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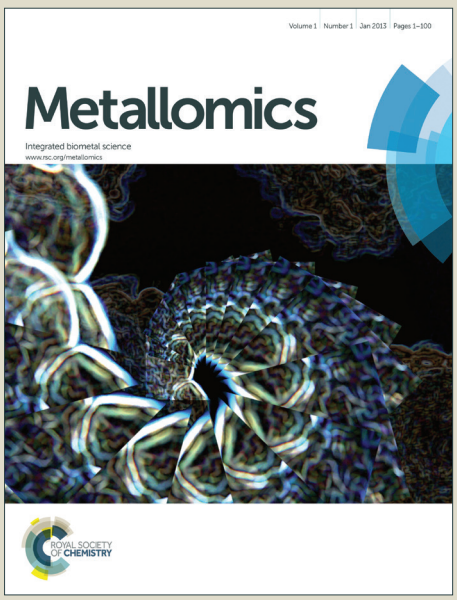

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Pt-based drugs: the spotlight will be on proteins

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Platinum-complexes represent one of the most successful groups of clinically used anticancer drugs. Their mechanism of action relies on the formation of stable DNA adducts occurring at the nitrogen in position 7 of guanine (N7) and involving one or two spatially closed residues. The stable DNA adducts are recognized as a DNA damage event and, ultimately, drive cell to death. Nevertheless, nucleobases are not the only reliable target of these drugs and other biomolecules can be involved. Among them large interest has been devoted to proteins since they contain several potential reactive sites for platinum (His, Met, Cys) and, in particular, because the reaction of the metal with sulfur containing groups is a kinetically favored process. As a result, the occurrence of protein adducts and DNA-protein cross-linkings must be further taken into account in order to fully define cisplatin mechanism of action. Herein, we will summarize the most recent experimental evidences collected so far on protein-cisplatin adduct formation to better dissect its correlation with the drug pharmacological profile. Indeed, in addition to modulation of drug bioavailability and toxicity, a potential role of proteins as reaction intermediates or reservoir systems in platinum drugs can be envisaged. Additionally, the effects of Pt-coordinating groups on the chemical reactivity of the metal complexes will be reviewed. From all these outcomes a general model for Pt-based drugs mechanism of action can be drawn which is more articulate than the one currently supported. It claims proteins as reactive intermediates for DNA platination and it defines them as relevant to fully describe the clinical potential of this class of anticancer drugs.

Introduction

After more than 30 years of clinical use, the success of cisplatin for the treatment of oncologic patients affected by different types of cancer is not concluded. As a matter of fact, this long practice evidenced several drawbacks connected to its use. This justifies the huge number of derivatives which were synthesized, tested and eventually entered into clinical trials. Unfortunately, the number of worldwide approved drugs is quite small.[1] Nevertheless, a critical analysis of the evolution of cisplatin derivatives generations, as summarized in Fig. 1, clearly highlights the main criticisms they were required to answer:

- Improved safety
- Broader spectrum of antitumor activity
- Oral activity
- Reduced cross-resistance
- Improved delivery

The chemical structures of the novel derivatives confirmed that they remain closely related to the leading drug. This is largely due to the aim to optimize the pharmacological profile by preserving the molecular basis of the mechanism of action of cisplatin. The common structural features of clinically active Pt-based drugs rests in the coordination around the metal ion (Pt(II) in the biologically active form) of two non-leaving/carries groups (N-Pt bonds) and of two leaving groups in *cis* (chloride or carboxylate). Generally, an activation step, corresponding to aquation, is required to allow efficient DNA platination, the rate of the process being modulated by the nature of the leaving groups (i. e. chloride are more efficient than carboxylate groups). The *cis*- geometry of the metal complex allows to form mono- or bi-dentate adducts on the nucleic acid with peculiar structural features distinct from those produced by *trans*- analogues. Such adducts, *per se* or due to the unique structural modifications they induce on the double stranded DNA, lead to activation of apoptotic stimuli and, ultimately, to cell death.[2]

In this picture DNA appears as the bright side of the story. Conversely, proteins have been considered the dark side. In fact, the bifunctional nature of these metal complexes does not limit the reactivity toward the double helix. Indeed, the presence of two leaving groups allows them to react with other target even before they reach the nucleus. This easily occurs with proteins since histidine, cysteine and methionine represent good reactive partners for platinum. However, consistently with several basic evidences, proteins have been considered mainly as responsible for reduced bioavailability and toxicity of platinum complexes.

Reactions on proteins can occur at different stages along the long pathway these drugs need to complete to exert their therapeutic DNA platination. Since all these drugs are generally intravenously administered, plasma represents the first potential target. Here, the drug finds several proteins among which human serum albumin (HAS) is the most abundant (in blood HSA concentration is 30-50 g/l).[3] It is easy to accept that an efficient interaction of platinum-based drugs with HSA contributes to reduce their blood

concentration and, consequently, their efficacy. To overcome this problem the drug dosage can be increased but this usually promotes the occurrence of off-target side effects. Moreover, since platinum has been demonstrated to cause HAS oligomerization, we can also relate this reaction to a specific toxic effect such as nephrotoxicity.[4]

At this point the problems are not ended. Indeed, the drug must cross the cell membranes to reach DNA. To do so the platinum complex can dissociate from the protein to use the same transport mechanisms of the intact metal complex. In turn, adducts may enter as well but the uptake efficiency of the protein-bound form may be remarkably different in comparison to the free drug. At this stage, reactivity can still occur on different cellular components relevant for the pharmacology of platinum anticancer drugs: these comprise membrane transporters or systems devoted to preserve the integrity of the membrane itself. This represents a further level for modulation of the drug distribution and efficiency.[5]

Once inside the cell, the environment changes again: the chloride concentration drops further promoting Pt(II) aquation and, likely, making the metal complex even more reactive. In this compartment, several further deactivation/detoxification pathways enter in action.[6] In particular, the intracellular concentration of glutathione (GSH) is extremely high (GSH 5-10 mM) and, again, promotes a sulfur-directed reactivity.[7] Indeed, the formation of cisplatin-GSH conjugates has been confirmed to occur and to cause a depletion of the intracellular GSH reservoir.[8] Remarkably, other important components actively involved in controlling cellular redox homeostasis have also been found to be directly targeted by the platinum complex (i.e. the thioredoxin reductase system which functions result impaired). The consequence of this deregulation is actually a beneficial promotion of the cytotoxic activity of the drug. However, the same unbalanced redox processes have been associated to side effects such as nephrotoxicity and hepatotoxicity.[6]

All these effects can be hardly simultaneously controlled. To overcome the problem the basic idea is to avoid formation of unwanted protein adducts. Several delivery strategies were applied with the aim to physically protect the drug from the physiological environment before it reaches the target. The most recent advances in this field are well summarized in two reviews.[9] They can be roughly divided into an active delivery of the drug by conjugation with units designed to selectively target cancer cells or to the use of passive nanovectors in order to take advantage of the enhanced permeability and retention (EPR) effect which is distinctive of the tumor tissues. Additionally, the use of Pt(IV) complexes, which are pro-drugs to be activated where the cytotoxic effect is required, has been pursued.[10]

Moreover, Pt(IV) complexes can be further conjugated. Positive results were obtained using monomethoxyl poly(ethylene glycol) (MPEG) based polymers to obtain conjugates that auto-assemble into nanomicelles. They are effectively internalized by the cells via endocytosis mechanism and once the conjugates are inside the endosome, the low pH and the high concentration of reducing agents allow the release of the complexes in their active form (Pt(II) complexes).[11] This approach is versatile and can be applied to more

complex structures, like dinuclear Pt(IV) complexes which otherwise may result highly toxic at systemic level.[12]

An alternative and extensively explored strategy is based on the co-administration of ameliorating agents. Indeed, the use of sulfur containing molecules is expected to compete with physiological proteins for platination thus preserving them. This is the case of sodium thiosulfate (STS) which addition to plasma has been confirmed to reduce platination of plasma proteins *in vitro*.[13] This experimental evidence correlates with *in vivo* studies which supported the reduction of cisplatin ototoxicity by the coadministration of STS.[14] One concern is connected to a potential parallel suppression of the cytotoxic activity of the drug upon reaction with the protective agent. However, these preclinical studies indicate that administration of the otoprotective agent STS is not compromising cisplatin efficiency against neuroblastoma until its administration is postponed with reference to the metal complex.[14] Unfortunately, high sulfur concentration in plasma alters the physiological balance of thiol vs disulfide and produces severe toxicity.[15] From this observation the proposal to prevent intrinsic toxicity associated with Pt-based drugs by administration of the disulfide form protective agents emerged. Consistently, one of these compounds entered Phase II clinical trials.[16]

All these approaches can definitely help to improve the response of patients undergoing chemotherapy. However, only in the very last years, the acquisition of a detailed knowledge of the molecular mechanisms ruling the reactivity of Pt(II) complexes toward proteins has been systemically pursued. The fascinating aspect is that this is not only leading toward the design of novel complexes with increased chances to enter into clinical trials but also to the discovery of unexpected potential for these metal complexes in fields that span from medicinal chemistry to technical applications.

