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CHAPTER 1

Introduction:
Transition metal complexes as anticancer drugs

The discovery of anticancer properties of cisplatin in the 1960s has sparked a wide array of research in the field of transition metal complexes as anticancer drugs. Through key examples of complexes in metal-based chemotherapy, photodynamic therapy, and photoactivated chemotherapy, the state-of-the-art in the field of anticancer metallo drugs is described.
### 1.1 A new hope: cisplatin

The story of cisplatin (cis-[PtCl\textsubscript{2}(NH\textsubscript{3})\textsubscript{2}], Figure 1.1) is remarkable. For the first time synthesized by Michele Peyrone in 1844,\textsuperscript{1,2} the actual structure of cisplatin was extensively described 50 years after its discovery by Alfred Werner’s ‘Beitrag zur Konstitution anorganischer Verbindungen’ in 1892.\textsuperscript{3} Finally, cisplatin played a central role in Werner’s theory of coordination chemistry, for which he was later awarded the Nobel Prize in Chemistry ‘in recognition of his work on the linkage of atoms in molecules by which he has thrown new light on earlier investigations and opened up new fields of research especially in inorganic chemistry.’\textsuperscript{4}

Attention for cisplatin settled until the 1960s, when its anticancer effects were discovered by Barnett Rosenberg. While originally studying the effect of an electric field on mitosis in prokaryotic cells, Rosenberg observed inhibition of cell division of Escherichia coli bacteria. After thorough research Rosenberg came to the conclusion that cisplatin was the prime candidate for this effect that in the initial experiments was formed as electrolysis product from a platinum electrode.\textsuperscript{5} After years of research it proved to be a promising anticancer drug candidate in both in vitro\textsuperscript{6} and in vivo studies.\textsuperscript{7} In these years Rosenberg not only investigated and developed a compound that has become one of the most successful chemotherapeutic agents; he also lay the foundation of a whole new field of research, that of metal-based anticancer drugs. Rosenberg’s work in the late 1960s and 1970s comprised platinum anticancer chemistry that included cisplatin analogues, different modes of action of cis and trans isomers,\textsuperscript{8} and the activation of platinum(IV) complexes by photoreduction,\textsuperscript{9} that all have present-day relevance.

![Chemical structure of cisplatin.](image)

As a chemotherapeutic agent that is still used in the clinics today, cisplatin is administered intravenously to cancer patients. Due to the high chloride concentration in the bloodstream (> 100 mM), the chloride ligands mostly remain coordinated.\textsuperscript{10} Cisplatin can then enter the cell either via passive diffusion or via copper(II) transporters.\textsuperscript{11} In the cell the chloride concentration is much lower (3–20 mM), thus allowing for hydrolysis of cisplatin into [Pt(NH\textsubscript{3})\textsubscript{2}(OH\textsubscript{2})Cl]\textsuperscript{+}. Once inside the nucleus this species can form a coordination bond with thymine, guanine, cytosine, or adenine, the bases of nuclear DNA, of which guanine is the preferred coordination site. Once coordinated, the second chloride ligand is replaced via coordination to another DNA base, ultimately forming interstrand or intrastrand crosslinks.\textsuperscript{13} These crosslinks bend and unwind duplex DNA and this distortion attracts high-mobility group proteins.\textsuperscript{14} The attachment of these proteins shields the platinated DNA from excision repair and consequently sensitizes the cell for apoptosis.\textsuperscript{15} This cisplatin mechanism of action (MoA), as presented here, is the main paradigm in place. However, there is extensive scientific debate if and how interaction with biomolecules, before binding to nuclear DNA, plays a crucial role in the MoA.\textsuperscript{16}

The development and clinical success of cisplatin have sparked wide interest and allocation of funds to develop new platinum-based anticancer drugs. Cisplatin has a cure rate of over 95% for patients diagnosed with testicular cancer, and is also used in the treatment of bladder and ovarian cancer.\textsuperscript{17} However, side effects associated with cisplatin include nephrotoxicity, neurotoxicity, ototoxicity, and nausea. Another drawback is that some cancers have or can acquire cisplatin resistances.\textsuperscript{18} Therefore, cisplatin analogues have been developed in the clinic, for example carboplatin or oxaliplatin, that have significantly less side effects (Figure 1.2). Other cisplatin analogues that have been approved in at least one country include nedaplatin, lobaplatin, and heptaplatin (Figure 1.2).\textsuperscript{19} All these cisplatin analogues contain two amine ligands coordinated to the platinum center in a cis configuration.
A new hope: cisplatin

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1.2 Changing metal: the rise of ruthenium

There has been extensive research in metal-based non-platinum anticancer agents, to avoid cisplatin related side effects, to overcome cisplatin resistance, and to improve selectivity. In this context ruthenium complexes have been the most investigated alternative. This attention can be explained by the following properties of ruthenium as metal center for building transition metal complexes: a distinct coordination geometry (octahedron vs. square plane), specific binding preferences, access and physiological stability of +II and +III oxidation states, redox activity, and similar ligand exchange kinetics compared to platinum(II).

