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Peptide-mediated vectorization of metal complexes: conjugation strategies and biomedical applications

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Abstract

The rich chemical and structural versatility of transition metal complexes provide numerous novel paths to be pursued in the design of molecules that exert particular chemical or physicochemical effects that could operate over specific biological targets. However, the poor cell permeability of metallodrugs represents an important barrier for their therapeutic use. The conjugation between metal complexes and a functional peptide vector can be regarded as a versatile and potential strategy to improve their bioavailability and accumulation inside cells, and the site selectivity of their effect. This perspective lies on reviewing the recent advances on the design of metallopeptide conjugates for biomedical applications. Additionally, we highlight the studies where this approach has been directed towards the incorporation of redox active metal centers in living organisms for modulating the cellular redox balance, as a tool with application in anticancer therapy.

Introduction

The rich chemistry of metal centers constitutes an opportunity for designing coordination complexes with biological and biomedical applications.¹⁻³ Metal complexes are chemically and structurally very versatile; the nature of the ligand and the metal ion can be chosen to endow complexes with diverse electronic, chemical, kinetic and photophysical properties.^{2,4} This versatility provides numerous novel paths to be pursued in the design of molecules that exert particular chemical or physicochemical effects that could operate over specific biological targets.^{5,6} However, biochemical processes in living organisms are very complex, with multiple interdependent connections. Therefore, basic understanding of the mechanisms of action and target sites of metal complexes in biological hosts are necessary before these compounds can eventually reach therapeutic applications.⁴⁻⁷

On the other hand, it is also currently recognized that the poor cell permeability of metallodrugs represents an important barrier for their therapeutic use.⁸ Tackling this problem is considered a key area in modern medicinal chemistry, since it is crucial for the drug efficacy, but also for the control of adverse effects and to overcome drug resistance.⁹⁻¹² Research efforts in the development of drug delivery systems have led to approaches useful to improve the efficacy of the existing metal complexes through enhancing their cellular uptake level and their targeted intracellular delivery. Besides,

another key point relies on addressing the metal complex to the tumor or tissue in a selective way through homing devices, thereby avoiding potential toxic side effects. Among multiple approaches, conjugation of metal complexes to peptide scaffolds is emerging as a promising strategy to enhance their uptake properties, cell-selective internalization and site-selective delivery.

Considering this frame, the focus of this perspective lies on reviewing the recent advances on metallopeptide conjugates used for biomedical applications. Given the large literature on the synthesis and biological evaluation of these conjugates, the scope of this review has been restricted to those including a non-peptidic pendant metal complex or metal-binding ligand linked to a peptide scaffold. Therefore, studies on catalytically active metallopeptides or integrated de novo metallopeptide designs have been excluded. Details about metallopeptide catalysts were reviewed by Lewis¹³ and interesting approaches involving metal-induced peptide self-assembly have been recently summarized by Tian and co-workers.¹⁴

Potential of metal complexes and peptide-based vectors: towards metallopeptides

Improving the selectivity and the therapeutic activity of the drugs is a major goal in the development of novel anticancer compounds. Even when several molecular-targeted drugs discriminate cancer cells from healthy cells, drug resistance and genetic mutations remain as key barriers in current cancer treatments.^{11,15} Bearing in mind these limitations, the targeting of biochemical alterations in cancer cells is one of the most promising strategies against drug resistance. A particularly interesting target element is the enhanced oxidative stress observed in cancer cells.¹⁶ In biological systems reactive oxygen species (ROS) are generated through different pathways and its homeostasis is essential for the normal cell growth.^{16,17} However, ROS enhancement is generally associated to a disruption in redox homeostasis, especially as a result from disruption of mitochondrial functions. Hence, the possibility to target the redox balance in cancer cells has emerged as an effective strategy to pursue selectivity over normal cells.^{16,18} Mitochondrial and nuclear compartments are particularly important subcellular target sites. The delivery of ROS-interacting drugs to the former is especially interesting since mitochondria regulate the redox balance and the apoptotic cell death.⁴⁻⁶

Owing to their redox properties, transition metal complexes can directly interfere with the cellular redox chemistry by creating or interacting with ROS, or indirectly by binding to biomolecules involved in redox homeostasis.⁴ This redox activity can be finely tuned by manipulating the oxidation state, electronics and redox properties of the complexes.² Indeed, a wide variety of transition metals can reach variable oxidation states and are susceptible to engage in reactions with ROS. The ligand can also play a key role in the expected biological activity because it can recognize and interact with the target site, and recognition may trigger a redox response. This control over the redox properties of the transition metal complexes can actually help avoiding undesirable toxicity in other biological sites.^{16,17,19}

Despite of the interesting properties that offer metallodrugs in current biomedical field, their enhanced and targeted delivery into the cell is challenging. In first place, little is known about their speciation in biological systems and their real involvement in cellular redox processes. Moreover, it should be taken into account that transition metal complexes can suffer drastic alterations inside cellular environments; ligand exchange or degradation and redox changes may occur, and some of these changes can result in release of the metal.⁵ If so, severe difficulties arise when elucidating mechanism of action of these metal-based agents in cellular redox homeostasis. Of note, Sadler and Lippard groups recently reported excellent reviews where the direct and indirect involvement of redox chemistry in the activity of a wide variety of metal-based anticancer drugs is discussed.^{4,5}

The conjugation of transition metal complexes to effective targeting vectors when pursuing an improved cellular uptake and targeted recognition is depicted as an excellent approach for taking advantage of ROS enhancement at specific sites. In this regards the conjugation between metal complexes and a functional peptide vector can be regarded as a versatile and powerful strategy. In fact, peptide-based approaches have been considered as useful tools to enhance cell entry and to deliver biologically active cargoes at the subcellular level.^{20,21} The so-called cell-penetrating peptides (CPPs) are short cationic and amphipathic sequences, containing less than 30 amino acids, which are able to cross the cellular membrane.^{22–24} These peptides have shown excellent properties for the intracellular delivery of a wide variety of drugs through the cell membrane.^{24–26} In the similar vein, other peptide sequences such as mitochondria-penetrating sequences (MPPs)²⁷ or more specific targeting sequences for nuclear (NLS)

and mitochondrial delivery (MLS) allow a broad range of possibilities to gain effective intracellular trafficking.²⁸ On the other hand, targeted drug delivery approaches are required to obtain selectivity against diseased tissues.⁹ Among target vectors for cancer cells, homing peptides (HPs) are employed to obtain a selective interaction with cell-surface receptors that are overexpressed in cancer cells.^{20,29} There are generally two kinds of HPs: (i) those with the capacity to recognize specific phenotypes but without cell-penetrating properties, and (ii) those which recognize the targeted cell but are also able to internalize inside the cell. The latter, also called cell-penetrating-homing peptides (CPHPs), are particularly challenging due to their suitability to be used as selective delivery vectors with reduced side effects.^{20,30}

However, the use of vectorized transition metal complexes as drugs is not free of problems. On the one hand, it is still difficult to determine to what extent the modulation of cellular redox processes is involved in the activity of transition metal complexes. On the other hand, the direct quantification of the cellular uptake is often controversial and complementary monitoring techniques are highly required. These limitations and the lack of clear knowledge of how the whole conjugate would work in a cellular environment constitute major problems when designing highly efficient systems based in transition metals.

