Trans-ruthenium(II) complexes for photoactivated cChemotherapy: from design to anticancer activity

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Chapter 1

Transition-Metal Complexes in Cancer Therapy: Mechanistic and Photochemical Perspectives

1.1 Anticancer drugs based on transition metals

1.1.1 Platinum chemotherapeutics

Transition metals including iron, zinc, copper, or manganese are essential in many biological processes. For example, iron is well-known for its crucial role in dioxygen transport in mammals, with a human body of 70 kg containing about 5 g of iron.[1] It is therefore not surprising that a large amount of research has been dedicated to applying metal-containing compounds in modern medicine. In fact, a large number of inorganic compounds are currently in clinical use as imaging agents, diagnostics, or chemotherapeutics such as antibacterial, antibiotic, antiviral or antiparasitic drugs.[2] In oncology, however, only a handful of "blockbuster" metallodrugs have been approved for anticancer therapy.

First recognized for its antiproliferation properties in 1965 by Barnett Rosenberg, Loretta van Camp and Thomas Krigas, *cis*-diamminedichloridoplatinum(II) (commonly referred to as cisplatin) is the most successful metallodrug for anticancer therapy (Figure 1.1).[3] After its clinical introduction in 1978 for the treatment of testicular and ovarian cancers, cisplatin has become part of standard-of-care treatments against many types of cancer. Despite its success, patients treated with cisplatin experience severe side effects which arise from the toxicity of this compound to virtually every organ, but more particularly acute to the liver, heart and kidneys. Additionally, resistances of tumors first treated with cisplatin is often observed during relapse.[4] These clinical limitations have led to an ever-evolving field of research targeted to the development of new platinum-based anticancer drugs.

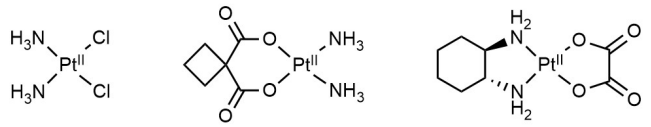

Figure 1.1 Selection of platinum-based metallodrugs for cancer treatment.

It is nowadays recognized that the curing power of cisplatin resides in its wide range of modes-of-action.[4] However, the activity of cisplatin is often primarily attributed to the two reactive chloride ligands dissociating in intracellular conditions, thus "releasing" an activated platinum (Pt) center that can subsequently bind to biomolecules such as proteins or deoxyribonucleic acid (DNA). Dissociation of the chloride ligands is a thermal process that is non-specific to cancer cells, which causes systemic toxicity. The chelating ligand cyclobutane-dicarboxylate in carboplatin (Figure 1.1) was introduced as a protecting group preventing thermal hydrolysis. *In vitro*, carboplatin requires an esterase to lead to active cleavage of the bidentate chelating ligand, reducing the toxicity of the compound and allowing for use of higher doses of the chemotherapy agent. Long-term use of cisplatin or carboplatin, however, both results in drug resistance, which led to the development of oxaliplatin, which bears a chelating diamine ligand (Figure 1.1). While oxaliplatin produces a comparable therapeutic effect, this compound showed no cross-resistance with cisplatin

or carboplatin, suggesting it works via a different mode-of-action than cisplatin and carboplatin. Overall, despite the recognized efficacy of these three worldwide-approved Pt-based drugs and their presence in ~50% of chemotherapy regimens, their overall toxicity remains a medical challenge in anticancer therapy.

1.1.2 Mode-of-action

The undeniable clinical success of cisplatin, carboplatin and oxaliplatin for cancer treatment has led to in-depth investigation into the mode-of-action (MoA) of these chemotherapeutics. For these Pt-based drugs, the binding of Pt to guanine bases in DNA is usually proposed as their principal MoA, forming intra-strand and inter-strand crosslinks, ultimately leading to apoptosis.[5] However, the reactivity of Pt towards sulfur, phosphorous and nitrogen-based ligands suggest interactions with biomolecules containing these elements that are not nuclear DNA. This hypothesis is consistent with the generally accepted idea that only 1-10% of cisplatin ends up in the nucleus.[6] In addition to causing nuclear DNA damage, cisplatin affects many other subcellular processes, reducing proliferation. These effects include the formation of high levels of mitochondrial reactive-oxygen species, an increase of the endoplasmic reticulum (ER) stress, impairment of the function of the cytoskeleton and acidification of the cytoplasm.[7–10] Apart from disrupting intracellular processes, cisplatin has also been shown to interact with the solid tumor microenvironment and to exhibit important immunomodulatory effects.[11] The major histocompatibility complex class I (MHC-I) is a recognition factor present on the cell surface, which activates a T-cell mediated immune response. As this represents a way to evade the immune system, MHC-I is down-regulated in many cancer cells. Interestingly, up-regulation of MHC-I surface expression upon treatment with cisplatin has been reported to occur in several human lung carcinoma cell lines.[12] This interaction with the cancer-immune interface highlights the capabilities of Pt-based chemotherapeutics to act as immunomodulators, as reviewed by Biasi et al. for cisplatin.[13]

The broad range of biological processes within a tumor that are affected upon treatment with Pt-based metallodrugs can be viewed as a double-edged sword. On the one hand, such multi-targeting characteristics allow these drugs to be “universally applicable”, i.e., they can be used to treat many cancer types with a lower susceptibility to genetic mutations that would cancel the activity of drugs specifically targeting a certain mutation in a given tumor. On the other hand, severe side effects remain common for these “broad-spectrum” anticancer drugs such as pain, nausea, or damage to the hearing system, which limits the drug dose that can be given to patients, thereby limiting their therapeutic efficacy.

1.1.3 Ruthenium-based anticancer complexes

The limitations of Pt-based chemotherapeutics in the clinics has sparked the search for novel metallodrugs based on alternative transition metals, including for example gold, palladium, iron, or ruthenium.[14,15] For ruthenium, a wide range of compounds have been described in the scientific literature as potential anti-tumor drugs, but only three of these

have made it to clinical trial. They can be divided into two types. On the one hand, photosensitizers, which require the action of light to deliver their cytotoxic load, which have been reviewed by McFarland et al.[16] A lead compound in this class, TLD-1433 (Figure 1.2), is currently evaluated in a phase II clinical trial for the treatment of invasive bladder cancer. On the other hand, cytotoxic prodrugs that do not require any external trigger but are activated spontaneously upon entering the body or cancer cells. The latter family of compounds consists of a class of compounds similar to Pt-based drugs, which affect one or more cellular processes, resulting in anti-tumor and anti-metastatic activity that are as effective, or better, as Pt-based compounds.