Moreover, early reports describing promising ruthenium complexes were already published in the eighties and nineties. This section is not intended to give a complete overview of ruthenium complexes that have been developed, reported, and tested in vitro or in vivo, but to describe a personal view on key ruthenium complexes that have been critical in the development of ruthenium anticancer chemistry. Ruthenium complexes in combination with phototherapy will be discussed more specifically in section 1.4.

For a long time NAMI-A developed by Sava and Alessio, and KP1019 developed by Keppler et al., have been the ambassadors to the field of ruthenium anticancer chemistry (Figure 1.3). In a Phase I-II study NAMI-A was given to 32 patients with advanced non-small lung cell carcinoma in combination with clinically approved gemcitabine. At the highest dose remission was reported for only one patient, and
stable disease was reported for six to eight weeks for ten patients. Patients experienced nausea, vomiting, constipation, diarrhea, fatigue, renal toxicity, and blister formation. These results proved not to be promising enough and NAMI-A was, after many years, qualified as ‘insufficiently effective for further use’.25 KP1019 was developed against colon cancer, caused no serious side effects in a Phase I study, and affected stability in five out of six patients up to 10 weeks.26 Due to limited solubility of the compound large infusion volumes were required.27 This low solubility ended the clinical testing of KP1019. However, the legacy of KP1019 lives on in the form of its more water-soluble sodium salt analogue NKP-1339 (Figure 1.3), which, according to its developers, is ‘the first ruthenium-based anticancer drug on the edge of clinical application’.28 In a phase I study NKP-1339 was administered to a total of 34 (heavily pre-treated) patients with various solid tumors. At the highest doses (780 mg.m⁻²), very minor side effects were observed, and a partial response was observed in one patient and disease stabilization in seven patients. These limited effects are a major advantage over current metal-based anticancer drugs, and justify further development of NKP-1339. Despite the fact that NAMI-A and KP1019 did not end up as clinically approved drugs, their development has greatly contributed to ruthenium anticancer chemistry.29

It is common to first assess the potential of any new complexes by evaluating their cytotoxicity. Cytotoxicity is commonly reported as the IC₅₀ or EC₅₀ value – the inhibiting or effective concentration at which 50% of the treated cells is dead, compared to untreated control cells. For the sake of clarity and uniformity in this thesis these values will be reported as EC₅₀ values. These values highly depend on...
the parameters of the cytotoxicity assay, one of the most important being the drug incubation time, which in the literature typically varies from 1 to 72 hours.

The two main types of compounds in ruthenium anticancer research are categorized as ruthenium arene complexes and ruthenium polypyridyl complexes. The first category of compounds contain a polyhapto-coordinated arene ligand. For example, RAPTA complexes developed by Dyson et al. comprise ruthenium η⁶-arene (RA, the core), the monodentate phosphine ligand 1,3,5-triaz-7-phosphoadamantane (PTA), and two chloride ligands. Dissociation of these chloride ligands is possible, thereby mimicking the first steps of the MoA of cisplatin. The compound RAPTA-C (Figure 1.4) has shown inhibition of tumor growth in vivo by approximately 75%, and inhibition of angiogenesis in the chicken chorioallantoic membrane model was demonstrated. Similar RAED (ruthenium arene ethylenediamine) complexes reported by Sadler contain a η⁶-arene ligand, a bidentate 1,2-ethylenediamine ligand, and a chloride ligand. From this series the compound RM175 (Figure 1.4) has exhibited growth inhibition against A2780 ovarian cancer cells (EC₅₀ = 9 µM) and 46% tumor growth inhibition with two doses of 25 mg.kg⁻¹ in A2780 xenografts and A2780cis xenografts.

![Chemical structures of RAPTA-C and RM175.](image)

Figure 1.4 Chemical structures of RAPTA-C and RM175.

