yan, X., Collins, S., Drescher, C.W., Urban, N.D., Hood, L., Lin, B., 2006. Proteins associated with cisplatin resistance in ovarian cancer cells identified by quantitative proteomic technology and integrated with mRNA expression levels. Mol. Cell. Proteomics 5, 433–443.
Streu, C., Feng, L., Carroll, P.J., Maksimoska, J., Marmorstein, R., Meggers, E., 2011. P-donor ligand containing ruthenium half- sandwich complexes as protein kinase inhibitors. Inorg. Chim. Acta 377, 34–41.
Sukumar, U.K., Bhushan, B., Dubey, P., Matai, I., Sachdev, A., Packirisamy, G., 2013. Emerging applications of nanoparticles for lung cancer diagnosis and therapy. Int. Nano Lett. 3, 1–17.
Sun, R.W.-Y., Ma, D.-L., Wong, E.L.-M., Che, C.-M., 2007. Some uses of transition metal complexes as anti-cancer and anti- HIV agents. Dalton Trans. 4884–4892.
Süss-Fink, G., 2010. Arene ruthenium complexes as anticancer agents. Dalton Trans. 39, 1673–1688.
Tabassum, S., Zaki, M., Afzal, M., Arjmand, F., 2014. Synthesis and characterization of Cu(II)-based anticancer chemotherapeutic agent targeting topoisomerase I*a*: *in vitro* DNA binding, pBR322 cleavage, molecular docking studies and cytotoxicity against human cancer cell lines. Eur. J. Med. Chem. 74, 509–523.
Talman, E.G., Kidani, Y., Mohrmann, L., Reedijk, J., 1998. Can Pt (IV) – amine complexes act as prodrugs? Inorg. Chim. Acta 283, 251–255.
Tan, C.-P., Lu, Y.-Y., Ji, L.-N., Mao, Z.-W., 2014. Metallomics insights into the programmed cell death induced by metal- based anticancer compounds. Metallomics 6, 978–995.
Tan, S.J., Yan, Y.K., Lee, P.P.F., Lim, K.H., 2010. Copper, gold and silver compounds

as potential new anti-tumor metallodrugs. Future Med. Chem. 2, 1591–1608.
Themsche, C.V., Parent, S., Leblanc, V., Descôteaux, C., Simard, A.-M., Bérubé, G., Asselin, E., 2009. VP-128, a novel oestradiol- platinum(II) hybrid with selective anti-tumour activity towards hormone-dependent breast cancer cells *in vivo*. Endocr. Relat. Cancer 16, 1185–1195.
Tiekink, E.R.T., 2002. Gold derivatives for the treatment of cancer. Crit. Rev. Oncol. Hematol. 42, 225–248.
Timerbaev, A.R., 2009. Advances in developing tris(8- quinolinolato)gallium(III) as an anticancer drug: critical appraisal and prospects. Metallomics 1, 193–198.
Tisato, F., Marzano, C., Porchia, M., Pellei, M., Santini, C., 2010. Copper in diseases and treatments and copper-based anticancer strategies. Med. Res. Rev. 30, 708–749.
Todd, R.C., Lippard, S.J., 2009. Inhibition of transcription by platinum antitumor compounds. Met. Integr. Biometal Sci. 1, 280–291.
Torti, S.V., Torti, F.M., 2013. Cellular iron metabolism in prognosis and therapy of breast cancer. Crit. Rev. Oncog. 18, 435–448.
Trejo-Solís, C., Jimenez-Farfan, D., Rodriguez-Enriquez, S., Fernandez-Valverde, F., Cruz-Salgado, A., Ruiz-Azuara, L., Sotelo, J., 2012. Copper compound induces autophagy and apoptosis of glioma cells by reactive oxygen species and jnk activation. BMC Cancer 12, 156.
Tripathi, L., Kumar, P., Singhai, A., 2007. Role of chelates in treatment of cancer. Indian J. Cancer 44, 62.
Trzaska, S., 2005. Cisplatin. Chem. Eng. News Arch. 83, 52. Vilmar, A., Sørensen, J.B., 2009. Excision repair cross-complementation group 1 (ERCC1) in platinum-based treatment of non-small cell lung cancer with special emphasis on carboplatin: a review of current literature. Lung Cancer 64, 131–139.Vock, C.A., Renfrew, A.K., Scopelliti, R., Juillerat-Jeanneret, L., Dyson, P.J., 2008. Influence of the diketonato ligand on the cytotoxicities of [Ru(6-p-cymene)(R2acac)(PTA)]$^{+}$ complexes (PTA = 1,3,5-triaza-7-phosphaadamantane). Eur. J. Inorg. Chem. 2008, 1661–1671.