Herein, we describe the most recent highlights following a journey which starts from the analysis of the platinum-protein reaction pathways at molecular level, proceeds along the structural features of the resulting adducts and ends with novel applications this knowledge suggests.

Modulation of the reactivity of Pt-based drugs toward proteins

As above introduced, the reactivity of Pt-based drugs is exploited at several different stages during their complex pharmacological cycle. In particular, since cisplatin-protein adduct formation has been extensively associated to toxicity and reduced efficacy, several derivatives have been synthetized in order to selectively reduce it. This is a hard task as both therapeutic and toxic effects are largely modulated by the same structural parameters of the platinum-based drugs. Nevertheless, this valuable work allowed building comprehensive, novel SARs which, in several instances, provided indications that are in contrast with the "canonical" postulates. To properly sustain these models, detailed studies on the effects of such modifications on the complexes reactivity at molecular level are currently undergoing. They are of great help since, as an example, it is not well defined yet if proteins and DNA platination follow the same ranking order or if they occur according to a common reaction scheme. In fact, whereas it is well stated that cisplatin reacts on DNA in its aqua-form, the same should not necessarily be expected on proteins. As an example, a correlation between hydrolysis rate and interference with DNA metabolism has been inferred but no clear data are available on proteins.[17] Additionally, the aminoacidic composition of target protein as well as the peculiar environments considered (plasma, cell membrane, cytosol or nucleus) may differently modulate the drug reactivity profiles.

To clarify these points, simple models for DNA and proteins, i.e. single nucleotide (guanine, GMP) and amino acid (Met, AcMet, N-AcHis), have been extensively employed to obtain detailed description of the reactions under investigation in terms of efficiency, kinetic and nature of reaction products. Additionally, the use of drugs systemically modified in single portions assisted in understanding how to modulate a selected reaction pathway.

Mechanism of reaction of platinum complexes with S-containing substrates and proteins: In order to identify proper strategies suitable to reduce protein platination, extensive works were directed in characterizing the reactivity of platinum-based drugs. Since histidine, cysteine and methionine have been confirmed to react with these drugs due to the high affinity of platinum for sulfur, these studies mainly focused on Pt-S adduct formation. Interestingly, this approach turned out to be useful to redesign some previously proposed reactivity restrictions.

Among them, a "postulate" was the assessment that reactivity of cisplatin should be limited to the substitution of one/two leaving groups. As a consequence, the proposal of platination of cytocrome c with the loss of an amine ligand was initially considered with suspicion.[18] This model is now unexpectedly sustained by pull-down studies aimed to identify the proteins (from cell lysate of several different mouse cell lines) that preferentially react with cisplatin causing toxicity in the cochlea and kidney.[19] To perform such an assay, two agarose-conjugates were designed, one with a (1-aminomethyl-1,2-

ethylenediamine)dichloroplatinum(II) complex and one with a (1-aminomethyl-1,2-ethylenediamine)diamineplatinum(II). Between them, the one exposing the two amino-groups reacted more extensively with the proteins, thus supporting that several proteins have higher affinity for the amino group side of cisplatin.

Interestingly, a detailed model of this reactivity was further integrated by studies on Pt(II) metal complexes showing a *trans*- geometry. The clinically relevant group of platinum-based drugs belongs to the *cis*- family but, recently, *trans*- derivatives raised a great interest since some of them are highly cytotoxic and not cross-resistant with cisplatin.[20] These differences are connected to the formation of different DNA-adducts. However, although few studies are currently available, it is rational to assume that, since *cis*- and *trans*-isomers differently react on DNA, the same may occur on proteins.[21]

The proposed pathways are schematically summarized in Fig. 2. In the presence of a sulfur containing substrate, like as Met, one chloride is replaced on both isomers. However, the resulting products are different because only in one instance the second chloride is in *trans* with reference to the S-Pt bound. This is extremely relevant due to the strong trans-labilizing effect of sulfur. As a consequence, the reaction proceeds always in *trans* and the two resulting disubstituted compounds are still distinct (see compounds cis-(3) and trans-(3) in Fig. 2). The reaction rates of these two steps are sensitive to salt composition and pH of the environment. But a key point is that at physiological condition (pH 7.0) a rapid head-to-tail isomerization of the coordinated Met can occur. As a result, the final products cis-(6) and trans-(6), in which two Met residues are cyclized around the platinum metal ion, share the same geometry. Despite this final analogy, the authors underline how this reaction scheme leads to a further modulation on the drugs reaction toward guanine too. Indeed, only in the *trans*- derivatives after addition of one Met, a chloride in trans to sulfur is retained, which accelerates the reaction toward a second nucleobase. Moreover, also in the Pt-protein bisadduct trans-(4) (see Fig. 2), the sulfur effect allows exchanging one Met with one guanine preserving both nitrogen carrier groups. Interestingly, this is an equilibrium reaction whose back rate is about 4 times slower than the forward. The final conclusion is that protein adducts represent suitable systems to actively deliver *trans*-complexes to DNA. This is consistent with previous suggestions on *trans*-derivatives and with data acquired on more complex systems such as proteins where a transfer of the metal complex form platinated proteins to DNA was actually monitored.[4, 22]

This study integrates the comprehensive work of Farrell and coworkers focused on TPAs, a class of *trans*-platinum amine derivatives as cytotoxic as the clinically active cisplatin.[20] This property is attributed to the aromatic nature of the substituted carrier group but large effort has been devoted to understand their reactivity profile towards different biological macromolecules.

A direct comparison among cisplatin, transplatin and two TPAs (SA1 and SA4 in Fig. 3), showed that the aromatic derivatives are more efficient in modifying model proteins such as bovine alpha-lactalbumin (α-LA), hen egg white lysozyme (HEWL) and HSA. Interestingly, the reactivity ranking order is not the same as

the one previously reported for nucleic acid.[23] In particular, SA1 and SA4 preferentially modify DNA and proteins, respectively. Thus, the reduced liability of the carboxylate vs chloride leaving groups, which correlates with the DNA reactivity, appears not to be directly related to the efficiency of protein adduct formation.[17] Distinctly from what observed in the presence of DNA, aquation is not the rate limiting step for TPA-protein adduct formation, both when the target is a single amino acid (i. e. Met) or a full length protein.[4, 17] Interestingly, although chloride-containing derivatives react efficiently with proteins, the carrier groups were found to be crucial in controlling reaction rate, stoichiometry and coordination modes and, ultimately, in driving distinct alterations of protein conformation. From these results a reflection must derive. Indeed, protein function is strictly connected to protein structure and flexibility: consequently, at physiological level, it is likely that the structural consequences of platination can be more crucial than the overall platination degree. Thus, a simple work devoted to only reduce the reaction rate of one metal complex on protein might not be useful without a detailed analysis of the folding of the modified protein. For this reason, studies performed on isolated amino acids must be integrated by analyses on full length proteins.

Novel platinum complexes designed to reduce protein adduct formation: Since protein platination has been largely related to cisplatin toxicity or resistance, a large synthetic effort was devoted in preparing novel compounds presenting reduced protein reactivity. Some of the most recent works were inspired by already approved drugs like carboplatin, which was confirmed to react less efficiently than cisplatin with plasma protein.[24] Alternatively, molecules in clinical trials were also used as starting models.[1] Among them Picoplatin (Fig. 1) represents an example of compound design to reduce the resistance connected to drug reaction with glutathione. This result is reached by Pt(II) coordination of a 2-methyl pyridine: the aromatic group sterically hinders the metal center thus reducing both aquation and reaction with sulfur containing sites on proteins.