Ruthenium polypyridyl complexes have been extensively studied as well, not only as anticancer compounds but also as (redox) catalysts or imaging agents. Due to the wide scope of mono-, bi-, tri-, tetra-, and pentadentate pyridyl ligands, an infinite number of different complexes is possible, and a vast amount of literature has been generated on this family of complexes. In 1995 the simple ruthenium polypyridyl complex [Ru(tpy)Cl₃] (tpy = 2,2':6',2″-terpyridine, Figure 1.5) was...
reported to have 50% growth inhibition of HeLa cells with 7 µM treatment after 72 hours of exposure in vitro, and was claimed to exhibit ‘significant antitumor activity’ in vivo. Another example is the cyclometallated ruthenium complex RDC11 (Figure 1.5). In vivo it reduced the tumor volume and weight by 40% and performed better than the control compound cisplatin, without causing severe side effects to the liver, kidneys, or the neuronal sensory system.

In conclusion, despite the early development of NAMI-A and KP1019 in the 1980s and clinical testing in the 1990s, the promise of (simple) ruthenium complexes has not been fulfilled yet. To date none of the second generation ruthenium complexes from the arene and polypyridyl groups have ended up in the clinics. The way to the clinic is complicated and lack of success can have many reasons, of which the investment climate is not the least important. Therefore, ruthenium-based anticancer drugs will only become clinically successful when they have superior properties, in terms of key issues such as selectivity, general toxicity, and/or resistance, compared to currently approved drugs and in particular cisplatin analogues.

1.3 No more side effects: in search of selectivity

Side effects caused by chemotherapy are primarily caused by a lack of selectivity. To sustain rapid cell division, cancer cells have a high uptake of molecules of all kinds, which may include anticancer agents. Such high uptake is not limited to cancer cells, but also includes fast-growing healthy cells such as hair cells, or microvilli located in the intestines. As the chemotherapeutic agent is usually distributed across the whole body, only a limited amount ends up in the tumor.
Therefore, improving selectivity would not only reduce side effects stemming from general toxicity, but also allow for more optimal treatment doses.

In inorganic chemistry several approaches have been developed to acquire selectivity in chemotherapeutic compounds. The first one is the prodrug approach, which involves chemical binding of the drug to a moiety that deactivates or cages the active compound. Ideally, the caged or inactive form does not interfere with the cell biochemistry, which prevents non-specific cytotoxicity and undesired side effects. Selectivity can then be induced by local activation, but also by equipping the active compound with targeting moieties. The Keppler group has oxidized oxaliplatin, and introduced axial maleimide ligands to selectively couple the maleimide to the thiol group of cysteine-34 of human serum albumin (HSA) as shown in Figure 1.6. HSA is known to accumulate in malignant and inflammatory tissues due to the enhanced permeability and retention effect. Mice treated with HSA-functionalized oxaliplatin(IV) exhibited no significant loss of body weight, significant reduction in tumor growth (CT colon xenograft), and disease stabilization.

The group of Lippard has developed a cisplatin-based platinum(IV) compound containing a (D)-1-methyltryptophan ((D)-1-MT) ligand in one of the axial positions (Figure 1.6). (D)-1-MT inhibits indoleamine-2,3-dioxygenase, an immunosuppressive enzyme in human tumors, leading to immunomodulation and enhanced T-cell proliferation in vitro. EC₅₀ values of these platinum(IV) complexes ranged from high nanomolar to micromolar concentrations against a panel of human ovarian cancer cell lines. In summary, square-planar cisplatin analogues can be oxidized into an octahedral platinum(IV) compound. After uptake of this prodrug, the platinum(IV) compound is subsequently reduced to the original cisplatin analogue, and then acts similar to the platinum(II)-based drugs. The two extra coordination sites can be used to couple the prodrug to ligands that have high target affinity. In the process alternative cell uptake mechanisms may be operative, overcoming acquired drug resistance that stem from lowered uptake.
An alternative strategy in targeting cancer cells is to disturb their distinct and delicate redox balance. Healthy cells commonly have tight regulation of reactive oxygen species (ROS) and metal homeostasis to maintain this intracellular redox balance. Enzymatic reactions performed by reductases, oxidases, and peroxidases, and non-enzymatic reactions involving glutathione (GSH) and thioredoxin, are employed by cells to preserve this redox balance. For example, the disturbance of homeostasis caused by cisplatin increases the intracellular ROS levels, and is associated with cisplatin-induced side effects ototoxicity and nephrotoxicity. Cancer cells are characterized by an imbalance in redox homeostasis that leads to enhanced intracellular ROS presence, and subsequently higher levels of oxidative stress. The higher concentrations of ROS are not only caused by a high metabolic activity to sustain rapid cell division, but are also caused by lower levels of antioxidants. Cancer cells, thus, have a lower ROS-buffering capacity and can deal less efficiently with extracellular stress compared to healthy cells. Anticancer compounds that are designed to induce ROS may thus be more cytotoxic toward cancer cells than toward healthy cells.