The first clinically evaluated Ru compound, NAMI-A, was discovered by Mestroni and Alessio in the early 1990's following the preparation of ruthenium(III) complex, Na[*trans*-$RuCl_4$(dmso-*S*)(imidazole)]. This complex was shown to have promising anti-tumor and anti-metastatic effects in a metastatic breast-cancer mice model.[17] The imidazolium salt NAMI-A (Figure 1.2) was developed as a more stable and water-soluble analogue of this complex.[18] Despite the promising pre-clinical results, the Phase I trial indicated severe side effects without a significant decrease in disease progression in several cancer types. In parallel, another class of structurally related anticancer ruthenium complexes were developed by Keppler and coworkers.[19] Of this family of compounds, KP1019 was the first candidate that was clinically evaluated (Figure 1.2). Unlike for NAMI-A, the phase-I study of KP1019 revealed no serious side effects and it was found to stabilize the disease for up to ten weeks in five of the six patients included in the trial, who had been diagnosed with varying cancer types.[20] Although good tolerability was shown for KP1019, the ending patent and sub-optimal solubility led to the development of the sodium salt analogue referred to as KP1339. This more soluble compound, commercially named BOLD-100, recently completed clinical phase II trial and has entered clinical phase III.

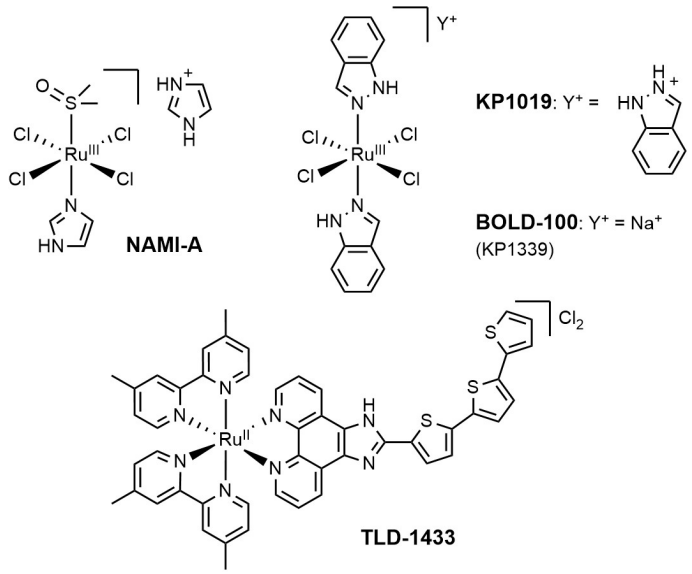

Figure 1.2 Selection of ruthenium-based metallodrugs for cancer treatment.

The Ru(III) coordination compounds NAMI-A and BOLD-100 are structurally related but exhibit distinct biological and pharmacological profiles. Compared to cisplatin, NAMI-A was found to be approximately 1000 times less cytotoxic in ovarian and breast cancer cell lines (EC_{50} > 500 µM).[21] The observed association with cell membranes contrasts with the typical cellular internalization found for many other compounds; it might be an explanation for the strong antimetastatic effect of NAMI-A, while its cytotoxicity is usually considered as low to negligible.[22] For KP1019 and its sodium salt analogue BOLD-100, slightly higher cytotoxicities were observed *in vitro* in several cancer cell lines (EC_{50} ≈ 100 µM).[23] Interestingly, BOLD-100 was shown to primarily induce ER stress in human patient-derived pancreatic ductal adenocarcinoma xenografts through inhibition of GRP78, a regulating chaperone protein of the unfolded protein response.[24] This MoA is completely different from DNA damage, which makes it intrinsically different from approved platinum drugs and hence interesting for clinical investigation and use. On the other hand, it seems more and more clear that BOLD-100 shows several cytotoxic modes-of-action, similar to platinum compounds. Clinically evaluated Ru compounds such as BOLD-100 contain only monodentate ligands and hence exhibit comparatively fast ligand-exchange reactions in physiological conditions. These thermal activation processes in living cells produce several metal-based species that can interact with biomolecules, thus resulting in a potentially large number of bioinorganic interactions. These interactions complicate mechanistic investigations, but they also offer several routes to harm cancer cells with diverse genetic backgrounds.

The anticancer efficacy of both NAMI-A and BOLD-100 is limited compared to that of approved chemotherapy agents; they are hence not ideal for application in monotherapy.

However, their low systemic toxicity opens promising opportunities for combination therapy. The latest clinical trials involving this ruthenium complex all consist of a combination therapy with other anticancer drugs. For example, a combination of NAMI-A and gemcitabine (a nucleoside analog) in non-small cell lung cancer patients after first line treatment was evaluated in a phase I/II study.[25] Unfortunately, the combination was found to be only moderately tolerated and less active than gemcitabine alone. For BOLD-100, on the other hand, synergistic effects were demonstrated in established preclinical models when combined with various anticancer therapies.[26] Therefore, a combination of BOLD-100 with the folinic acid, fluorouracil, and oxaliplatin (Figure 1.3, FOLFOX) regimen was recently evaluated in patients with advanced biliary duct or gastric cancer.[27,28] This phase II study concluded that the BOLD-100 + FOLFOX combination was well-tolerated and more effective than the FOLFLOX treatment alone. These studies highlight the importance of investigating drug combinations, which increases the efficacy of the so-called combination therapy, especially for anticancer metallodrugs.