Metallopeptides: Synthetic approaches

The preparation of metallopeptides is challenging because of the chemical functionality of peptides and the lability of metal complexes. Researches have taken advantage of the solid-phase peptide synthesis (SPPS) to prepare these compounds. These SPPS procedures were reviewed by Dirscherl and König in 2008.³¹ In particular, different approaches have been envisioned to obtain these conjugates using SPPS. One of them involves the attachment of the metal complex to the resin-bound peptide. In this strategy the use of an excess of the metal complex is required and, moreover, the resulting metallopeptide has to be stable to the final cleavage step from the resin. Alternatively, the metal complex can be linked in solution to the peptide which has been previously prepared on solid-phase. This protocol is especially useful for metallopeptides incorporating a very sensitive metal complex. Another versatile approach involves the coupling of the ligand moiety to the resin-bound peptide, followed by cleavage of the metal binding peptide and subsequent metallation in

solution. Following these approaches, different methods have been used to conjugate the metal complex or the ligand moiety to the peptide. One of the most extensively used methods is based on the formation of an amide bond. However, other strategies have been employed, such as Cu(I)-catalyzed azide-alkyne cycloadditions (Cu-AAC) or Pd-catalyzed Sonogashira cross-couplings. To illustrate these different synthetic approaches, herein we have selected a survey of recent examples of the preparation of metallopeptides.

The conjugation of metal complexes to a peptide sequence through amide bond formation was first described by Metzler-Nolte group in pioneering studies in this field. On the one hand, this group prepared metallopeptides that incorporate a metallocene bound to a nuclear localization sequence (NLS). These metallocene-NLS conjugates were synthesized by coupling cobaltocenium or ferrocene carboxylic acid to the resin-bound NLS, followed by their cleavage from the resin (Figure 1a).^{32,33} On the other hand, these authors also reported the synthesis of metallopeptides incorporating a NLS and a complex of Zn(II) or Cu(II) with a modified *N,N*-bis(2-picoly)amine (bpa) ligand (Figure 1b).³⁴ The bpa-NLS metal binding peptide was obtained using standard SPPS methods, whereas the metalation step was performed in solution. These early studies opened up the possibility to deliver biologically important metal ions at the subcellular level.

Later, this methodology was used by other authors to prepare new metallopeptides. For instance, Artaud and coworkers described the synthesis of metallopeptides by anchoring a tris(2-pyridylmethyl)amine (tpa) or a 2,2'-dipicolylamine (dpa) ligand to a resin-bound peptide followed by cleavage and metallation with a Zn(II), a Co(III) or a Cu(II) salt (Figure 1c).^{35,36} The peptides used in this study were the short antimicrobial peptide analogue RWRW-OBn and the efflux pump modulator PAβN. Recently, it has been reported the synthesis of Zn(II) and Cu(II) metallopeptides based on PyTACN and (*S,S'*)-BPBP tetradentate aminopyridine ligands in the context of ROS generation in cancer therapies (Figure 1d).³⁷ The preparation of these metallopeptides relied on the solid-phase synthesis of the corresponding metal binding peptide, subsequent cleavage from the solid support and metallation with a Zn(II) or Cu(II) salt. These two metal binding peptides were obtained following two different approaches. The synthesis of the metal binding peptide

incorporating a PyTACN ligand required the previous preparation of this ligand in solution, which was then coupled to the resin-bound peptide. In contrast, the (*S,S'*)-BPBP aminopyridine ligand was assembled to the peptidyl resin in a stepwise manner, including the attachment of a nicotinic acid derivative followed by derivatization with the corresponding secondary amine.

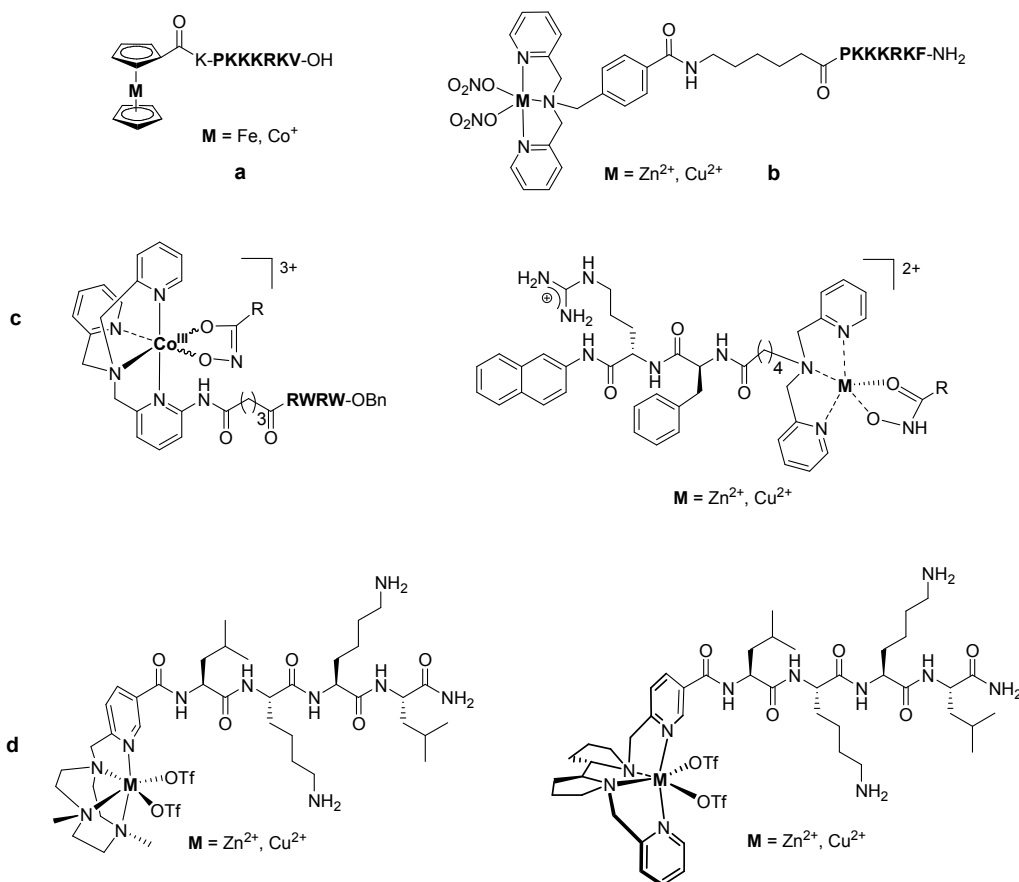


Fig.1 Metallopeptides obtained by conjugating the metal complex to the peptide through an amide bond.