Drawing inspiration from transfer hydrogenation catalysis by ruthenium-arene complexes, Sadler et al. have developed complexes capable of pushing the coenzyme NAD$^+$ + H$^-$ ⇌ NADH equilibrium towards NADH in the presence of an excess sodium formate acting as the hydride donor. Excess NADH results in reductive stress causing the cells to die. Treatment of A2780 cancer cells with [Ru(p-cym)(MsEn)Cl] (MsEn = N-(2-aminoethyl)methanesulfonamide, Figure 1.7, left) without formate resulted in EC$_{50}$ values of 11.9 µM. When co-treated with formate, an enhanced antiproliferative activity, characterized by an EC$_{50}$ lower...
than 1.0 μM, was measured. In contrast, \([\{\eta^5-\text{Cp}^{\text{xbiph}}\}\text{Ir}^{\text{phpy}}\text{py}]\text{PF}_6\) (\(\text{Cp}^{\text{xbiph}} = \text{biphenyltetramethyl-cyclopentadienyl, Hphpy} = \text{phenylpyridine, py} = \text{pyridine, Figure 1.7, right}) in vitro acts as an oxidation catalyst and pushes the NAD\(^+\) + H\(^-\) ⇌ NADH equilibrium towards NAD\(^+\), resulting in higher levels of H\(2\text{O}_2\).\(^{47}\) Co-treatment with \(L\)-buthionine sulfoximine (BSO), an inhibitor of \(\gamma\)-glutamylcysteine synthetase, results in a two-fold increase of the activity of this compound, to EC\(_{50}\) values of 60 nM. As BSO lowers the ability of cells to scavenge ROS, the enhanced cytotoxicity of the iridium complex is explained by its capacity to interfere with the redox balance of the cancer cell.

\[
\begin{align*}
\text{Ru reduction catalyst} & \quad \text{Ir oxidation catalyst}
\end{align*}
\]

Figure 1.7 Chemical structures of ruthenium reduction catalyst and iridium oxidation
catalyst developed by Sadler.

### 1.4 Phototherapy in anticancer treatment

Phototherapy is a rapidly developing method in cancer research to increase the selectivity of chemotherapeutic drugs. It relies on the local activation of a prodrug by in vitro light irradiation of the tumor. Ideally, the prodrug is non-toxic and becomes highly toxic after activation in the irradiated tumor tissue. Phototoxicity is quantified as the ratio of the EC\(_{50}\) value obtained in a dark control and that obtained after light irradiation. This factor is commonly defined as the Photo Index (PI), and it should ideally be as large as possible. In phototherapy, light activation by lower-energy light (red light) is preferred over activation by high-energy light (UV- and blue light), because low-energy light is less scattered, less damaging to cells, and less absorbed in vivo, meaning that it can penetrate further in biological tissues.\(^{48}\) In contrast, UV light is carcinogenic in itself,\(^{49}\) and does not penetrate deeply in tissues, which limits in vivo applications. Several features determine the potential efficiency of phototherapy agents: (i) water solubility, (ii) stability in the
Photodynamic therapy (PDT) relies on absorbance of light by a photosensitizer, and transfer of this energy to ground state triplet oxygen (‘O3) to form highly reactive excited state singlet oxygen (‘O2). The photogenerated ‘O2 can induce damages to biomolecules, or react with biomolecules to form other ROS species such as H2O2. This type of PDT is commonly referred to as PDT type 2. PDT type 1 involves the direct reaction between a photosensitizer and biomolecules without the direct involvement of dioxygen, but it is much less common than PDT type 2. To date, PDT photosensitizers approved in the clinic are the organic molecules Photofrin, Foscan, Levulan, and Metvixia cream. Among inorganic PDT photosensitizers the water-soluble palladium porphyrin complex, Padeliporfir (WST11), developed by Scherz and Salomon, is the only compound clinically tested and even approved in some countries (Figure 1.8, left).50 Phase II clinical trials demonstrated that 4 mg/kg WST11, and light doses of 200 J.cm⁻¹ (λirr = 753 nm), are the optimal treatment conditions for patients with localized low-risk prostate cancer. Evaluation six months after treatment demonstrated that > 80% of patients were tested negatively for prostate cancer. WST11 has passed Latin America Phase III clinical trials, and is currently clinically available in Mexico. Results of a European Phase III clinical trial have been submitted to the European Medicine Agency in January 2016 and approval of WST11 is pending. In the field of ruthenium chemistry a highly promising PDT photosensitizer, TLD1433, has been developed by McFarland et al. (Figure 1.8, right). In vitro this compound has an EC50 value after light activation by red light (λirr = 625 nm) of 19 μM, while in the dark it remains higher than 300 μM in HL-60 cells.51 In 2016 TLD1433 finished Phase I-II clinical trials with promising outcomes as treatment for bladder cancer, and it is currently lining up for Phase III clinical trials.52
Similarly, aldehyde, sulfoxide, or 50-λ ch areas in the field of PACT focuses on ruthenium 50-λ of PACT relies on a 63-λ - - - - - 54-λ - - - - - treatment this is a prodrug that after its photoproducts is a biologically active molecule. Typically in 1.5 - - - - - 1.5.1 Introduction photoactivated chemotherapy Many sections of tumors are poorly oxygenated (hypoxic), while PDT type 2 relies on the presence and activation of dioxygen. PhotoActivated ChemoTherapy (PACT) is an alternative phototherapy that takes advantage of local photochemical activation of a prodrug to acquire high specificity. The concept of PACT relies on a (non-toxic) prodrug that upon light activation undergoes photolysis or photosubstitution. Whereas the prodrug has no biological activity, at least one of its photoproducts is a biologically active molecule. Typically in anticancer treatment this is a prodrug that after light activation becomes cytotoxic.