1.2 Combination therapy

Chemotherapy is the main modality for cancer treatment. Conventional, small-molecule chemotherapeutics can be subdivided into four classes based on their mode of action. Firstly, anti-microtubule agents based on taxanes such as paclitaxel or vinca alkaloids like vinblastine, interact with tubulin to disrupt the function of microtubules and inhibit mitosis.[29] Secondly, the topoisomerase (Top) inhibitors cause DNA damage by blocking DNA unwinding enzymes. Top inhibitors include camptohecin analogues, anthracyclines such as doxorubicin and semisynthetic natural product derivatives such as etoposide.[30] Alkylating agents are another important class of anticancer drugs, which reduce tumor growth by inducing DNA damage through chemical modification (e.g. cyclophosphamide, temozolomide), intercalation (anthracyclines such as doxorubicin) or crosslinking (e.q. Pt-based metallodrugs) of nucleotides.[31–33] The last class involves antimetabolites including 5-fluorouracil and gemcitabine, that disrupt DNA transcription/translation by displacing natural nucleosides.[34]

Although countless small molecular chemotherapeutics have been developed for the treatment of cancer, single agent therapies generally result in time-limited remission due to drug resistances as discussed above for platinum drugs. Therefore, most chemotherapy regimens used to date consist of a combination of two or more drugs. The combined drugs generally exhibit different individual mode of actions and the combination is thus expected to be less vulnerable to oncogenic resistance. The POMP regime (Figure 1.3 A), combining antimetabolite 6-mercaptopurine (**P**urinethol), anti-microtubule agent Vincristine (**O**ncovin®), antimetabolite **M**ethotrexate and immunosuppressor **P**rednisone, was one of the first combination chemotherapies for the treatment of adult acute leukemia.[35]

Although the mode-of-action remains largely unknown, the POMP therapy combines antimetabolites and an anti-microtubule agent, which results in an efficacy increase compared to monotherapies of the individual components. To date, POMP is still used for maintenance therapy in adult acute lymphoblastic leukemia.[36] For the treatment of metastatic colorectal cancer, the FOLFOX (**Fol**inic acid, 5-**F**luorouracil and **O**xaliplatin) regime combines antimetabolites with Pt-based chemotherapeutics (Figure 1.3 B).[37] Despite being the standard-of-care, a reduced therapeutic effect of the FOLFOX regimen is regularly observed and has been related to oxaliplatin resistance.[38] These chemotherapeutic regimes highlight the clinical potential of combinatorial therapy for the treatment of cancer, while also emphasizing that they are not almighty.

Besides circumventing drug resistance, the combinatorial cancer-therapy approach may result in additional benefits for patients. To achieve a certain therapeutic effect, the dose needed of a synergistic drug combination is usually intrinsically lower than for monotherapy to realize the same effect. Furthermore, the lower dose of the more potent combination increases the selectivity towards cancerous cells, which in turn reduces side effects. With the ever-increasing understanding of biological processes in oncology, which enables development of highly selective anticancer drugs, targeting two or more specific cellular mechanisms can be achieved nowadays. The combination Ibrutinib and Venetoclax (Figure 1.3 C) for the treatment of chronic lymphocytic leukemia (CLL), is a highly successful example of such a synergy. Ibrutinib is a Burton's tyrosine kinase (BTK) inhibitor that blocks the B cell receptor pathway and is used for the treatment of lymphocytic cancers.[39] When BTK inhibition fails, Bcl-2 inhibitor Venetoclax is used as an alternative therapy.[40] Blocking Bcl-2 prevents this apoptosis-regulatory protein from promoting cancer-cell survival.[41] As Ibrutinib-resistant cells were shown to express high levels of Bcl-2, combining Ibrutinib with Venetoclax synergistically suppressed *in vitro* proliferation of Ibrutinib-resistant lymphoma cells. Recently, the effectivity of this combination therapy was translated into a clinical setting, with a phase II trial showing no relapse in 75% of CLL patients with a 3-year overall survival of 96%.[42]

While the results of the Ibrutinib/Venetoclax combination are encouraging, rational design of drug combination remains challenging as it requires extensive understanding of the involved cellular mechanisms and of their interactions.[43] High-throughput screening (HTS) has shown promising results in identifying relevant drug combinations.[44] HTS can, however, only be utilized for initial drug selection as large scale screening in *in vivo* using animal-tumor models is financially unviable and unethical. Alternatively, predicting synergistic drug combinations with computational methods is expected to become more prevalent with the rapidly expanding field of artificial intelligence and application of machine learning in health sciences.[45]