The relevance of this effect is now further sustained by studies aimed to monitor the reaction kinetic of cis-diamino Pt(II) complexes bearing amine ligands of variable steric hindrance (compound SH1 in Fig. 3).[25] Interestingly, steric clashes turned out to impair the reactivity with different efficiency accordingly to the chemical structure of the target. In particular, SH1 represses to a larger extent the reaction with N-AcMet in comparison to N-AcHis. As a consequence, it is possible to design ligands which preferentially platinate a set of proteins according to their aminoacidic composition.

A severe limit of this approach often rests in a parallel reduction of the reactivity required for the cytotoxic activity, i. e. DNA platination. To overcome such a drawback, the advantage of a cooperative action of multicentric drug was assessed. Brown et al. connected two reactive moieties by inert flexible linkers of different lengths (compound SH2 in Fig. 3).[26] A direct comparison of the reaction rate vs GMP, GSH and HSA confirmed that the sterically hindered derivatives have a slower reaction rate in comparison to the

unprotected analogues. Unfortunately, this was still occurring towards all tested substrates, thus involving DNA as well. However, the work suggested cooperativity between the two reactive units since the length of the linker was shown to play a crucial role in defining the cytotoxicity. This opens now the chance to play with the nature of the reactive groups in a concerted way with the features of the linker in the attempt to optimize novel molecular tweezers.

These data showed also that there is a quite high flexibility in terms of chemical structure of the carrier groups. Thus, a methodology derived from the delivery techniques can be merged. In this connection, the hindered amine can be selected to represent a good ligand for a receptor overexpressed in defined tumor cells. As it was confirmed using translocator protein as target, this approach allows obtaining metal complexes with improved uptake in cell lines overexpressing the selected receptor.[27] The incremented intracellular concentration of the resulting bioconjugate compensates for its reduced intrinsic activity. As a result, relevant cytotoxicity was coupled to poor unselective protein reactivity and good cancer cell selectivity.

Reversible interaction of platinum complexes with proteins: Recently, the paradigm of nitrogen not labile ligands as a requirement for cytotoxicity has been broken and other atoms have been used to coordinate the metal ion. Among them phosphor was considered since P-Pt coordination bonds are quite stable. In a more radical approach the coordination sphere of a PtP_2 system has been fulfilled with other non-conventional groups. This is the case of derivatives of the type PtP_2X_2 where P_2 are two monodentate or one bidentate phosphane ligand and X_2 derives from coordination of a dithiolate ligand or of two chlorides (compounds P1 and P2 in Fig. 4).[28] By comparing the reactivity of cisplatin to PtP_2Cl_2 and PtP_2S_2 on cytochrome c, it emerged that the most reactive are derivatives of the PtP_2Cl_2 type. Indeed, they easily lose the chlorides and react with the protein. Interestingly, adducts at three protein sites were identified, all likely located at His residues. This is in contrast to what observed with cisplatin which forms only a monoplatinated specie. Conversely, the PtP_2S_2 derivatives, due to their coordination pattern, are stable to hydrolysis and, they only slowly undergo to other substitution reactions. Nevertheless, they turned out to be the most cytotoxic group and to be not cross-resistant with cisplatin.

Similar behavior was shared by a related compound [Pt(dmba)(N9-9AA)(PPh3)]PF6 (compound P3 in Fig. 4) which was found to be 30- fold more cytotoxic than cisplatin in HL-60 cell cultures.[29] ESI-TOF MS and fluorescence measurements ruled out any strong metal binding to proteins or covalent interaction with the oligonucleotides. In this case, DNA recognition was preserved mainly thought π staking likely involving the 9-aminoacridine moiety coordinated to the metal center.

This result invites to look at the protein platination event from a different perspective. This novel approach refers to the development of Pt(II) complexes inert to substitution but able to recognize DNA and/or proteins according to reversible processes. Clearly these novel derivatives, although still contain a Pt^{2+}

atom, must be considered as novel pharmacological entities, likely directed toward different targets (including non-genomic target) and endowed with unique distribution and uptake properties.

A recent example is reported by Bernocchi and coworkers who analyzed the behavior of a platinum complex containing a dimethylsulfoxide/dimethylsulphide (DMSO/DMS) system in the coordination sphere and a O,O'-acetylacetonate (O,O'-acac) group as carrier ligand.[30] As expected, its activity is related to its intracellular concentration. Interestingly, although this drug crosses the blood-brain barrier more efficiently than cisplatin and also its intracellular uptake is incremented, it does not alter the integrity of the barrier itself and it only modestly affects the fundamental events connected to the normal CNS development. Clearly this is connected to the different mechanism of action of the drug and it allows predicting a favorable outcome in terms of neurotoxicity thus foreseeing its application in pediatric oncology.

In this perspective, also the substitution of nitrogen ligand with biphosphine ligands was considered.[31] Among the tested compounds five coordinated Pt(II) complexes were found to bind to DNA and proteins reversibly (compound P4 in Fig. 4). Although a direct comparison of the efficiency of these two recognition processes is not yet available, their high cytotoxicity has been described as the result of a carrier mechanism by HSA and of a direct inhibition of the proteasome.

Cationic polynuclear Pt(II) complexes: A further evolution of cisplatin is represented by polynuclear Pt drugs (Fig. 5). They were initially designed to keep the presence of leaving groups in order to preserve DNA reactivity. Obviously, this chemical feature preserves also the reactivity towards proteins which is usually incremented thanks to the net positive charge of these compounds. Moreover, generally, one amine carrier group was used to insert the linker moiety in order to connect more Pt(II) units. Due to the strong trans-labializing effect of sulfur, in the presence of thiolates (Met, Cys), a rapid cleavage occurs which causes the loss of the polynuclear structure and, consequently, of the cytotoxic activity. In virtue of these effects high toxicity and poor cytotoxic activity are obtained *in vivo*. To limit these collateral reactions two main approaches were followed.

In one instance the leaving groups (usually chlorides) were substituted with not-leaving group (amine groups) thus providing inert metal complexes. This strategy was applied to the polynuclear platinum compounds BBR3464 which was accordingly converted into TriplatinNC.[32] The transformation of BBR3464 into a non-reactive form makes the cytotoxicity of resulting TriplatinNC unaffected by GSH. At the same time, GSH-dependent drug efflux mechanisms are not altered. Consistently, also the reversible complex formed with HSA does not impair drug accumulation, cellular localization and ultimately cell cytotoxicity. Thus, reversible binders have a pharmacological profile clearly distinct from the one displayed by the chloride containing homologues.

Alternatively, the use of a tridentate bridge has been pursued (compound TP1 in Fig. 5). In this case it was possible to maintain the leaving chlorides as substituents, since the geometry of the system does not allow

drug decomposition upon reaction with sulfur containing reactants.[33] As a result, the polynuclear complex reacts with HSA mainly according to reversible π-π stacking and hydrophobic bonds, thus preserving its cytotoxic activity during blood circulation. Indeed, it was confirmed to produce DNA adducts and DNA-protein crosslinked species.

Structural and functional studies on platinated proteins

In contrast to the extensive literature dealing with the characterization of the reactivity of platinum-based drugs towards proteins, only few X-ray or NMR structural studies are currently available. However, together with computational calculations, they are extremely valuable in addressing the consequences of Pt–protein species formation at molecular level. The most appropriate subjects would be proteins of biological relevance. However, due to experimental restrictions, several of these works take advantage of the use of model proteins like as HEWL.

The first crystallographic structure of HEWL-cisplatin adduct was reported by Casini et al. (PDB entry 2I6Z).[34] Along with structural studies carried out with superoxide dismutase, it highlights the relevance of histidine as interaction site for platinum drugs complexes.[35] Indeed, the Nε of the imidazole ring of His15 was found to coordinate the Pt^{2+} ion with occupancy close to 50%. In this case the two amine groups are conserved and no further platination sites were identified. This crystal was obtained by soaking pre-grown "native" lysozyme crystals with an excess of the metal complex. Unfortunately, this protocol did not allow obtaining crystals with different platinum complexes.