One of the key research areas in the field of PACT focuses on ruthenium polypyridyl complexes. Upon irradiation, this type of metal complexes first is excited to a singlet metal-to-ligand charge transfer (1MLCT) state, from which very fast (femtosecond time scale) intersystem crossing populates the corresponding triplet MLCT state (3MLCT). From this long-lived 3MLCT state the system can then either relax to the ground state by emission of a photon (phosphorescence) or thermally populate triplet metal-centered states (3MC) if these states are low enough in energy. 3MC states have a dissociative character because an antibonding dσ* orbital of the metal center is occupied by one electron. 3MC population hence leads to increased lability of at least one of the ligands that subsequently may be photosubstituted. In other words, such light-activatable ruthenium complexes can undergo ligand photosubstitution. Ligands that can be photosubstituted are
typically bound to a ruthenium(II) center via nitrile, amine, pyridine, sulfoxide, or thioether functional groups. In the following sections several examples of ruthenium complexes will be described that have been reported as light-activatable anticancer drugs in vitro.

1.5.2 PACT based on cis Ru complexes

The complex [Ru(bpy)2(dmbpy)]Cl2 (bpy = 2,2’-bipyridine, dmbpy = 6,6’-dimethyl-2,2’-bipyridine) developed by Glazer et al. was the first reported ruthenium-based PACT complex (Figure 1.9). Upon visible light irradiation ($\lambda_{irr} > 450$ nm) [Ru(bpy)2(dmbpy)]Cl2 releases its sterically demanding dmbpy ligand. In vitro this light activation increases cytotoxicity of the compound from an EC50 of over 150 $\mu$M in the dark to an EC50 to 1.1 $\mu$M after light activation, which represents a 136-fold increase in cytotoxicity. Similarly, [Ru(biq)(phen)2]Cl2 (biq = 2,2’-biquinoline, phen = 1,10-phenanthroline), displayed in Figure 1.9, was also shown by Glazer et al. to be a potential PACT compound. Photoejection of the biq ligand in vitro is accompanied by lowering of the EC50 from 52.5 $\mu$M in the dark to 1.2 $\mu$M after light activation with white light ($\lambda_{irr} > 400$ nm). Glazer attributed the enhanced toxicity of the compound to the two available cis coordination positions that become available upon photosubstitution of biq by two labile aqua ligands. Binding of the light activated ruthenium complex to DNA similar to cisplatin-DNA binding was suggested as a possible cause for light-enhanced cytotoxicity.