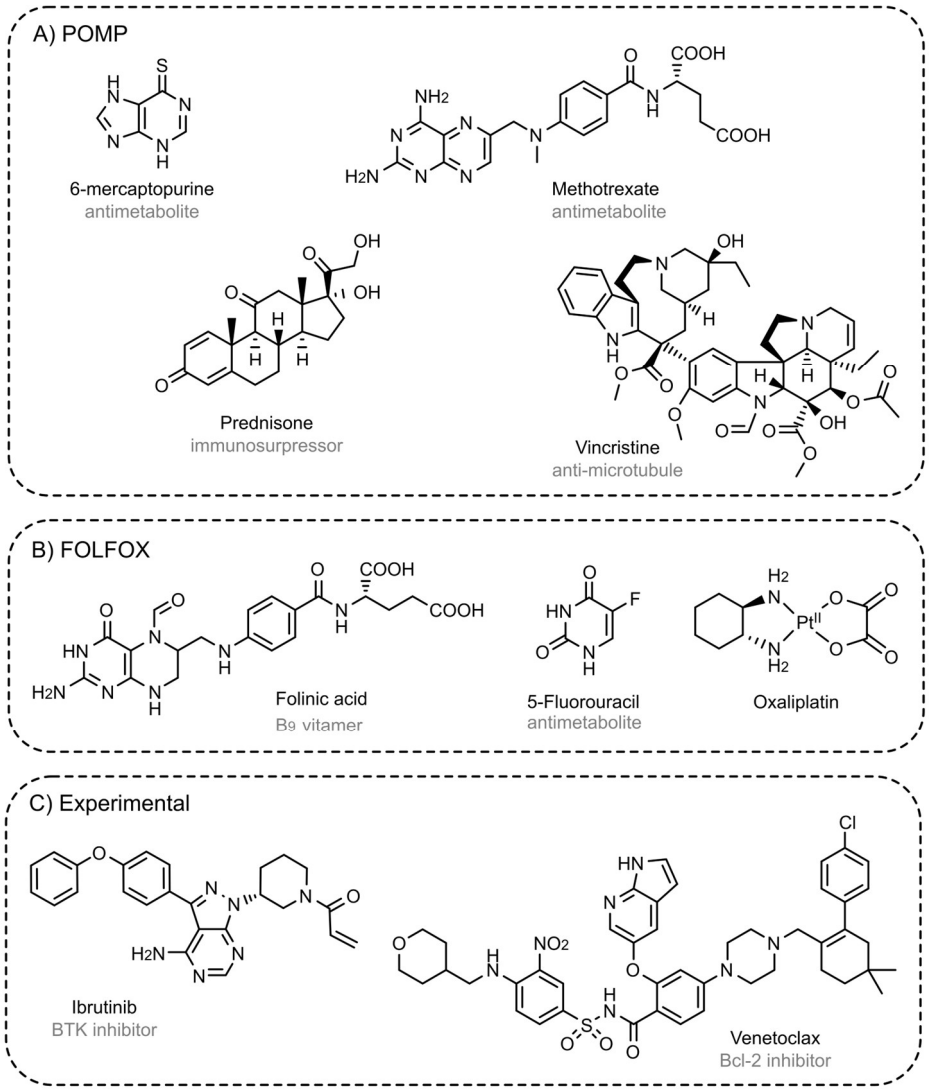

Figure 1.3 Examples of approved and experimental chemotherapy regimens for cancer treatment. A) The POMP regime was the first clinically used combination therapy for adult acute leukemia. B) The FOLFOX regime is the current first-line treatment for advanced colorectal cancer. C) Experimental combination of Ibrutinib and Venetoclax that successfully completed phase II clinical trial for the treatment of chronic lymphocytic leukemia.

1.3 Ruthenium(II)-based prodrugs for phototherapy

1.3.1 Activation by light

The activation of a prodrug with light irradiation is an excellent way to gain spatiotemporal control of the cytotoxic action of a chemical compound in the body. For cancer treatment, phototherapeutic polypyridyl-ruthenium(II) complexes can be divided into two categories: those for photodynamic therapy (PDT) and those for photoactivated chemotherapy (PACT). These two strategies have been extensively reviewed.[16,46,47] In PDT, light-induced excitation of a photosensitizer results in formation of reactive oxygen species (ROS) including $O_2^{\bullet -}$, H_2O_2 and/or $OH^{\bullet}$ (PDT type I), or of singlet oxygen ($^1O_2$, PDT type II). While the cytotoxicity of a PDT compound such as TLD-1433 arises from the oxidation of biomolecules induced by massive ROS generation,[16] PACT operates through a different mechanism. Light activation of a PACT compound triggers a photosubstitution reaction, thus dissociating a ligand from the first coordination sphere of the metal center. This photocleavage reaction produces cytotoxicity either via the action of the released ligand, or via the action of the ruthenium-based photoproduct, or via both. The highly tunable photophysical and photochemical properties of polypyridyl-ruthenium complexes therefore offers remarkable opportunities for Ru-based PACT, which are described in more detail below.

1.3.2 Photochemistry of polypyridyl-ruthenium(II) complexes

Upon irradiation of a Ru(II)-polypyridyl complex with ultraviolet or visible light, a triplet excited state is populated through intersystem crossing from the initially generated singlet excited state (Figure 1.4). Depending on the electronical properties of the complex, these triplet excited states can have an intra-ligand (IL), intra-ligand charge transfer (ILCT) or metal-to-ligand charge transfer (MLCT) character. In PACT compounds, a metal-centered (^{3}MC) state must be accessible to be thermally populated through internal conversion of the ^{3}MLCT state, leading to elongation of the ruthenium–ligand bond and facilitating ligand dissociation. If the ^{3}MLCT–^{3}MC energy barrier cannot be overcome thermally, other photophysical/photochemical processes may occur such as phosphorescence, electron transfer (PDT type I) or energy transfer (PDT type II). Importantly, the energies of the different excited states of Ru(II)-polypyridyl complexes are strongly dependent on the electronical features of the ligands and the geometrical constraints in the metal complex. These properties allow for exquisite tunability of the photochemistry of Ru(II)-polypyridyl complexes through modification of their molecular structure. So far, ligand photosubstitution from Ru(II)-polypyridyl complexes has been reported for a variety of ligands including pyridine, pyrazine, imidazole, nitriles, primary amine, thioether and sulfoxide (Figure 1.5).

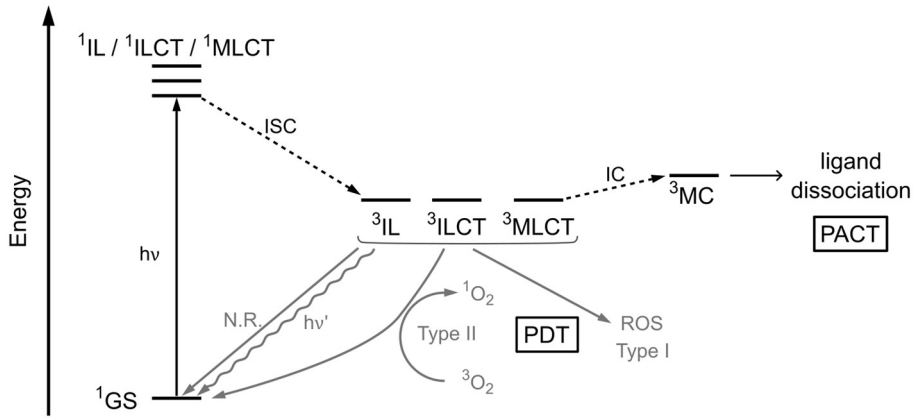

Figure 1.4 Jablonski energy diagram of the photophysical processes involved upon excitation of ruthenium(II)-polypyridyl complexes. When the complex is excited from the ground state (^{1}GS) to the first singlet excited state, which can be of metal-to-ligand-charge-transfer (^{1}MLCT), intra-ligand (^{1}IL) or intra-ligand charge transfer (^{1}ILCT) character, intersystem crossing (ISC) occurs to populate a triplet excited state (^{3}IL, ^{3}ILCT or ^{3}MLCT). Relaxation from this triplet state to the ^{1}GS can occur thermally (i.e. non-radiative decay, N.R.) or be accompanied by phosphorescent emission, electron transfer to form radical-based oxygen species (ROS; PDT type I), or energy transfer to form singlet oxygen ($^1O_2$; PDT type II). However, a triplet metal-centered state (^{3}MC) can be populated through internal conversion from the ^{3}MLCT if the associated energy barrier can overcome thermally, which subsequently leads to ligand dissociation.