To fill this gap, more recently, Tanley et al. further extended the investigation using a cocrystallization protocol of cisplatin or carboplatin with HEWL in both aqueous and DMSO containing conditions (PDB entries: 4DD0; 4DD1; 4DD2; 4DD3; 4DD4; 4DD6; 4DD7; 4DD9; 4DDA; 4DDB; 4DDC).[36] Their starting results suggested that both Pt(II) complexes do not bind to HEWL in aqueous media whereas they succeeded in obtaining crystals of platinated protein in the presence of DMSO. Distinctly from Casini's results, in these experimental conditions, two molecules of cisplatin or carboplatin coordinated His15 (Fig. 6A). The two Pt(II) atoms bind on either N atoms of the imidazole ring. The authors suggest this may result from the tautomeric equilibrium existing in the aromatic residues. Alternatively, the loss of one N-hydrogen promoted by the crystallization conditions and leading to the formation of an imidazolyl anion has been considered. An intriguing point highlighted in this work rests in the role of DMSO. It is accepted that it rapidly reacts with cisplatin through the favorable Pt-S bond formation.[37] Here, although no DMSO was found coordinated to the Pt(II) in the HEWL-cisplatin structure, it was found to promote

cisplatin/carboplatin reactivity towards the protein. These findings suggest that DMSO can cause platination of protein which is not occurring in physiological conditions. Consequently, if used as co-solvent for drug administration, it may produce an increase in the toxic effects.

Interestingly, comparable crystallographic data were collected in aqueous conditions but only when crystals underwent a 15 month-long chemical exposure (PDB entries: 4G4A, 4G4B, 4G4C, 4G4H, 4G49).[38]

An additional controversial point of Casini's structure rested in a poor definition of all the four Pt(II) coordinating groups: the drug bound at the surface of the protein showed the metal ion in an unusual tri-coordinated mode with no or weak density at the fourth coordination site.

To better discriminate between the real occurrence of a tri-coordinated species with unexpected T-shape geometry or an experimental limitation, computational studies have been carried out.[39] Calculations were performed with pure quantum mechanical (QM) calculations on cluster models as well as with hybrid quantum mechanics/molecular mechanics (QM/MM) on the cisplatinated HEWL. The authors confirmed that stable trigonal planar geometries with T-shaped configuration exist as minima on the gas phase potential energy surface. In comparison to the tetra-coordination, stronger Pt-His interactions have been calculated for the trigonal form, which derive from a compensation for the lack of the fourth ligand. However, the cost of dehydration of the tetra-coordinated system is quite high ruling out the possibility it occurs spontaneously. Additionally, QM/MM calculations did not identified any impairment to the formation of a square planar adduct, thus suggesting it as the most probable form in physiological conditions. In agreement with Casini's, these results allow to conclude that the T-shaped configuration observed in the experimental system is likely to be due to crystallographic limitations in identifying the fourth ligand of the metal. Nevertheless, in this work only the diaquo- form of the drug has been considered. Thus, result on different species like as dichloro- ones will help in fully define a potential physiological relevance for the tri-coordinated configuration of Pt(II) systems.

Cysteine proteases: Once a protein is platinated, a modulation of its functions likely occurs, mainly when interactions with the metal complex involve residues in the active site. Cysteine proteases (e.g., papain, cathepsin B, *etc.)* contain a cysteine in the active site and consistently, several reports confirmed that Pt(II) complexes can bind them. Not in all instances this binding results in a full inhibition of the enzymatic activity thus leaving open the question on the interaction mode between the protein and the drugs. Interestingly, cysteine proteases and hTrxR1 contain similar residues in the catalytic site (cysteine or selenocysteine, histidine and glutamate or asparagine). Targeting of selenoprotein thioredoxin reductase (TrxR1) by cisplatin has been confirmed to efficiently trigger apoptosis likely through a covalent cross-linking of TrxR1 with Trx1 and TRP14 (Trx-related protein of 14kDa), a mechanism not shared by Au- or Pd-containing compounds.[40]

Starting from these evidences and from their successful inhibition of human TrxR1 by terpidine-Pt(II) (TP-

Pt(II))[41], Lo et al. extended their investigation toward a panel of cysteine proteases of pharmacological relevance.[42] Indeed, the authors hypothesized that Pt(II) complexes may interact with many cysteine proteases and inhibit their functions. In their paper, they focused on three cysteine proteases with distinct biological activities: 3Cpro, involved in the proteolytic processing of large polyproteins and with an essential role in viral replication and maturation; the N-terminal amidase domain of the redox regulator GspSA (GspA), a papain-like cysteine protease that hydrolyzes trypanothione or glutathionylspermidine to spermidine and glutathione; and PGP I, which removes the N-terminal pyroglutamyl residue which protects oligopeptides and proteins from aminopeptidases digestion. All these proteins contain the same catalytic triad but they are differently folded. Moreover, their active sites are of different shapes and with different hydrophobic components. The study showed that the TP-Pt(II) complex is able to covalently interact with the active-site cysteine of all tested cysteine proteases, regardless of their structural folds. The X-ray characterization of the resulting adducts performed on PGP I (PDB entries: 3RO0, Fig. 6B), allowed rationalizing the reduced efficiency of inhibition in comparison to the previously reported human TrxR1 (PDB entries: 2ZZB). Indeed, in the PGP 1 complex only hydrophobic interactions are conserved between the terpidine moiety and the protein, whereas stacking on a tryptophan residue (present in TrxR1) is lost.

Copper proteins: A special mention must be deserved to metalloproteins. Indeed, metal ions are important proteins cofactors where they have structural (they can modulate the protein folding) or enzymatic (they can be required along the catalytic cycle) roles. Metal coordination sites can often accommodate metal ions of different nature. As a consequence, the physiological metal cofactor can be exchanged upon treatment with platinum-based drugs resulting in altered protein functions.

In humans, copper covers fundamental roles: it is found in the active sites of many proteins and it is involved in the maintenance of the intracellular redox balance.[6, 43] At the same time, it can be toxic due to redox reactions with subsequent ROS production. As a consequence, in healthy organisms, this metal is constantly protein-bound and distributed by a direct transfer process from one protein to the other. A common requirement for copper binding in proteins is the presence of peculiar domains rich in histidine, cysteine or methionine which properly coordinates it. Thus, the idea that platinum may compete for the same binding site emerged. The consequences of such an event might be multiple: a reduction of drug bioavailability, a modulation in drug cellular uptake/efflux, a modification of the functions of copper protein and, last but not least, a misregulation of the copper homeostasis with loss of the physiological redox balance. At the same time, the knowledge of these processes is relevant since the development of small molecules aimed to target redox systems might have important therapeutic application for several diseases, including cancer.

Copper distribution and intracellular concentration are finely tuned by a complex system of transporters and chaperons. Among them, a key role is played by Atox1: it delivers copper to proteins which ultimately

organize its excretion. Studies performed using Atox1+/+ and Atox1-/- cells confirmed the interaction of cisplatin with Atox1 in physiological conditions. This result was further supported by two crystallographic structures one referring to a monomeric and one to a dimeric form of the protein (PDB entries: 3IWX and 3IWL).[44]

Recently, in solution spectroscopic studies showed that protein-cisplatin interaction not affected by the presence of copper. Indeed, the two metal ions were found in close proximity when bound to the protein, as indicated by changes in the near-UV circular dichroism spectrum of the protein.[45] Upon platination, the protein is destabilized (the unfolding rate being greatly incremented by the presence of copper) and aggregation of the unfolded protein fractions was observed. The kinetic of these reactions are largely modulated by ligand type and orientation around the Pt-center. Interestingly, only complexes with two good leaving groups in *cis*- produced adducts with significant Cu–Pt interactions.[46]

The authors were also able to demonstrate that the unfolding of Atox1 promoted by distinct metal complexes follows different patterns: they proposed that only the cisplatin-like protein platination may stop the transfer of Pt(II) to the nucleus thus justifying in-cell mechanisms for cisplatin resistance.