A representative example of the synthesis of metallopeptides through the conjugation of metal complexes to peptides via the formation of a 1,2,3-triazole ring was reported in 2011 by Todd and co-workers for the preparation of Cu(II), Ni(II) and Zn(II) cyclam-tripeptide complexes (Figure 2a).³⁸ The methodology involved: (i) the solid-phase synthesis of an azidotriptide; (ii) the click reaction with the propargylcyclam derivative in solution; and (iii) the metalation of the resulting triazolyl conjugated with a Cu(II), a Ni(II) or a Zn(II) salt. The authors extended this procedure to the preparation of cyclam-(G)KLVFF hybrids, and their Zn(II) and Cu(II) complexes.³⁹ They observed that these compounds protect neurons from amyloid β

toxicity. Metzler-Nolte and co-workers also reported the use of click chemistry in standard SPPS for the conjugation of an azidomethylruthenocene moiety to resin-bound alkynyl peptides (Figure 2b).⁴⁰ This report shows the suitability of this ruthenocene moiety as a convenient building block to be introduced into peptide sequences under mild conditions. The same authors described a silyl-based linker useful for the synthesis of C-terminal acetylene-derivatized peptides which they reacted afterwards with azidomethylferrocene in solution to obtain the corresponding ferrocene-peptide conjugates (Figure 2c).^{41,42}

The usefulness of the CuACC and also of the Pd-catalyzed Sonogashira cross-coupling for the preparation of metallopeptides has been explored by Schatzschneider group (Figure 2d).⁴³ Towards this end, a tris(pyrazolyl)methane (tpm)-alkynyl complex ($[\text{Mn}(\text{tpm-L1})(\text{CO})_3]\text{PF}_6$) was prepared and then conjugated in solution to peptides carrying an azido or a iodoarene moiety. These functionalized peptides were previously prepared on solid-phase. Similarly, a Pd-catalyzed Sonogashira cross-coupling in solution between a ferrocene alkyne and a 4-iodophenylalanine-containing peptide was employed for the synthesis of ferrocene peptide derivatives by Metzler-Nolte group (Figure 2e).⁴⁴

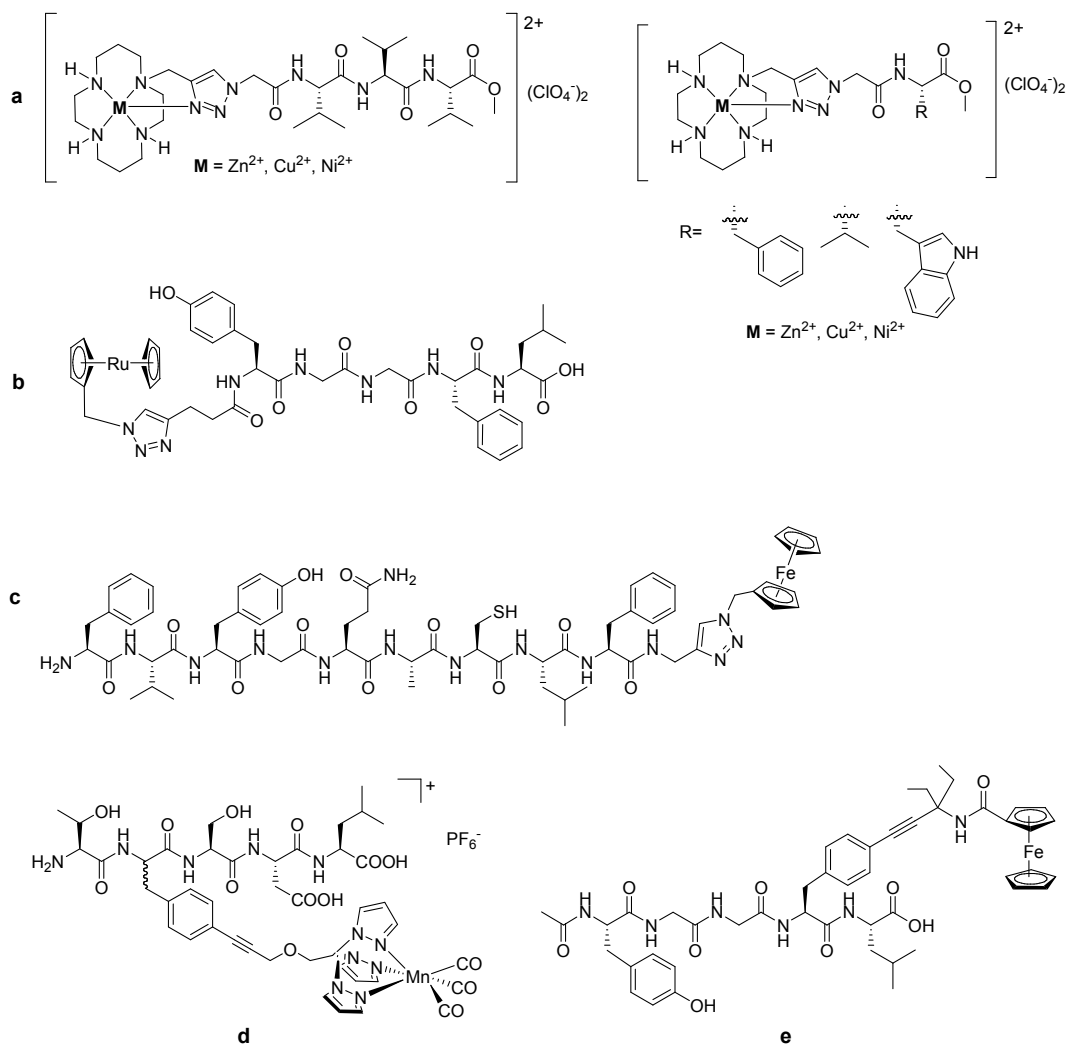


Fig. 2 Metallopeptides prepared using a Cu-AAC or a Pd-catalyzed Sonogashira cross-coupling.

In recent studies, Vázquez and co-workers took advantage of the standard SPPS procedures and of the intrinsic chirality of peptides to achieve the stereoselective formation of chiral metallopeptides. This work was prompted by the crucial role of chirality in recognition processes of biological targets.⁴⁵ For this purpose, following standard SPPS protocols, the authors synthesized metal binding peptides incorporating two bpy coordinating units joined by three to five amino acids that include a [D/L]-Pro-Gly β -turn-promoting moiety (Figure 3a). After the cleavage from the resin and metallation with Zn(II), Co(II) and Ni(II) salts, the corresponding chiral metallopeptides were obtained. This methodology was further extended to the preparation of chiral metallopeptide helicates with DNA-binding properties (Figure 3b).⁴⁶ The corresponding peptidic ligands were synthesized on solid-phase and contained six bpy units arranged

in three sets of two consecutive bpy moieties joined through two short loops that incorporate the [D/L]-Pro-Gly sequence. The peptide ligands were cleaved from the support and incubated with a Fe(II) salt. It was observed that the Pro residues direct the folding of the ligands and also encode the chirality of the helicates. Vázquez and co-workers also exploited the use of the bpy chelator for the preparation of dinuclear Ru(II) metalloptides (Figure 3c and 3d).⁴⁷ The synthesis of the bis-chelating peptide ligands and also their metalation were performed on solid-phase. This methodology easily allowed the incorporation of an octaarginine tail to these DNA-binding metalloptides to promote their cellular uptake.