For PACT compounds, ligand dissociation should only occur upon excitation, while the Ru-ligand bond should be thermally stable in the dark. Furthermore, the photosubstitution quantum yield (φ_{ps}), i.e., the fraction of absorbed photons that lead to a photoreaction, is an important parameter for PACT compounds. Etchenique and coworkers reported a series of *cis*-$[Ru(bpy)_2(L)(ACN)]^{2+}$ complexes $[\mathbf{1a}]^{2+} - [\mathbf{1d}]^{2+}$, where bpy = 2,2'-bipyridine, ACN = acetonitrile, and L = ACN, triphenylphospine (PPh_3), trimethylphosphine (PMe_3) or NH_3, and investigated their stability and φ_{ps} (Figure 1.5).[48] While all complexes exhibited relatively high φ_{ps} between 0.34 and 0.46 upon blue light (405 nm) irradiation in aqueous solution, only the PMe_3-containing complex was shown to be thermally stable in the dark, underlining the importance of the spectator ligands in fine-tuning the properties of PACT compounds. Using this scaffold, photorelease of neurotransmitter γ-aminobutyric acid (GABA) or Janus kinase inhibitor Ruxolitinib was achieved from complex $[\mathbf{2a}]^{+}$ and $[\mathbf{2b}]^{2+}$, respectively.[49,50] The Glazer group investigated the influence of steric and electronic effects in a series of $[Ru(tpy)(NN)(Py)]^{2+}$ complexes on the stability of the compounds and φ_{ps} of pyridine (Py) photodissociation (tpy = 2,2':6',2''-terpyridine and NN = bpy $[\mathbf{3a}]^{2+}$; 6,6'-dimethyl-2,2'-bipyridine (dmbpy) $[\mathbf{3b}]^{2+}$; 2,9-dimethyl-1,10-phenanthroline (dmphen) $[\mathbf{3c}]^{2+}$; 2,2'-biquinoline(biq) $[\mathbf{3d}]^{2+}$.[51] These results showed an increase in φ_{ps} upon increasing steric bulk around the metal center, unfortunately at the cost of thermal stability in aqueous solution. For $[\mathbf{3d}]^{2+}$, the increased aromatic surface of the biq ligand resulted in a

bathochromic shift of 60 nm of the ^{1}MLCT absorbance band compared to bpy-containing complex [**3a**]$^{2+}$.

Red-shifting of the activation wavelength is important for PACT compounds, due to the deeper penetration of lower-energy light in biological tissues.[52] Furthermore, the cytotoxicity of complexes in HL-60 cells were also evaluated and indicated that biq-containing complex [**3d**]$^{2+}$ was the most toxic, both before and after photoactivation. These results underline the importance of the molecular design of PACT compounds on not only their photochemical, but also their biological properties. When the Ru(II)-complex is intended as a photocage for a photolabile and biologically active ligand, the Ru(II)-photoproduct should be biologically inert such as for compounds [**4**], [**5**]$^{2+}$ and [**6**]$^{2+}$ shown in Figure 1.5.[53–55] In the case of a toxic Ru(II) photoproduct, like compounds [**7**]$^{2+}$, [**8**]$^{+}$ and [**9**]$^{2+}$, the dissociating ligand is typically a biologically inert spectator ligand (for example, dmso in [**8**]$^{2+}$).[56–59] Combination of these two approaches may lead to synergy of the toxicity of the released ligand and that of the Ru(II)-photoproduct, resulting in a more potent PACT compound. For example, red-light photoactivation of compound [**10**]$^{2+}$ was recently shown to result in synergy between released STF31, a nicotinamide phosphoribosyl transferase (NAMPT) inhibitor, and the [Ru(tpy)(biq)(OH$_2$)]$^{2+}$ photoproduct in hypoxic glioblastoma cells (U87MG).[60] Another approach was reported by Turro et al., who combined PDT and PACT by incorporating dimethylbenzo[i]dipyrido[3,2-a:2′,3′-c]phenazine (Me$_2$dppn) in compound [Ru(tpy)(Me$_2$dppn)(L)]$^{2+}$ ([**11**]$^{2+}$), where L is a pyridyl-based cytochrome P450 3A4 inhibitor.[61] With a φ_{ps} of 0.024 in acetonitrile and $^1O_2$ generation quantum yield of 0.59 in methanol, the cytotoxicity (EC_{50}) of compound [**4**] upon blue light irradiation increased from >25 µM to 2.8 µM in human prostate cancer cells (DU-145). These results indicate the potential of such dual-action compounds that can generate both PDT and PACT action in cancer cells.

While most PACT compounds reported to date contain a single photolabile ligand coordinated to a Ru(II)-based photocage, a few examples have been reported wherein two photolabile ligands are bound to a single Ru(II) complex. The photorelease of two protease inhibitors from complex [**12**]$^{2+}$ was one of the first examples reported, although it delivered two molecules of one drug.[62] In such complexes the second photosubstitution process has a much lower quantum yield that the first one, so that the second light-induced delivery only occurs at very high light doses. Photosubstitution of symmetrical bidentate ligands such as dithioethers in complex [**7**]$^{2+}$ has been studied, indicating a two-step substitution mechanism. Alternatively, dissymmetric bidentate N,S ligands such as 2-methylthiomethylpyridine (mtmp) were also photosubstituted upon blue light irradiation from tris-heteroleptic complex [**13**]$^{2+}$, resulting in a cytotoxic bis-aqua Ru-based photoproduct.[63] A detailed computational study on the photorelease of mtmp from [**13**]$^{2+}$ revealed the mechanism of the photoreaction, indicating the involvement of multiple excited states depending on the first released functionality, either the thioether or

pyridine.[64] These results underline the differences in photoreactivity of different bidentate ligands and the possible high selectivity of photosubstitution reactions in tris-heteroleptic ruthenium(II) complexes.