To fully export these models into a physiological environment, Arnesano et al. merged in solution ESI-MS and NMR data with in-cell NMR spectroscopy.[47] They confirmed that in E. coli cells, cisplatin binds to Atox1 at a CXXC motif causing minimal alteration of protein folding but reducing its DNA platination efficiency. Additionally, they were able to monitor the initial formation of the cisplatin-Atox1 adduct and to show that in a physiological environment cisplatin retains the ammine ligands, the binding occurring at the S atoms of Cys12 and Cys15 coordinate in *cis*- positions. Since ammine retention by Pt(II) was observed only in the crystal corresponding to Atox1 dimer, they suggest it as an intermediate species.

The reaction of several copper-proteins with platinum-complexes has been carefully addressed in a recent review paper.[43] It deals with wild-type proteins. However, by biotechnological approaches, the copper binding site can be engineered to shift the preferential coordination of platinum vs copper. This result has been pursued on azurin, a small protein from *Pseudomonas aeruginosa* which contains only one metal ion binding site.[48] A single mutation (H117G) allowed to largely increment the binding affinity for Pt(II). Interestingly, the binding did not cause any irreversible change of the protein structure.

Zn-finger proteins: In the last years the attention has been moved also toward different metal ion binding domains. The breast cancer suppressor protein 1 (BRCA1) has an important role in the maintenance of genome integrity by taking part in DNA repair, cell cycle checkpoint, protein ubiquitination and transcriptional regulation. Preclinical and clinical studies revealed that its inactivation in cancer cells leads to chemosensitivity.[49] BRCA1 contains a N-terminal Zn^{2+} finger RING domain with two conserved metal-binding sites. Recently, Atipairin et al demonstrated that cisplatin promotes *in vitro* platination of the

BRCA1 N-terminal Zn^{2+} finger RING domain.[50] SDS-PAGE and mass-spectrometric analysis revealed that cisplatin forms monofunctional and bifunctional BRCA1 adducts and promotes protein dimerization to some degree. The Pt-binding site is His117. The structure of the apo form of BRCA1 is preserved and the content of secondary structure incremented upon cisplatin binding thus resulting in an enhanced thermostability of the treated protein. Interestingly, the structure of the Zn^{2+} bound form of BRCA1 is fully preserved during cisplatin addition but a drug excess confers additional thermal stability. This leads to assume interaction of the drug with other residues beyond the Zn^{2+} binding sites. The authors extended their study to a number of platinum complexes and demonstrated that the most effective to inactivate the BRCA1 ubiquitin ligase function is transplatin, followed by cisplatin, oxaliplatin and finally carboplatin.[51]

These results further underline that zinc-finger proteins represent druggable targets for platinum complexes. Consistently, Pt(II) complexes containing a guanine in their coordination sphere have been applied to reversibly inhibit the nucleocapsid Zn finger NCp7 protein of human immunodeficiency virus type 1 in order to prevent viral infection.[52] In a more evolved approach the effect of platinated-DNA on the protein has been proposed thus combining the physiological recognition elements (stacking of purines with aromatic amino acids) with the overlap enhancements provided by platination. Thus, the C-terminal Zn finger domain (34-52 residues) of the protein was investigated in the presence of a platinated single-stranded hexanucleotide.[53] The platinated oligonucleotide ((Pt-(dien)d(5'-TACGCC-3'))) has been characterized by mass spectrometry and NMR spectroscopy and the solution structure of the protein domain alone and in the presence of the oligonucleotide both in its unmodified and platinated forms calculated from the NOESY-derived distance constraints. Upon platination the Gua4-Trp37 interaction weakened whereas new Cyt6-Met46 and Cyt5-Trp37 contacts appear. Theoretical QM/MM calculations supported this rearrangement being connected to the bulkiness of the platinated DNA and to a partial conformational change in the peptide backbone.

Advanced analytical tools for Pt(II) protein adducts characterization

In addition to X-ray crystallography or NMR spectroscopy, which are used to deeply investigate the structural features of Pt-protein adducts, mass spectrometry has been widely applied to provide direct sequence-specific information on the position and on the type of the drug-mediated protein modifications. At the same time, the peculiar chemico-physical properties of platinum prompted the setting-up of novel protocols which can find applications in several analytical fields.

To make the identification of Pt-binding site on target proteins much more feasible and realistic, the most recent progresses combine mass spectrometry and proteomic technologies. There are essentially two methods that are currently applied: the "bottom-up" (peptide level) and the "top-down" (intact protein level) approaches. "Bottom up" strategies are based on the cleavage of the treated protein by proteolytic enzymes before mass spectrometry analysis and subsequent characterization of the peptide fragments. "Top-down" methods identify protein species by measuring the mass of the whole protein. In this approach, tandem mass spectrometry exploiting different dissociation methods (such as collision activated dissociation (CAD), electron capture dissociation (ECD) or infrared multiphoton dissociation (IRMPD)), can be used to produce the fragmentation of intact proteins, so that all modifications are generally detected and identified. This means that no protein processing before MS analysis is required. However, the complexity of the acquired mass spectra limits the analysis to small proteins and requires the use of high resolution instruments. The first question to address is if the currently used analytical approaches used up-to-date, are appropriate. The crucial point focuses on the preservation of Pt-protein adducts throughout the analysis phase (i. e. during sample preparation or ionization) and the suitability of MS techniques in terms of data interpretation and analysis performance. As an example, it was demonstrated that electrospray ionization (ESI) is more suitable than matrix-assisted laser desorption-ionization (MALDI) due to the excessively energetic ionization process involved in the latter which may lead to the loss of labile ligands during the analysis.

With the aim to dissect the advantages/disadvantages of these two approaches, they were applied to insulin, a suitable model protein due to its low molecular mass and to the presence of potential platinum reaction sites such as histidines and cysteines (both in their reduced and oxidized forms) in its aminoacidic sequence.

In principle, the top-down method should be expected to provide the best results thus Gómez-Gómez's group applied it to cisplatin-insulin adducts.[54] Size exclusion chromatography was then coupled to inductively coupled plasma mass spectrometry ICPMS (SEC-ICPMS), matrix-assisted laser desorption ionization- time of flight mass spectrometry (MALDI-TOF MS) and electrospray ionization-linear ion trap tandem mass spectrometry (ESI-LIT-MS/MS). This protocol allowed the identification of several different platinum binding sites in the protein which were located at His5, probably His10 residues and Cys7, all belonging to B chain. However, minor Pt-binding sites are not easy to assign in the MS spectra generated when the intact platinated protein is fragmented by MS/MS. This uncertainty is further incremented by the preservation of the disulfide bonds, linking A and B chains, in the gas-phase which impairs the detection of the contiguous ion fragments, required for an accurate sequence assignment. To fully characterize the system, the same authors moved to the bottom-up methodology where the contribution of platinum isotopic pattern is more significant thus making easier the identification of Pt-containing peptides resulting from protein digests.[55] Surprisingly, new reactive sites on insulin, not previously found by the top-down

approach, were assigned (His5, His10, Cys7 and Cys19 at the N-terminus of the B chain; Cys6, Cys7 and Cys20 in the A chain) thus shifting the choice towards this strategy.

This conclusion was strengthened by an insight into the stability of the Pt–protein adducts during the enzymatic digestion performed before MS detection. Indeed, several reagents used along the sample preparation, such as ammonium bicarbonate buffer, denaturing (Urea), reducing (dithiotreitol, DTT) and alkylating (iodoacetamide, IAA) agents can remove the platinum moiety from proteins, thus preventing the identification of the Pt-binding sites. Direct analysis of the digestion products by nESI-LIT-MSn, confirmed the stability of the Pt–insulin bonds during each steps of the enzymatic digestion procedure. Even when the metal ion was bound to a cysteine, it remained linked to the carbamidomethylated residue after the reducing and alkylating steps. Additionally, similar platinum localization was obtained when the enzymatic digestion of Pt–protein adducts was performed by high-intensity focused ultrasound (HIFU) instead of the conventional overnight digestion protocol at 37 °C. Only small differences were observed: in the case of the HIFU-assisted digestion, a monodentate ligand $Pt(NH_3)_2Cl$ was preferentially found linked to the protein, whereas overnight incubation produced the bidentate $Pt(NH_3)_2$ as the main protein-bound moiety. This was mainly related to the change in the pH of the medium (from acidic to basic), although the occurrence of slow kinetic processes should be taken into account.