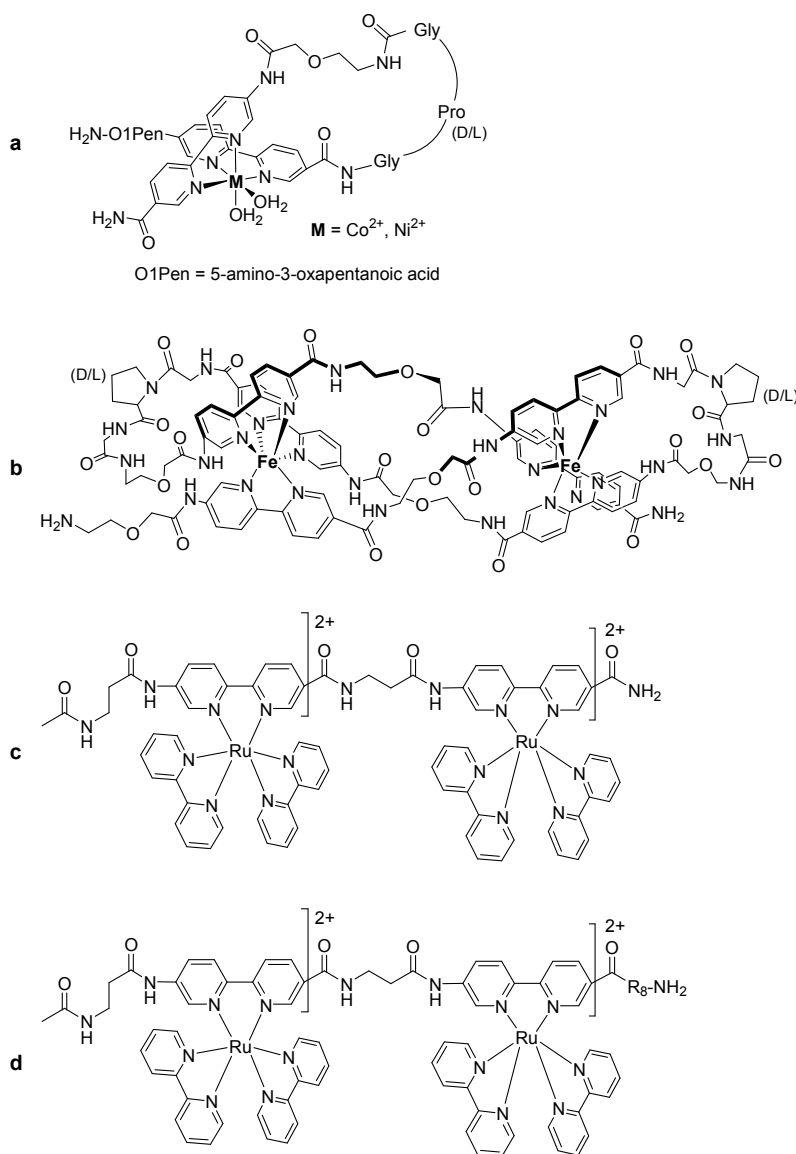


Fig. 3 Co and Ni chiral metallopeptides (**a**),⁴⁵ Fe(II) chiral metallopeptide helicates (**b**)⁴⁷ and dinuclear Ru(II) metallopeptides (**c** and **d**)⁴⁷ reported by Vázquez and co-workers.

Peptide-based delivery of metal complexes

Peptide-based delivery of bioactive metal complexes is specially challenging when seeking an improved stability, cell selectivity and a targeted subcellular localization. Therefore, the improvement in the cellular uptake properties of these compounds could lead to highly efficient metal-based systems where the final targeting and function is defined in the cellular environment. The examples covered in this

section deal with the conjugation of metal complexes to cell-penetrating sequences (CPPs), mitochondria-penetrating sequences (MPPs) or organelle-specific targeting sequences, such as nuclear or mitochondrial localization sequences (NLS and MLS, respectively).

Since CPPs effectively enhance the cellular uptake and physical properties of many cargoes, several studies have been described in the literature exploring the properties of metal complexes containing appended CPPs. Concerning DNA targeting studies with metalloptides, pioneering examples were described by Barton's group and have been recently reviewed.⁷ In light of this, metal-peptide conjugates of ruthenium and rhodium intercalators were extensively explored in an effort to evaluate DNA sequence selectivity, DNA hydrolysis as well as DNA cross-linking. Given the interesting DNA targeting properties of these metalloptides, Barton group functionalized a $[\text{Rh}(\text{bpy})(\text{chrysi})(\text{phen})]^{3+}$ complex with a D-octaarginine, which acts as a CPP (Figure 4a).⁴⁸ The results established that this CPP efficiently enhanced the cell internalization of the Rh^{3+} complexes and facilitated their nuclear localization. Although the presence of the CPP increased the non-specific binding to DNA, it did not notably influence the site specificity of the photocleavage. Taking advantage of these cellular uptake studies, Barton group evaluated the cell-penetrating properties of luminescent Ru(II) dppz complexes conjugated to D-octaarginine or to RrRK, and compared them with those of the corresponding fluorescein-labeled metalloptides (Figure 4b).^{49,50} This work emphasizes the importance of exploring the fluorophore effect, since it was noticed that the cellular uptake properties of the non-labeled metalloptide differed significantly from those of the labeled one.

CPPs based on arginine have also been conjugated to other metal complexes to search for an enhanced biological activity. Sadler and Brabec groups reported the conjugation of the Os(II) arene anticancer complex $[(\eta^6\text{-bip})\text{Os}(\text{pico})\text{Cl}]$ (bip = biphenyl, pico = picolinate) to an octa- or a pentaarginine sequence (Figure 4c).⁵¹ Despite the presence of a CPP did not improve the cytotoxic activity of the parent metal complex in cancer cells,^{52,53} it significantly enhanced its cellular uptake and its DNA binding. In support to these targeted delivery works, Keyes group exploited the capacity of Ru(II) polypyridyl peptide conjugates as probes for cell imaging.^{54,55} To this end, metalloptides based on $[\text{Ru}(\text{bpy})_2\text{pic}]^{2+}$ and $[\text{Ru}(\text{dppz})_2\text{pic}]^{2+}$ complexes conjugated to an octa- or pentaarginine sequence were prepared (Figures 4d and 4e). In particular, it

was shown that the peptide conjugate $[\text{Ru}(\text{dppz})_2\text{pic-Arg}_8]^{10+}$ (Figure 4e) is a versatile probe for combined confocal luminescence and resonance Raman imaging capable of selectively imaging the subcellular organelles.