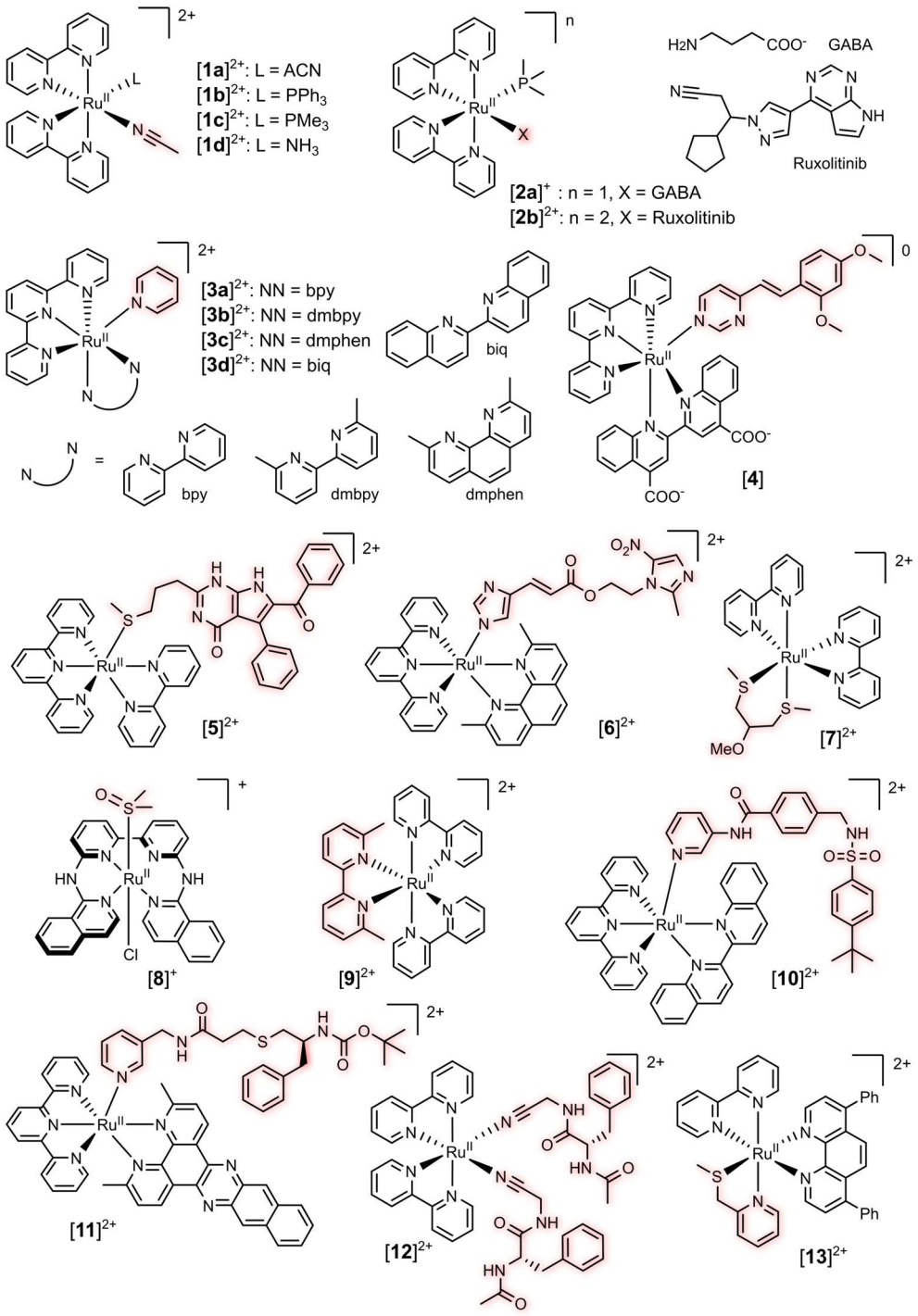

Figure 1.5 Selection of polypyridyl-ruthenium(II) based PACT compounds with the photolabile ligands highlighted in red. See text for details and references.

1.4 Coordination isomerism in metal-based chemotherapeutics

1.4.1 *Cis* versus *trans* platinum(II) complexes

While cisplatin and its derivatives are widely used in the clinic, its *trans* isomer, *trans*-$[PtCl_2(NH_3)_2]$ (also called transplatin) is deemed clinically ineffective.[65] From a chemical point of view, the two isomers exhibit distinct differences in reactivity towards hydrolysis of the chloride ligands (Figure 1.6). While the exchange of the first Cl^- ligand by H_2O from *trans*-complex occurs relatively fast compared to the *cis*-isomer, the second hydrolysis step occurs much slower due to the H_2O in *trans* position. The accepted order of the *trans* effect is $Cl^- > NH_3 > OH^- > H_2O$.[66] In contrast, both chlorido ligands in the *cis*-isomer are trans to the amine ligands, ensuring similar exchange rates. The varying exchange rates of transplatin also leads to potential deactivation by coordination to thiol-containing moieties such as human serum albumin and glutathione, which may contribute to the reduced antitumoral activity of this complex.[67] Furthermore, for d^8 square-planar platinum complexes different isomers also affect DNA binding modes of the platinum complexes and therefore their cytotoxicities. While cisplatin predominantly forms 1,2-GG intra- and interstrand crosslinked adducts with DNA, transplatin crosslinks 1,3-GG intrastand and 1,1-GC interstrand.[68] However, the slower ligand exchanges rates prevent the conversion of monofunctional to bifunctional adducts, which may account for the observed decrease in interstrand crosslinks induced by transplatin, compared with cisplatin.