Overall, the insulin case of study seemed to choose the bottom-up approach over top-down one. However, a work by Møller and coworkers on the determination of the binding site of oxaliplatin on insulin, opened another scenario where bottom-up method failed in the univocal assignment of the original binding sites.[56] Indeed, upon protein digestion of the platinated insulin, the author found that Pt-complex was released. At the same time, after proteolysis, the accessibility of amino acids changes. As a result, the released drug can rebind to different amino acids thus leading to the identification of a binding sites distribution distinct from the original one.

An additional comparative work was also performed on calmodulin (CaM).[57] In this case, up to 10 specific binding sites for mononuclear (cisplatin and [Pt(dien)Cl]Cl) or dinuclear ($[cis\text{-}PtCl_2(NH_3)]_2(\mu\text{-}NH_2(CH_2)_4NH_2)$) complexes were identified on CaM. Nevertheless, the global assignment of the Pt-protein binding sites was made possible only by a proper combination of the top-down and bottom-up mass spectrometric (MS) strategies, thus actually promoting their complementarity as the most successful approach.

It is worth to underline that a more comprehensive characterization of this system took advantage of a further contribution.[58] Indeed, only a novel computational method designed to simulate protein flexibility allowed to completely rationalize the crosslinking pattern that cisplatin generates on CaM. It evidenced that CaM motions play a fundamental role in allowing the drug to bring together the reactive sites of apparently distant Met residues. This result supports that computational methods can be easily applied to predict crosslinking pairs in proteins and to facilitate MS data analysis.

The critical issue in the preservation of metal-protein bond moves to another difficult level when the complexity of physiological matrices, such as plasma, is taken into an account. To assess the potential application of MS-based approaches on these systems, the behavior of a mixture of platinated proteins (equine cytochrome and myoglobin, bovine carbonic anhydrase, human serum albumin and transferrin) has been monitored by a gel-based bottom-up approach; in particular, the effect of in-gel protein digestion, as well as of their separation by the well-known sodium dodecyl sulfate polyacrylamide gel electrophoresis SDS-PAGE technique, on the preservation of Pt-protein binding sites has been evaluated.[59] The model proteins processed by in solution and in-gel digestion protocols without the use of reducing and alkylating agents were considered. The in-gel digestion method was found to be appropriate for the characterization of Pt-binding sites when performed in the absence of steps which require DTT and IAA. Additionally, this work underlined the potential effects of solvent. In particular, the recurring use of acetonitrile (ACN) during the digestion protocol was found to cause the exchange of nitrogen ligands. Nevertheless, platinum complex was not released from the original binding sites and thus, the position of platinum along the protein seems not to be impaired. This implies that the whole bottom-up approach starting from a 2D electrophoresis separation of proteins may be suitable for the identification of Pt-binding proteins in biological samples.

Finally, it was taken into an account that different delivery approaches can be applied in order to protect the drug from interaction with off-target counterparts: obviously, the formulation of the drug can contribute to lower the sensitivity of the protein-Pt adducts detection. This can be highly relevant with the use of liposomes which are specifically designed to not dissociate in order to prevent leakage of the active compound upon interactions with blood components. In this condition an analytical tool able to identify the drug and discriminate among encapsulated, free and protein-bound fraction would be greatly useful for drug release/stability studies and for the analysis of *in vivo* samples. This goal has been realized through the set-up of a capillary electrophoresis inductively coupled plasma mass spectrometry (CE–ICP–MS) method.[60] This was specifically designed to perform a simultaneous monitoring of the metal-based cisplatin and of the phosphorous-containing liposome, thus providing information on the behavior of both the drug and the vehicle. Additionally, the applied method resulted highly sensitive and thus it requires a small sample amount, is not time consuming and it is automatable. These properties made it suitable to be applied in drug formulation design and development.

In addition to the identification of protein platination sites, mass spectrometry technology is now able to provide protein structure information too. This is the case of one of the latest evolution of the instrumentation called ion mobility-mass spectrometry (IM-MS) which evaluates the collision cross-section (Ω) of proteins. Studies performed on ubiquitin in the presence of cisplatin evidenced up to three different conformations which are generated upon platination.[61] The collision cross-sections for each conformation suggest that the platinated proteins are contracted in size when compared to the unmodified ubiquitin

(with generally smaller Ω values), thus further empathizing how cisplatin-induced changes may have a significant effects on protein functions *in vivo*.

Further perspectives for platinum-protein complexes

So far platinum has been considered as the reactive species that we need to characterize by applying the most advanced tools of mass spectrometry. However, from these works, platinum has been turned into a valuable support for the mass spectrometry methodologies themselves.

As an example, cisplatin itself has been proposed as crosslinking agent useful in defining protein structure and interactions by MS techniques.[62] Indeed, it provides a wide range of advantages in comparison to other canonical reagents which rest in its ability to crosslink proteins as well as DNA and to target thioether and imidazole groups. Not less relevant, its unique isotopic pattern facilitates peaks identification whereas the two positive charges of Pt(II) promote the detection of cross-linked products .

In a different approach, platinum nanoparticles (PtNPs) have been reported to improve the analysis of amino acids, peptides, proteins and microwave digested proteins (lysozyme and bovine serum albumin) by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).[63] In this case the PtNPs were confirmed to increase the detection sensitivity by reducing the background associated to the organic matrices.

Moving even further from the pharmacological field, platinum-protein interactions can now find applications in very different fields. Recent examples are the use of platinum-protein complexes to realize novel self-assembled organometallic material endowed with unique chemico-physical properties,[64] to produce structured surfaces functionalized with enzyme for electroenzymatic measurements[65] or biosensor for quantitative protein detection[66] ending up with the use of platinum complexes in the design of selective artificial metalloproteases.[67]

Despite these appealing technical applications, it is worth to conclude this overview with a different perspective in medicinal chemistry. Indeed, we extensively discussed the interaction of platinum complexes with proteins as a step that sometimes competes but sometimes integrates their DNA-targeted mechanism(s) of action. However, we cannot forget that proteins represent powerful targets in medicinal chemistry. Keeping this in mind, we can imagine shifting our efforts from the aim of reducing protein reactivity toward the promotion of Pt-adduct formation on selected proteins relevant for defined pathologies.

Several examples are now emerging. Among them, one allows to summarize several concepts above discussed in this new light. It is represented by the use of Pt-based drugs in the treatment of Alzheimer disease (AD).[68] A principal actor in the development and progression of this pathology is the aggregation of β-amyloid peptides (Aβ): these aggregates may deposit as plaques in the brain parenchyma providing the most prominent pathological feature of AD. Additionally, soluble oligomeric forms are toxic and their concentration correlates with the disease progression. The Aβ aggregation process is driven by metal ions like Zn^{2+}, Fe^{2+} or Cu^{2+} which bind to these peptides at a metallo-binding motif containing three histidines located in the hydrophilic N-terminal domain (His6, His13 and His14).[69] Based on these physiological data, the currently explored strategies are devoted to reduce the conversion of Aβ into their oligomeric forms.

Thus, we have a metalloprotein as potential target - and Pt(II) can compete with the physiological/pathological ions involved in the aggregation process- and a histidine-rich binding site - and Pt(II) complexes can bind to these residues. As a consequence, the idea that Pt(II) complexes can interfere with the neurodegeneration caused by Aβ aggregates emerged. The pivotal paper on this topic led to the identification of 1,10-phenanthroline-Pt(II)Cl_2 complexes as promising agent able to prevent β-amyloid aggregation (Fig. 7).[70] Interestingly, it was found that the binding of platinum on the peptide does not induce the release of Cu^{2+} or Zn^{2+}. Nevertheless, the presence of the platinum complex shifts them to a secondary coordination site on Aβ causing an overall structural change in the peptide and producing a reduction of the metal-induced Aβ aggregation and toxicity.[71] This effects derives from the occupancy by platinum predominantly of two His, leaving one His residue available and sufficient for Cu^{2+} binding.[72]

Structural studies allowed rationalizing the selectivity towards Aβ shown by these complexes which is not shared by cisplatin.[73] Indeed, stacking interactions between the aromatic carrier and the aromatic residues present in the binding site (Phe, His, Tyr) turned out to be the responsible of such a selectivity. Moreover, as a consequence of this different interaction mode, 1,10 phentrantroline-based complexes were found to coordinate the imidazole group of His6 and His14 whereas cisplatin preferentially binds to Met35.[74]

The great enthusiasm associated to such a discovery is now prompting the research to the design of metal complexes suitable for clinical applications, again proficiently fishing out from the experience collected in the anticancer field. This implies the use of novel carrier groups which are more soluble, stable and selective for the target or the use of Pt(IV) complexes as orally bioavailable prodrugs.[75]

Conclusions

The results herein summarized are just some recent examples of the worldwide work that is currently undergoing to better understand the platinum-protein interactions. As far as we get into it, it becomes clearer that it is not possible to classify this process into a single category, i.e. cisplatin-associated toxicity. Conversely, it is a multifaceted topic which deserves a wide analysis in order to properly develop all its potential.