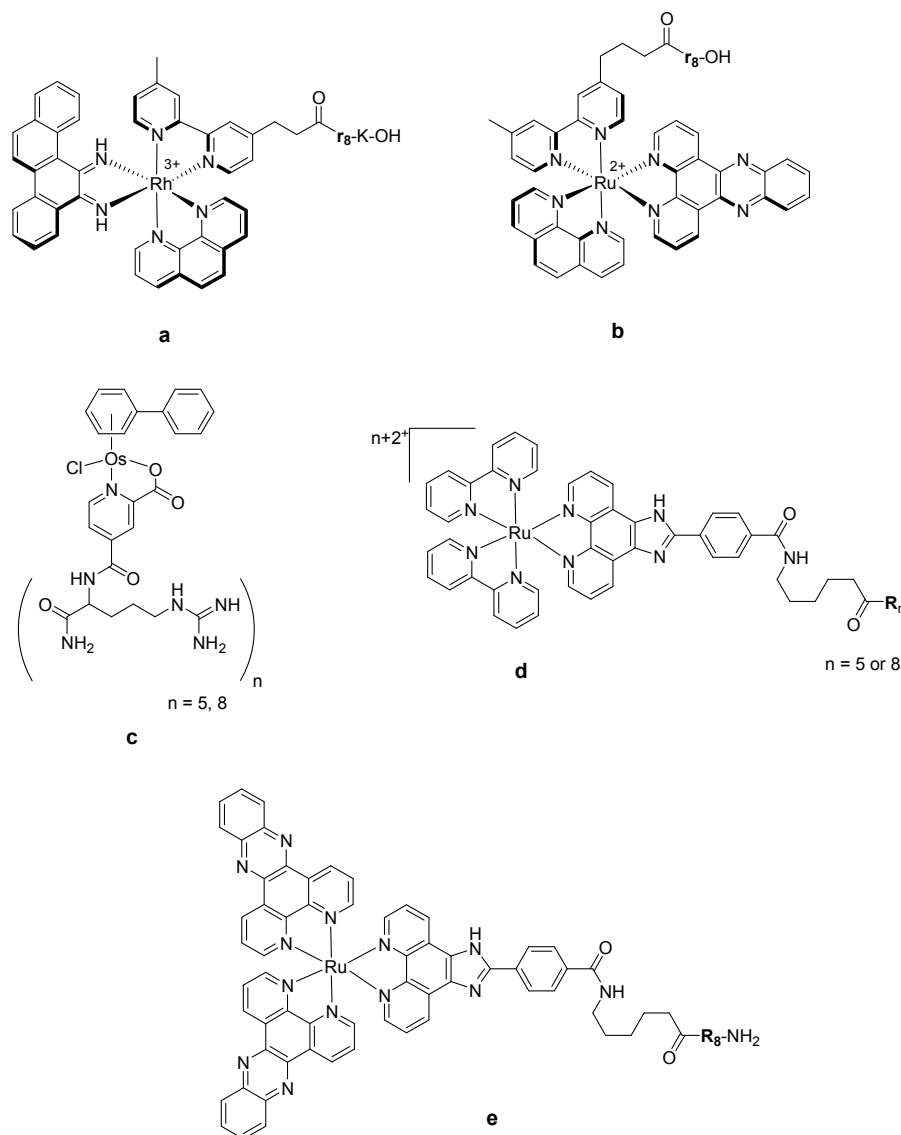


Fig. 4 Metallopeptides resulting from the conjugation of a metal complex and a CPP based on arginine.

Metallopeptides incorporating other CPPs such as Tat₄₇₋₅₇, sC18 or Penetratin have also been described. For instance, Keppler group reported the conjugation of a Pt(IV) analogue of oxaliplatin prodrug to the Tat₄₇₋₅₇ peptide (YGRKKRRQRRR-NH₂).⁵⁶ The resulting mono- and difunctionalized conjugates were found to be more cytotoxic than the parent platinum complex. Similarly, Gasser group conjugated a luminescent Re(I) tricarbonyl polypyridine-based complex to the myristoylated Tat₄₇₋₅₇

peptide via a CuACC reaction (Figure 5a).⁵⁷ This conjugation improved the cellular uptake and the cytotoxicity of the metal complex, being the resulting metallopeptide as cytotoxic as cisplatin against HeLa cells. Moreover, Neundorf and co-workers reported the attachment of cymantrene ($\text{CpMn}(\text{CO})_3$) carboxylic acid derivatives to the cell-penetrating peptide GLRKRLRKFRNKIKEK-NH₂ (sC18), which was previously identified by the same authors (Figure 5b).⁵⁸ This work rendered novel cymantrene-sC18 conjugates with promising antitumor activities against different cancer cells that were attributed to their efficient internalization. The biological activity could be further improved by incorporating a cathepsin B cleavage site (GFLG) next to the cymantrene moiety.

Metal chelators that are unable to access intracellular metal ions have also been efficiently internalized into cells by conjugation to a CPP. Recently, Esposito and coworkers reported metal binding peptides obtained by covalent attachment of the iron chelator desferrioxamine (DFO) to Tat₄₇₋₅₇ and to Penetratin (RQIKIWFQNRRMKWKK) (Figure 5c).⁵⁹ The resulting conjugates retained the iron binding abilities and the antioxidant properties of DFO, and showed an enhanced cell internalization capacity.