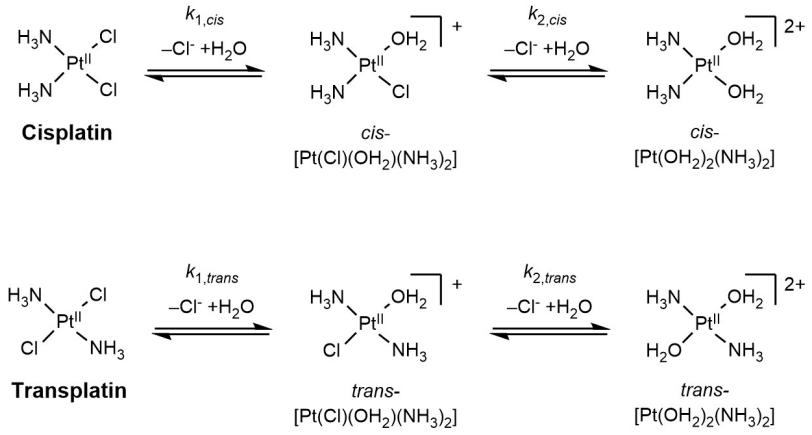

Figure 1.6 Proposed scheme for the hydrolysis of cisplatin and transplatin.

Although transplatin exhibits no clinically relevant anticancer activity, similar cytotoxicity to that of cisplatin can be achieved with *trans*-platinum(II) complexes by incorporation of various ligands to modulate numerous pharmacokinetic properties of the resulting complex, including lipophilicity, hydrolysis kinetics and mode-of-action.[69–72] Substitution of one or both NH_3 ligands with a planar, heterocyclic amine ligand such as in compound **14** and **15** (Figure 1.7), enhances their toxicity compared to the "parent" transplatin, while they exhibit no cross resistance with cisplatin or oxaliplatin.[69,73] However, these complexes are

poorly water soluble and suffer from high levels binding to serum proteins due to increased hydrolysis reactivity induced by the planar amine ligands. Replacing the chloride with carboxylate ligands has been shown to significantly reduce the undesirable reactivity of these compounds.[74] Additionally, *in vitro* evaluation of complexes **16**–**18** suggested production of DNA-protein crosslinked adducts, a striking difference in mode-of-action compared to cisplatin which predominately yields DNA-DNA crosslinked adducts.[75] In order to further mitigate the reactivity of the Pt(II)-complex while maintaining its toxicity, Fabra et al. reported two Pt(II) complexes containing one acetate ligand in *trans* position to either chloride (complex **19**) or acetate (complex **20**; Figure 1.7).[76] While both complexes where shown to be sufficiently stable in DMSO or in a 1:1 (v/v) mixture of DMSO and 0.9% (w/w) NaCl aqueous solution, complex **20** was much more reactive towards *N*-acetyl-methionine, a frequently used model to investigate the role of biological S-donor molecules in Pt(II) drug-protein binding. On the other hand, complex **19** showed higher anticancer activity and selectivity against multiple human cancer cell lines. Further investigation of the cellular uptake mechanism of both complexes also suggested that **20** is taken up actively while **19** enters the cell passively. With no cross-resistance observed for cisplatin and oxaliplatin, both complexes likely induce anticancer activity through non-DNA-associated mechanisms. These studies emphasize how the geometric isomerism of a complex can lead to distinct differences in chemical and biological effects, offering ample possibilities in the development of more effective anticancer therapeutics based on transition metals.

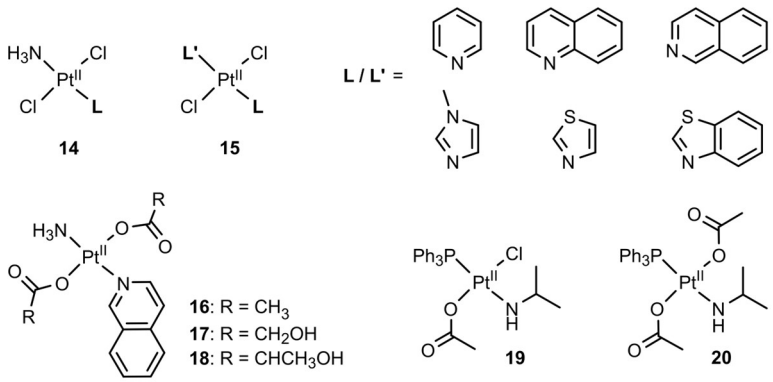

Figure 1.7 Selection of *trans*-platinum(II) complexes. See text for details and references.

1.4.2 Ruthenium(II) polypyridyl complexes

The octahedral geometry of ruthenium(II)-polypyridyl complexes provides ample opportunity for using the *trans* effect to influence the kinetics of ligand substitution processes. For example, the substitution of H_2O by acetonitrile (ACN) from $[Ru(tpy)(4,4'\text{-}(X)_2\text{-}bpy)(H_2O)]^{2+}$, where X is H, CH_3, OCH_3, NH_2, $N(CH_3)_2$, has been shown to be dependent on the electron-donating character of substituent X.[77] Because of their effect on ligand substitution kinetics, and similar to Pt(II) compounds, *trans*-Ru(II)-polypyridyl complexes

might also exhibit different cytotoxic profiles than their *cis*-isomers. For example, the Glazer group reported a study on the cytotoxicity of *cis*-[Ru(bpy)$_2$Cl$_2$] and *trans*-[Ru(qpy)Cl$_2$] (qpy = 2,2':6',2'':6'',2'''-quaterpyridine) in human leukemia (HL-60) and lung cancer (A549) cell lines.[78] Although both ruthenium complexes could not match the toxicity of cisplatin, the *trans*-Ru(II) complexes was about 10 times more toxic than the *cis*-derivative. Interestingly, the *trans*-complex also exhibited a 49-fold increase in cellular accumulation than the *cis* analogue in these cancer cell lines. It was therefore hypothesized that the slower exchange rate of the Cl^- ligand in the *trans* complex prevents interaction with media or non-essential biomolecules, while facilitating its cytotoxic behavior. These results underline the importance of geometric conformation when fine-tuning the pharmacological properties of ruthenium(II)-polypyridyl complexes for anticancer therapy.