Wang, B., Qian, H., Yiu, S.-M., Sun, J., Zhu, G., 2014a. Platinated benzonaphthyridone is a stronger inhibitor of poly(ADP- ribose) polymerase-1 and a more potent anticancer agent than is the parent inhibitor. Eur. J. Med. Chem. 71, 366–373.
Wang, N.X., von Recum, H.A., 2011. Affinity-based drug delivery. Macromol. Biosci. 11, 321–332.
Wang, X., Guo, Z., 2008. Towards the rational design of platinum (II) and gold(III) complexes as antitumour agents. Dalton Trans. 1521–1532.
Wang, Y., He, Q.-Y., Sun, R.W.-Y., Che, C.-M., Chiu, J.-F., 2005. GoldIII porphyrin 1a induced apoptosis by mitochondrial death pathways related to reactive oxygen species. Cancer Res. 65, 11553–11564.
Wang, Z., Qian, H., Yiu, S.-M., Sun, J., Zhu, G., 2014b. Multi- targeted organometallic ruthenium(II)–arene anticancer complexes bearing inhibitors of poly(ADP-ribose) polymerase-1: a strategy to improve cytotoxicity. J. Inorg. Biochem. 131, 47–55.
Wheate, N.J., Walker, S., Craig, G.E., Oun, R., 2010. The status of platinum anticancer drugs in the clinic and in clinical trials. Dalton Trans. 39, 8113–8127.
Wong, D.Y.Q., Ang, W.H., 2012. Development of platinum(iv) complexes as anticancer

prodrugs: the story so far. COSMOS 08, 121–134.
Wong, E., Giandomenico, C.M., 1999. Current status of platinum- based antitumor drugs. Chem. Rev. 99, 2451–2466.
Xie, W., Wei, S., Liu, J., Ge, X., Zhou, L., Zhou, J., Shen, J., 2014. Combination anticancer therapy activity studies for the complex of hypocrellin A and gallium ion. Dyes Pigm. 101, 43–50.
Yi, T., Pathak, M.K., Lindner, D.J., Ketterer, M.E., Farver, C., Borden, E.C., 2002. Anticancer activity of sodium stibogluconate in synergy with IFNs. J. Immunol. 169, 5978–5985.
Yu, L., Ma, Y.-Q., Liu, R.-C., Wang, G.-C., Li, J.-S., Du, G.-H., Hu, J.-J., 2004. Synthesis, characterization and *in vitro* antitumor activity of some arylantimony ferrocenylcarboxylate derivatives and the crystal structures of [C5H5FeC5H4C(CH3) = CHCOO]. Polyhedron 23, 823–829 (see abstract).
Zang, D.Y., Lee, K.H., Lee, J.S., Lee, J.H., Kim, W.K., Kim, S.H., Kim, W.D., Kim, D.S., Kim, J.H., Kim, B.S., Cho, Y.B., Kim, D.K., Kim, K.H., 1999. Phase II trial of a novel platinum analog, SKI 2053R, in patients with previously untreated extensive-stage small-cell lung cancer. Am. J. Clin. Oncol. 22, 495–498.
Zerzankova, L., Kostrhunova, H., Vojtiskova, M., Novakova, O., Suchankova, T., Lin, M., Guo, Z., Kasparkova, J., Brabec, V., 2010. Mechanistic insights into antitumor effects of new dinuclear cis PtII complexes containing aromatic linkers. Biochem. Pharmacol. 80, 344–351.
Zhang, J.Z., Bryce, N.S., Lanzirotti, A., Chen, C.K., Paterson, D., de Jonge, M.D., Howard, D.L., Hambley, T.W., 2012. Getting to the core of platinum drug bio-distributions: the penetration of anti-cancer platinum complexes into spheroid tumour models. Met. Integr. Biometal Sci. 4, 1209–1217.
Zhong, H.-J., Leung, K.-H., Liu, L.-J., Lu, L., Chan, D.S.-H., Leung, C.-H., Ma, D.-L., 2014. Antagonism of mTOR activity by a kinetically inert rhodium(III) complex. ChemPlusChem 79, 508–511.