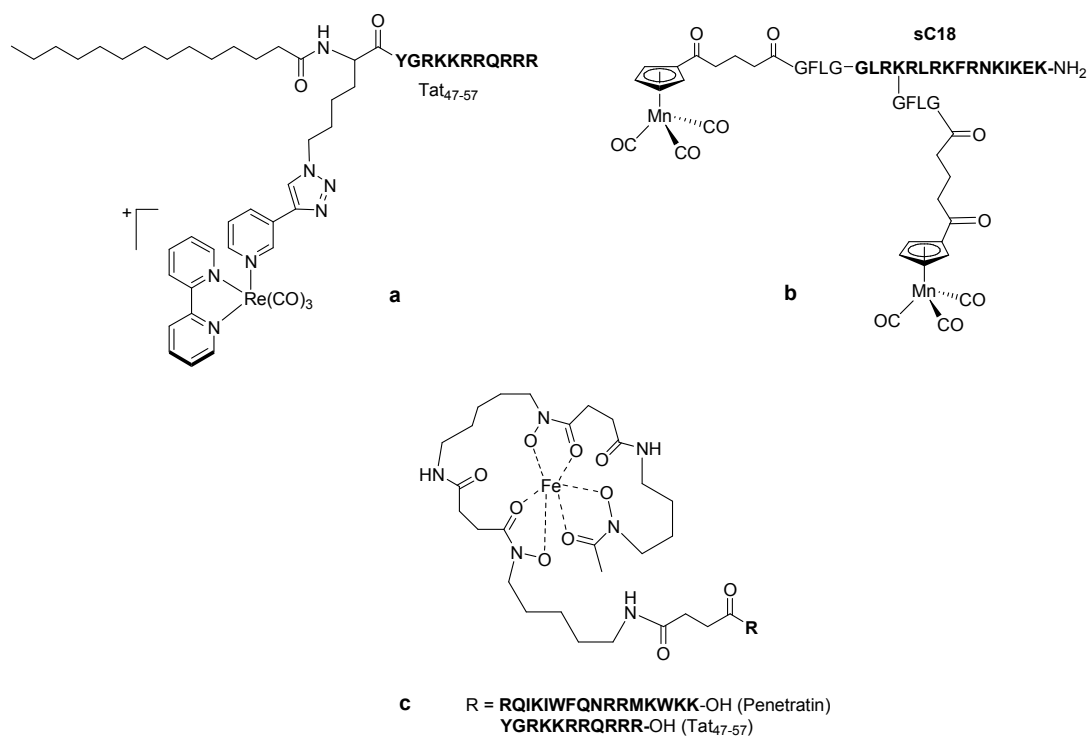


Fig. 5 Metallopeptides resulting from the conjugation of a metal complex and a CPP such as Tat₄₇₋₅₇, sC18 or Penetratin

Since DNA is the cellular target of many bioactive metal complexes, these compounds have been appended to a nuclear localization sequence (NLS). These sequences are small peptides that enable the active transport of drugs into the cell nucleus. Hence, the conjugation of a metal complex to a NLS constitutes a feasible approach to obtain a subcellular localization of these compounds. As previously mentioned, Metzler-Nolte group reported metallopeptides incorporating a metallocene covalently bound to a NLS (Figure 1a).^{32,33} It was found that whereas the metallocene moiety facilitates the cellular internalization, the NLS plays a key role in the nuclear localization of the conjugate. Moreover, these metallopeptides resulted to be non-toxic. Similarly, Keyes group achieved the selective delivery to the cell nucleus of two luminescent Ru(II) polypyridyl complexes, [Ru(dpp)₂pic]²⁺ and [Ru(bpy)₂pic]²⁺, by conjugating them to the NLS peptide VQRKRQKLMP-NH₂, which derives from the transcription factor NF-κB.⁶⁰

Moreover, the unique properties of transition metal complexes have been also employed for monitoring the intracellular redox status. Since in mitochondria the redox balance plays an important role, the vectorization of a metal complex into this organelle has been used to monitor changes in oxygen concentration as well as ROS generation. In this context, Keyes and co-workers reported the mitochondrial vectorization of a dinuclear Ru(II) polypyridyl complex.⁶¹ A metallopeptide was designed consisting of two luminescent Ru(II) complexes, based on [Ru(bpy)₂phen]²⁺, bridged across the mitochondrial penetrating peptide (MPP) FrFKFrFK-NH₂ (Figure 6a). It was demonstrated that the resulting conjugate [(Ru(bpy)₂phen-Ar)₂-MPP]⁷⁺ selectively accumulates inside the mitochondria, being able to monitor changes in oxygen concentration within this organelle. This study highlights the usefulness of metallopeptides for in-cell sensing purposes.

On the other hand, metal complexes initially designed for photodynamic therapy have also been explored in anticancer treatment via their targeted delivery. In this regard, Gasser group recently published several studies where phototoxic Re(I) tricarbonyl *N,N*-bis(quinolinoyl) complexes were conjugated to a NLS (Re-NLS) and to a derivative of the neuropeptide bombesin (Re-bombesin) in order to improve the

selectivity of these complexes (Figure 6b).⁶² The Re-NLS conjugate significantly accumulated into the cell nucleus and both conjugates induced DNA damage via production of $^1\text{O}_2$, as shown by DNA photocleavage studies. The activity of these conjugates was improved by using a photocaged strategy.⁶³ An *o*-nitrophenyl photolabile linker (PLPG) was incorporated between the Re(I) complex and the corresponding peptide sequence (Figure 6c). Re-NH₂ was selectively photoreleased from Re-PLPG-NLS and Re-PLPG-bombesin conjugates and, upon irradiation, both metalloptides showed IC₅₀ values similar to those of cisplatin against HeLa cells. Following a similar approach, the same group described the photocaging of a cytotoxic ferrocenyl derivative (Fc-NH₂) which acts as a ROS-generation catalyst in cancer cells. This photocaging was achieved with an *o*-nitrophenyl or an *o*-nitrobiphenyl photolabile protecting group (PLGP) and the photocaged conjugates were further linked to the MLS CrFK-NH₂ (Figures 6d and 6e).⁶⁴ Both metalloptides were less cytotoxic than Fc-NH₂ in the dark and, upon irradiation, the conjugate incorporating the *o*-nitrobiphenyl cage was the most active against the cancer cell lines tested. Besides, upon irradiation, the two conjugates displayed poor cytotoxic effects in non-malignant cells.