1.4.3 Photoactivated *trans*-ruthenium complexes

While *trans* effects on the reactivity of metal complexes in the ground state are well studied, reports on such effects in the excited state are quite rare. This is unfortunate, as *trans* effects could potentially be used to tune the photochemical and phototherapeutic properties of PACT compounds. The Etchenique group reported on the photo-isomerization of [Ru(bpy)$_2$(PMe$_3$)(H$_2$O)]$^{2+}$ from the *cis* to *trans* isomer with 405 nm light, and the reverse reaction with 532 nm light. Distinct differences in photochemical properties were observed with different irradiation wavelengths. Firstly, the MLCT absorbance band of the *trans* isomer exhibited a bathochromic shift with a higher absorptivity than that of the *cis* isomer. In addition, conversion from the *trans* to the *cis* isomer at elevated temperature was observed in absence of light, indicating thermal instability of the former. Interestingly, green (532 nm) light irradiation of the imidazole (ImH) derivative *trans*-[Ru(bpy)$_2$(PMe$_3$)(ImH)]$^{2+}$ resulted in photosubstitution of the ImH ligand in aqueous solution with much higher quantum yield than the *cis* isomer (0.23 and 0.10, respectively). Although these compounds were mainly investigated for their photochemical properties, these observations emphasize the importance of conformation on the photoreactivity of ruthenium complexes.

Similar to cisplatin and transplatin, therapeutic application of photoactivated ruthenium(II) complexes may be significantly influenced by their conformation. For example, from *trans*-[Ru(biqbpy)(Amet)$_2$]$^{2+}$ a single κS coordinated *N*-acetyl-methionine (Amet) ligand can be readily substituted by H$_2$O upon green light irradiation.[79] From the resulting mono-aqua complex [Ru(biqbpy)(Amet)(H$_2$O)]$^{2+}$, however, photosubstitution of the second Amet ligand does not occur in the reported conditions. Furthermore, [Ru(biqbpy)(Amet)(H$_2$O)]$^{2+}$ was also shown to be less cytotoxic than the bis-aqua complex [Ru(biqbpy)(H$_2$O)$_2$]$^{2+}$ in several human cancer cells lines. Since minor molecular changes of the formula of a ruthenium polypyridyl complex, such as a *trans* vs. *cis* conformation can significantly influence the photochemical and biological properties of the complex, we foresee that *trans* effects in the

excited state may also take place and could potentially be utilized for improved PACT compounds.

1.5 Aim and outline of this thesis

The aim of this thesis is to advance the understanding of wavelength-dependent photosubstitution reactions in *trans*-ruthenium(II) polypyridyl complexes and to explore their application in the development of dual-targeting PACT compounds. In particular, this work focuses on photoactive ruthenium(II) complexes designed to enable the controlled release of two distinct cytotoxic agents. To address these objectives, the research is structured along two complementary lines. The first research line involves the synthetic and photochemical investigation of *trans*-Ru(II)-based photocages, together with *in vitro* studies assessing their ability to sequentially photorelease a pyridine-based nicotinamide phosphoribosyltransferase (NAMPT) inhibitor and a thioether-based microtubule polymerization inhibitor. The second research line focusses on the synthesis and evaluation of *trans*-Ru(II)-based photocages targeting heme oxygenase-1 (HO-1), either through functionalization of the tetrapyridyl ligand or via coordination of an organic imidazole-based HO-1 inhibitor to the metal center.

In Chapter **2**, the development of a novel methodology is reported for mono- or di-amination of polypyridine *N*-oxides. The approach combines activation with tosyl chloride and nucleophilic substitution using potassium phthalimide, followed by hydrolysis to afford the aminated product. This work establishes access to aminopyridine building blocks that are relevant for the synthesis of coordination complexes, including the PACT compounds investigated in subsequent chapters.

In Chapter **3**, a study is reported focusing on the design, synthesis and photochemical investigation of a series of *trans*-ruthenium(II) complexes based on the tetrapyridyl scaffold di([2,'2-bipyridin]-6-yl)amine HL and its *N*-methylated analogue MeL. The $[Ru(HL)(X)(Y)]^{2+}$ and $[Ru(MeL)(X)(Y)]^{2+}$ complexes are designed, with methyl(2-thioethanol) (MTE), ACN or pyridine coordinated to the two *trans* axial positions. The synthesis and characterization of the symmetric complexes (X = Y) and *trans*-dissymmetric complex (X ≠ Y) is reported, and their photosubstitution behavior is examined under irradiation with visible light of different wavelengths. In particular, the differences in ligand photosubstitution kinetics are compared and the potential influence of *trans* effects in the excited state evaluated. These results provide a foundation for the development of the photoactive ruthenium(II)-based complexes investigated in this thesis.

Chapter **4** investigates the applicability of the *trans*-ruthenium(II) photocages developed in Chapter **3** for dual-targeting PACT. Here, a pyridine-based NAMPT inhibitor called STF31, and the thioether-based microtubule polymerization inhibitor MTI are coordinated to the tetrapyridyl-ruthenium(II) scaffold, providing two symmetric complexes and one

dissymmetric complex. The photochemical characteristics of these complexes are investigated under different irradiation conditions, and their capacity for sequential photorelease of the inhibitors is assessed. Furthermore, the phototoxicities of caged inhibitors are evaluated *in vitro* in human skin melanoma, lung adenocarcinoma and glioblastoma cancer cell lines in both normoxia (21% O_2) and hypoxia (<1% O_2). This study explores the potential dual-targeting PACT compounds based on the photorelease of multiple anticancer agents.

In Chapter **5**, two synthetic approaches are explored for the development of PACT compounds designed to release a Ru(II)-based inhibitor of HO-1 upon photoactivation. The first approach focuses on the functionalization of the tetrapyridyl ligand HL with carboxylic acid substituents using cross-coupling or pyridyl C–H functionalization methodologies. After coordination, the resulting Ru(II)-complexes are intended to mimic heme, the enzymatic substrate of HO-1, and as a result inhibit HO-1. The second approach involves the preparation of a series of *trans*-Ru(II) tetrapyridyl complexes incorporating QC82, a known organic imidazole-based HO-1 inhibitor, leading to dissymmetric photocages bearing additional monodentate pyridyl-type ligands. The photochemical behavior of these complexes is investigated under visible light irradiation, and their capacity to generate ruthenium–aqua species is examined. Furthermore, computational docking studies and preliminary *in vitro* evaluations are employed to assess the potential of the photoactivated products as Ru(II)-based HO-1 inhibitors.

The findings of the research presented in this thesis are summarized and discussed in Chapter **6**, which also contains an outlook on dual-targeting photoactivated prodrugs based on ruthenium and its implementation in modern combination therapy.

1.6 References

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