Legend to the figures

Fig. 1: Cisplatin derivatives worldwide approved (solid arrows) or in clinical trials (dashed arrows). In ProLindac, R corresponds to a 25 kDa linear polymer delivery vehicle based on hydroxypropylmethacrylamide (HPMA) functionalized with a linker in 10:1 ratio.

Fig. 2: Schematic mechanism for cisplatin or transplatin derivatives with methionine. Compounds in the box share the same geometry.

Fig. 3: Chemical structures of TPAs (SA1 and SA4) and of sterically hindered complexes (SH1 and SH2).

Fig. 4: Chemical structures of platinum complexes containing Pt-P bonds.

Fig. 5: Chemical structures of trinuclear platinum complexes.

Fig. 6: Tridimensional structures of HEWL cocrystallized with cisplatin (Panel A, PDB code: 4DD4) and of PGP 1 with terpyridine platinum complex (Panel B. PDB code 3RO0). Platinum complexes are in ball, bound aminoacids in orange (Hys15 and Cys144 for HEWL and PGP 1, respectively).

Fig. 7: Chemical structures of platinum complexes with potential application in Alzheimer disease.

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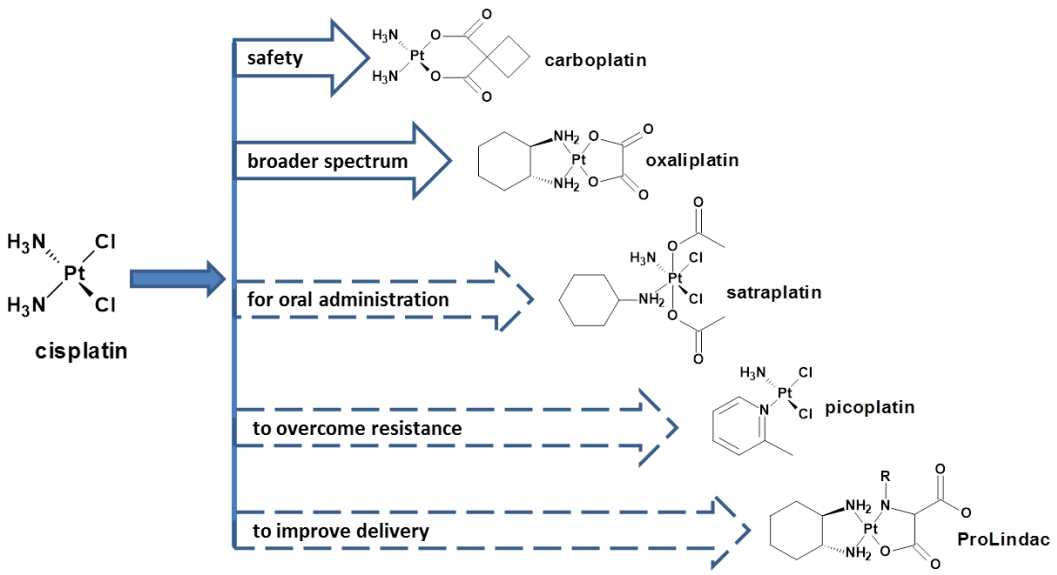


Fig. 1: Cisplatin derivatives worldwide approved (solid arrows) or in clinical trials (dashed arrows). In ProLindac, R corresponds to a 25 kDa linear polymer delivery vehicle based on hydroxypropylmethacrylamide (HPMA) functionalized with a linker in 10:1 ratio.

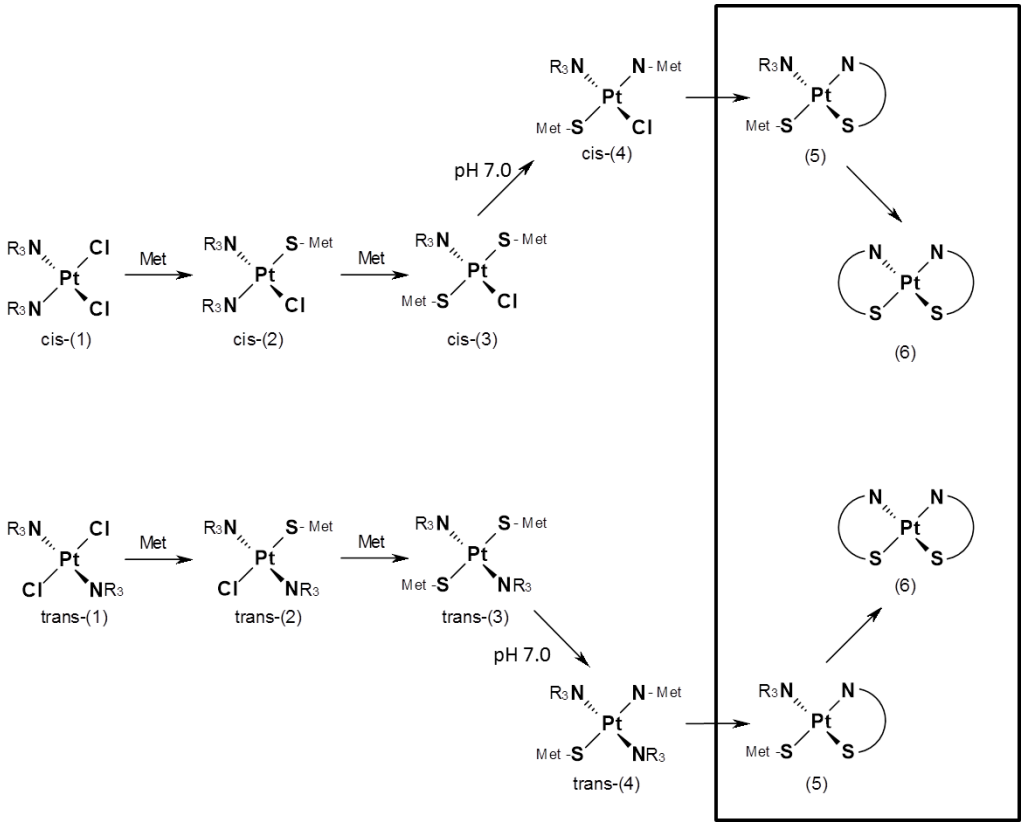


Fig. 2: Schematic mechanism for cisplatin or transplatin derivatives with methionine. Compounds in the box share the same geometry.