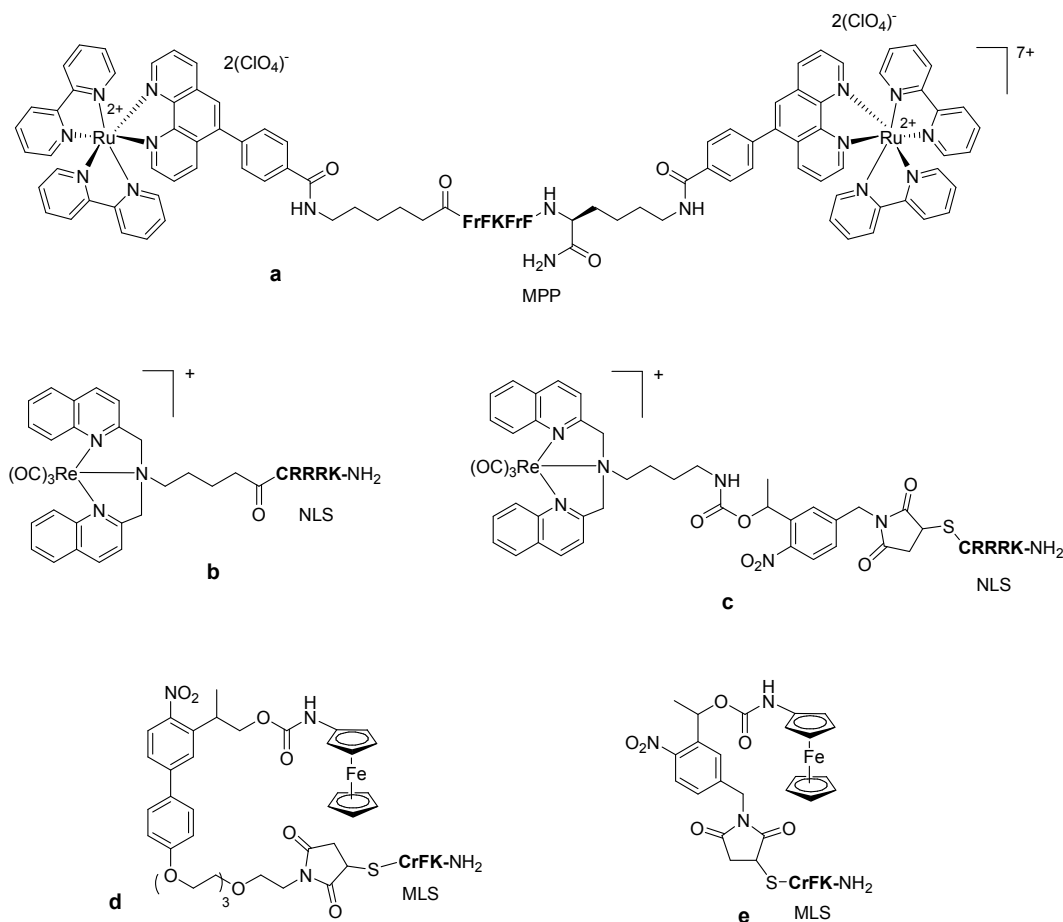


Fig. 6 Metallopeptides resulting from the conjugation of a metal complex and a NLS, a MPP or a MLS.

Cell-selective delivery of metal complexes for anticancer therapy

In order to overcome harmful side-toxic effects of metal-based anticancer complexes, cell-targeting strategies could be also rationally designed using peptides as site-selective delivery devices. Selected recent examples covering this concept based on the conjugation of metal complexes to cell-penetrating-homing peptides (CPHPs) are described in the following lines.

Proof of concept of this approach can be found in work by Lippard and co-workers, who described for the first time mono- and difunctionalized Pt(IV) metallopeptides containing RGD and NGR motifs (Figure 7). RGD and NGR peptides render selective recognition to malignant cells through binding to the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins and to cells expressing aminopeptidase N (CD13), respectively.^{30,65} This study highlighted the opportunity to obtain a tumor-selective delivery through integrin and

amino-peptidase N (APN) receptors. Remarkably, Pt(IV)-RGD conjugates were found to be more active than non-targeted Pt(IV) compounds and displayed significant inhibitory effects against different cell lines.^{66,67}

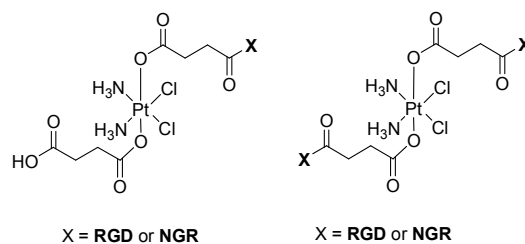


Fig. 7 Some representative examples of the Pt(IV)-RGD and NGR conjugates synthesized by Lippard group.⁶⁶

Along the same line, the groups of Marchán and Sadler recently reported on the biological potential for anticancer treatment of multiple ruthenium and platinum-based prodrugs conjugated to receptor-binding peptides. Two metallopeptides were first synthesized based on the conjugation between a ruthenium(II) arene complex and two homing peptides, a dicarba analogue of octreotide and the RGD peptide (Arg-Gly-Asp) (Figure 8).⁶⁸ Octreotide is a potent cyclooctapeptide agonist of the endocrine hormone somatostatin, which is overexpressed in cell membrane of various malignant cells, and it can be envisioned that their corresponding conjugates may display cell-selective antitumor properties. In the dicarba analogue of octreotide, a disulfide bond is replaced by the CH₂-CH₂ linkage. This modification increases its stability in the reductive cellular environment, without altering the binding affinity toward its somatostatin receptor. Upon irradiation with visible light, both metallopeptides were selectively photodissociated from the ruthenium complex and the resulting aqua species $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{bpm})(\text{H}_2\text{O})]^{2+}$ (cym = $\eta^6\text{-}p\text{-cymene}$, bpm = 2,2'-bipyrimidine) reacted preferentially with guanine nucleobases. Their mechanism of action towards DNA was extensively studied by UV-Vis and NMR spectroscopy, suggesting the preference of ruthenium(II) active species to react with the guanine moiety rather than peptide ligands. Bearing in mind this pioneering design, a thorough follow-up study reported by the same groups aimed at evaluating the biological activity in cancer cells lines of octreotide conjugates containing $[\text{PtCl}_2(\text{dap})]$ (dap = 1-(carboxylic acid)-1,2-diaminoethane), $[(\eta^6\text{-bip})\text{Os}(4\text{-CO}_2\text{-pico})\text{Cl}]$ (bip = biphenyl, pico = picolinate), $[(\eta^6\text{-}p\text{-cym})\text{RuCl}(\text{dap})]^+$ and $[(\eta^6\text{-}p\text{-cym})\text{RuCl}(\text{imidazole-CO}_2\text{H})(\text{PPh}_3)]^+$ ($p\text{-cym}$ = p -

cymene).⁶⁹ Although the prepared compounds displayed lower cytotoxic activities compared with their parent complexes, this study emphasized the opportunity to target tumor cells due to the conjugation to a homing peptide. In an effort to further improve this design, the authors recently described the preparation of a photoactivable Pt(IV)-c(RGDfK) and its biological evaluation in cells overexpressing $\alpha v \beta_3$ integrin (Figure 9a).⁷⁰ The RGD motif was tethered to the cytotoxic moiety *via* a polyethyleneglycol spacer. Preliminary phototoxic studies were carried out in the presence of 5'-GMP and further investigation was performed in different cancer cell lines, highlighting the selective phototoxicity observed upon irradiation in SK-MEL-28 melanoma cancer cells overexpressing the desired receptor. In the same direction, the conjugation of the Pt complex to the tetrameric RAFT-RGD peptide (RAFT = regioselectively addressable functionalized template cyclodecapeptide scaffold) increased by 20-fold the antitumor efficacy in comparison to cytotoxicity observed by picoplatin alone.⁷¹ This enhanced activity was explained by the selective binding of the RGD motif, which led to a higher intracellular accumulation of the Pt(IV) prodrug in cancer cells. On the other hand, the conjugation of different Ru(II) polypyridyl complexes to the linear RGD peptide was described by Keyes group. The resulting Ru metalloptides exhibited binding to targeted integrins present in live Chinese hamster ovary (CHO) living cells as observed by colocalization studies and, therefore, they are potential peptide probes in the biomedical field.⁷²