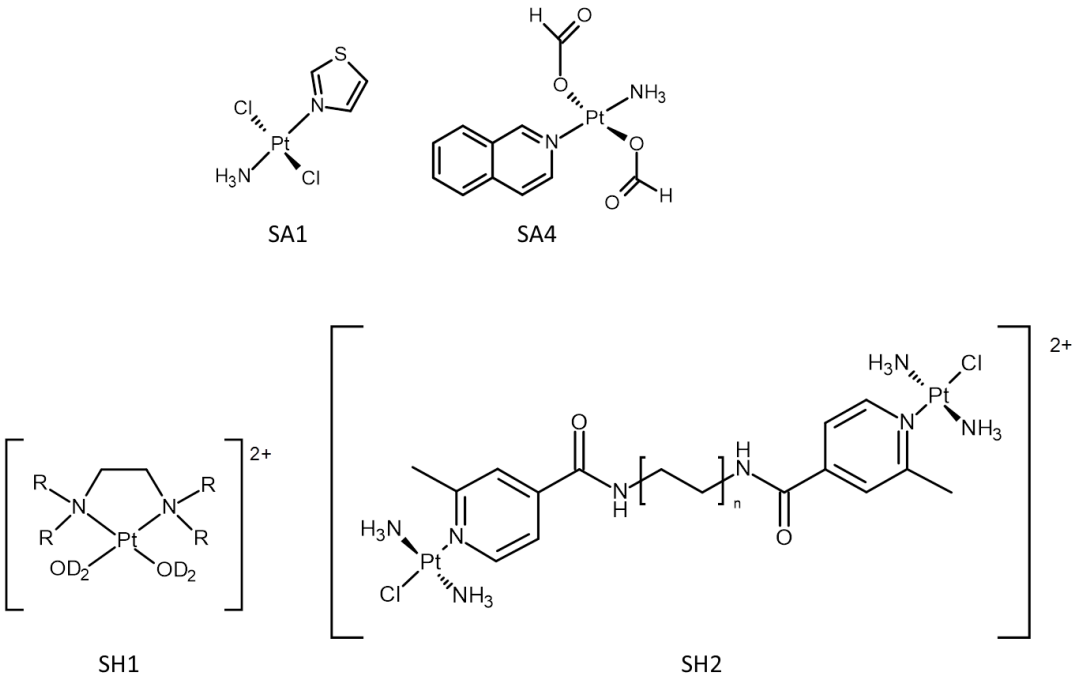


Fig. 3: Chemical structures of TPAs (SA1 and SA4) and of sterically hindered complexes (SH1 and SH2).

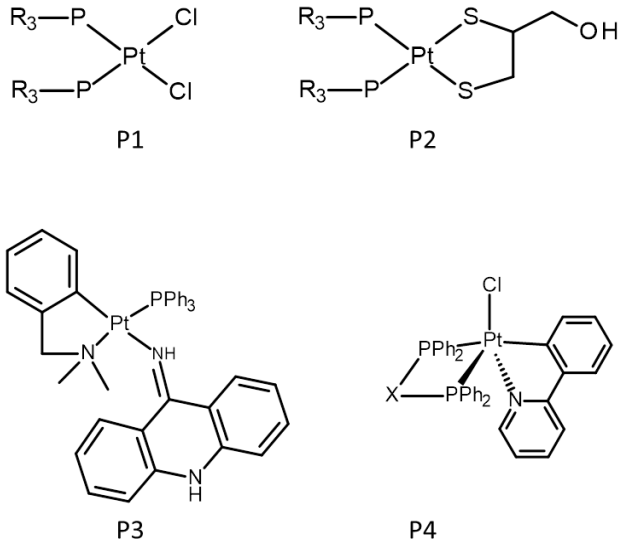
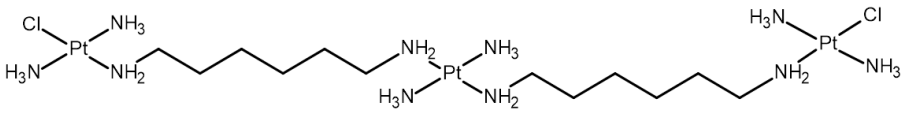


Fig. 4: Chemical structures of platinum complexes containing Pt-P bonds.



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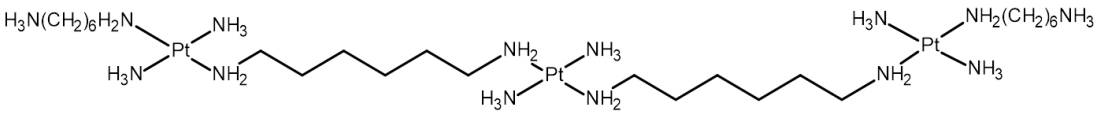
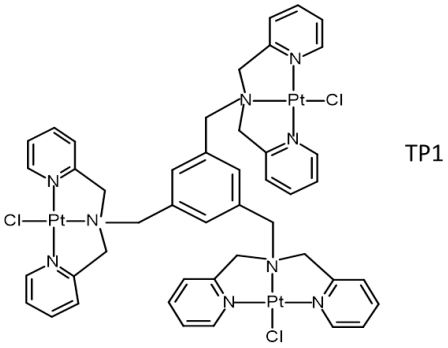


TriplatinNC



TP1

Fig. 5: Chemical structures of trinuclear platinum complexes.

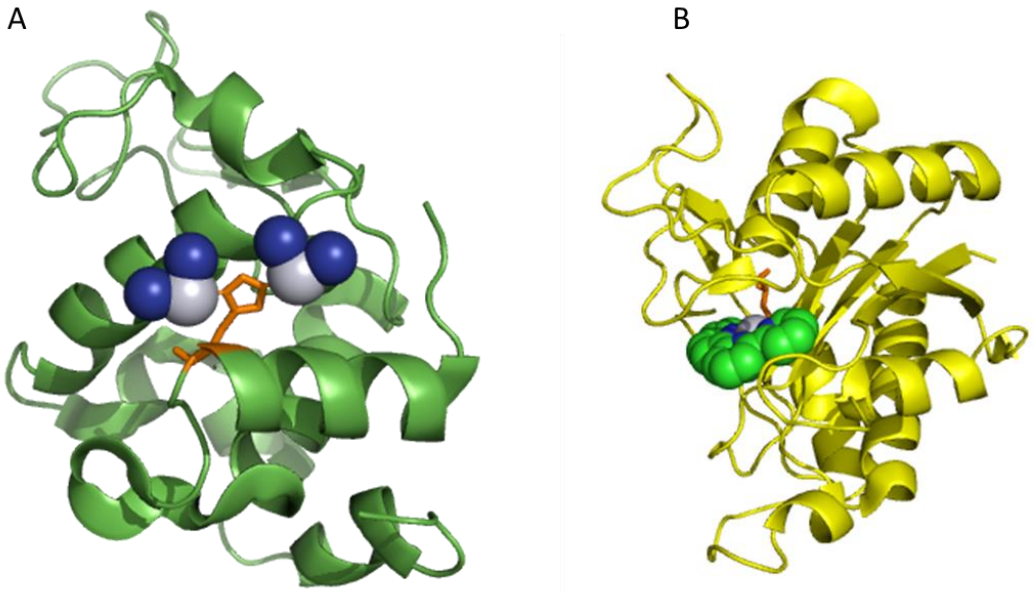


Fig. 6: Tridimensional structures of HEWL cocrystallized with cisplatin (Panel A, PDB code: 4DD4) and of PGP 1 with terpyridine platinum complex (Panel B. PDB code 3RO0). Platinum complexes are in ball, bound aminoacids in orange (Hys15 and Cys144 for HEWL and PGP 1, respectively).

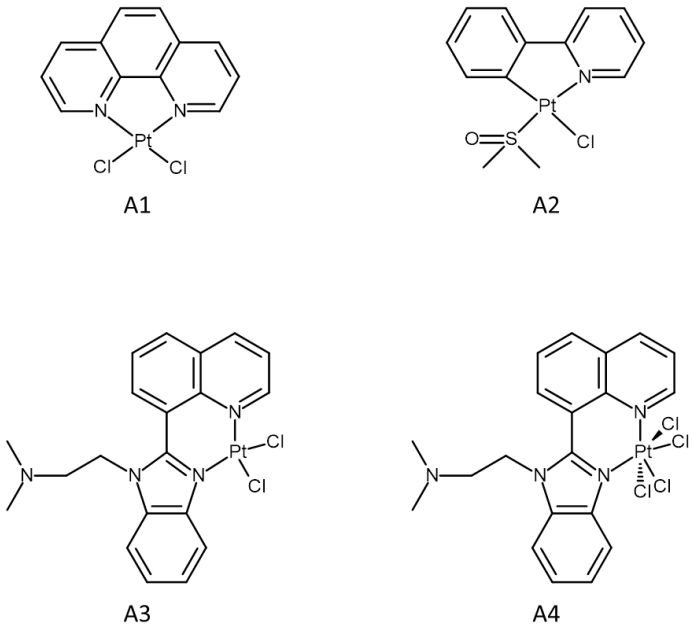


Fig. 7: Chemical structures of platinum complexes with potential application in Alzheimer disease.

Pt-based drugs: the spotlight will be on proteins

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The mechanism of action of platinum-based drugs derives from different contribution on DNA and proteins.