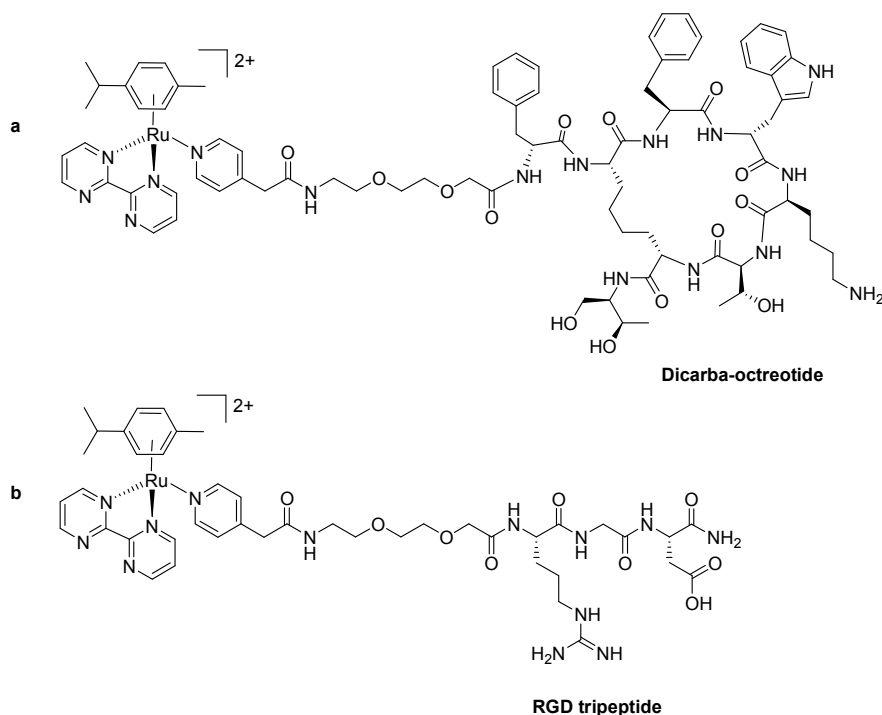


Fig. 8 Structure of the octreotide (a) and RGD (b) conjugates synthesized by Marchán and Sadler groups.⁶⁸

Beyond the somatostatin and integrin receptors, the conjugation of metal complexes to bombesin peptide (BBN), which shows high affinity for the gastrin-releasing peptide receptors (GRPR), has also attracted attention. For example, different conjugates containing Re(I) tricarbonyl *N,N*-bis(quinolinoyl) complex, zinc phthalocyanine or Ru(II)-polypyridyl complexes have been synthesized and screened for their photodynamic efficacy against different cancer cell lines expressing different levels of GRPR.^{63,73,74}

In an effort to overcome drug resistance, especially towards the multiple anti-apoptotic signaling pathways that hamper the biological action of conventional chemotherapeutics, the Ang group has recently reported the design of a biphasic mode of action based on Pt(IV)-conjugates.⁷⁵⁻⁷⁷ The authors introduced a HER2-targeted Pt prodrug design, which consists on the conjugation of cisplatin and oxaliplatin to the AHNP (anti-HER2/neu peptide) targeting peptide moiety.⁷⁸ In light of this, this design is meant to selectively internalize through HER2 expressing cells and further induce a rapid killing through targeted necrosis instead of apoptosis, thus avoiding apoptosis resistance mechanisms. The Pt(IV) prodrug scaffolds were effectively conjugated to a

AHNP peptide *via* a chemoselective oxime ligation strategy and also to a small tripeptide spacer KGG (Figure 9b). Both conjugates exhibited enhanced cellular uptakes compared to the ones observed by their parent Pt drugs and, moreover, displayed selective cytotoxic activities. Thus, this strategy represents an excellent example in which an alternative design is capable to overcome apoptotic resistance inducing HER2-targeted necrosis in cancer cells.

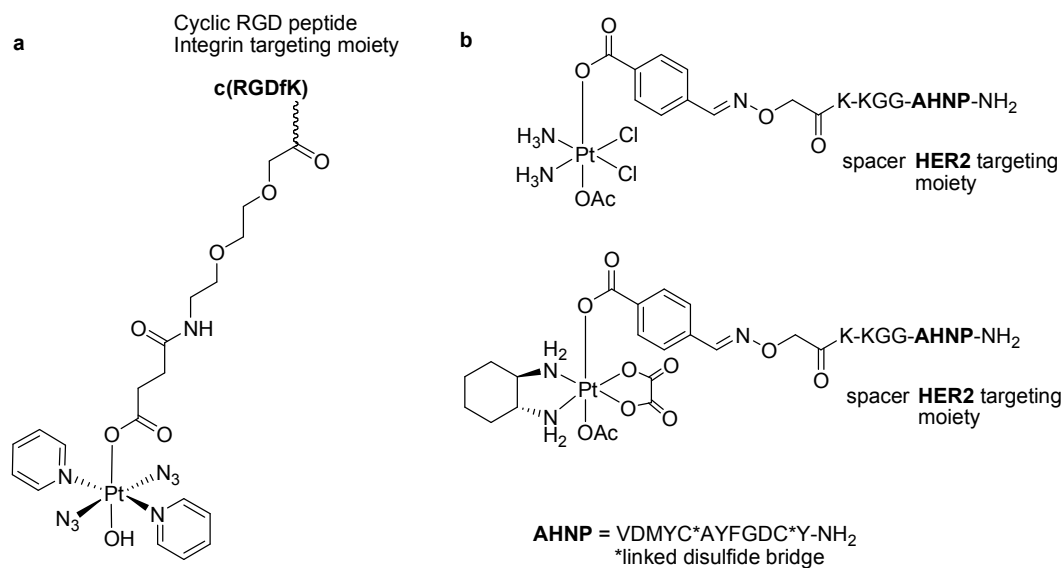


Fig. 9 Structures of platinum-based peptide conjugates; Pt-c(RGDfK) conjugate (a)⁷⁰ and HER2-targeted Pt anticancer prodrugs (b).⁷⁸

Outlook

Metal complexes provide a highly versatile platform to design metal-based drugs. To improve their cellular uptake, efficacy and stability, their conjugation to peptide-based delivery vectors is depicted as a potential strategy in the field of modern medicine. This perspective is a survey of selected works that report pioneering designs and synthetic strategies within the context of peptide-based drug delivery and biologically active metallodrugs. Moreover, considering the toxic side effects in therapeutic treatments, it is evident that the pioneering results found for the examples highlighted herein are certainly highly motivating for scientists working to design metal-based prodrugs with cell selective specificities. Although using peptides as targeting devices in *in vivo* studies may lead to some drawbacks related to chemical instability when exposed to plasma enzymes,^{22,28} other approaches such as using protease-resistant D form of peptides, backbone stabilization or side-chain derivatization

are meant to extend their safety as well as their efficacy while pursuing very low toxicity.^{22,79} Thus, given the biological potential of metallopeptide conjugates, undoubtedly these designs will attract more attention and play a key role in synthetic chemistry as well as in medicinal chemistry in the coming years.

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Amino acid nomenclature: Alanine: Ala, A; Arginine: Arg, R; Asparagine: Asn, N; Aspartic acid: Asp, D; Cysteine: Cys, C; Glutamic acid: Glu, E; Glutamine: Gln, Q; Glycine: Gly, G; Histidine: His, H; Isoleucine: Ile, I; Leucine: Leu, L; Lysine: Lys, K; Methionine: Met, M; Phenylalanine: Phe, F; Proline: Pro, P; Serine: Ser, S; Threonine: Thr, T; Tryptophan: Trp, W; Tyrosine: Tyr, Y; Valine: Val, V

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