The cyclometallated compound RDC11 – described in section 1.2 – also exhibited a 14-fold increase in cytotoxicity after red-light activation ($\lambda_{irr} = 690$ nm), from an EC50 in the dark of 1.0 $\mu$M to an EC50 after light irradiation of 70 nM. The Turro lab has also developed PACT compounds such as [Ru(bpy)(dppn)(CH3CN)](PF6)2 (dppn = benzo[b]dipyrido[3,2-a:2’,3’-c]phenazine, Figure 1.9) that photosubstitutes both CH3CN ligands in H2O. In vitro on HeLa cells the toxicity of the complex increases from an EC50 of 331 $\mu$M in the dark to an EC50 of 0.47 $\mu$M after blue-light ($\lambda_{irr} = 466$ nm) activation. According to Turro the enhanced cytotoxicity is induced for this compound both by the generation of $^1$O2 (PDT type 2) and by photosubstitution of the CH3CN ligands (PACT). A double mechanism of action is always possible. The examples discussed in this section are all ruthenium(II) compounds with bidentate ligands similar to bpy. Photosubstitution of one of the bpy ligands offers available coordination sites in the cis configuration that are able to bind to DNA in a similar mode as cisplatin. These complexes also have in
common that from the two photoproducts that are produced by photosubstitution reactions, i.e., the ruthenium bis-aqua complex and the free photosubstituted ligand, the metal fragment is usually believed to act as the active species, i.e., the metal-based drug is caged by an organic ligand that can be photosubstituted.

**Figure 1.9** Chemical structures of [Ru(bpy)$_2$(dmbpy)]Cl$_2$, [Ru(biq)(phen)$_2$]Cl$_2$, and [Ru(bpy)(dppn)(CH$_3$CN)$_2$](PF$_6$)$_2$, and photosubstitution reaction with [Ru(bpy)$_2$(dmbpy)]Cl$_2$.

1.5.3 Photocaging
The concept of PACT relies on light activation of a prodrug resulting in photosubstitution. In case of a mononuclear complex for example photosubstitution will lead to a metal-based and an organic fragment. In previous sections a PACT strategy was described wherein the metal fragment acts as the cytotoxic drug. An alternative PACT approach is a strategy wherein the photosubstituted organic part acts as the drug and the metal fragment as the caging agent. In light of this strategy Turro has coordinated 5-cyanouracil (5-CNU), the nitrile analogue of the clinically approved anticancer drug fluorouracil, to [Ru(bpy)$_2$(OH)$_2$](PF$_6$)$_2$. Binding of [Ru(bpy)$_2$(5-CNU)$_2$](PF$_6$)$_2$ to DNA in gel
electrophoresis experiments could be photocontrolled ($\lambda_{\text{irr}} > 395$ nm), but no phototoxicity data was reported. In another example reported by Turro [Ru(tpy)(OH)$_2$)$_2$ was utilized as cage, to form [Ru(tpy)(5-CNU)$_3$]Cl$_2$. Activation using visible light ($\lambda_{\text{irr}} > 395$ nm) in water showed the formation of the bis-aqua species [Ru(tpy)(5-CNU)(OH)$_2$]Cl$_2$ via the mono-aqua species [Ru(tpy)(5-CNU)$_3$(OH$_2$)]Cl$_2$ (Scheme 1.1). When HeLa cells were treated with [Ru(tpy)(5-CNU)$_3$]Cl$_2$ a qualitative fluorescence (SYTOX) assay confirmed that cells were dying to a higher degree upon light activation ($\lambda_{\text{irr}} > 400$ nm), compared to a dark control. When in a control experiment cells were treated with 1 molar equivalent of free 5-CNU, the same cytotoxic activity was observed as with the photoactivated compound [Ru(tpy)(5-CNU)$_3$]Cl$_2$. Caging of existing drugs to improve selectivity could be a viable path to the development for new therapies, as pharmacological data on the existing drugs is present. Apart from chemotherapy this could also include other medication where selectivity and dosage are crucial such as antibiotics.

![Scheme 1.1 Photochemical reaction of the PACT compound [Ru(tpy)(5-CNU)$_3$]Cl$_2$.](image)

Photocaging is not limited to breaking coordination bonds between a metal and a ligand, but may also occur within one of the ligands. For example, Gasser has developed the compound [Ru(dppz)$_2$(Cpp-DMNPB)]([PF$_6$]$_2$) (dppz = dipyrido[3,2-a:2',3'-c]phenazine, Cpp = 2-(2-pyridyl)pyrimidine-4-carboxylate, DMNPB = 2-(4,5-dimethoxy-2-nitrophenyl)butene, Scheme 1.2) in which the DMNPB moiety can be cleaved off upon UV irradiation ($\lambda_{\text{irr}} = 350$ nm), to result in the cytotoxic carboxylate ruthenium complex and a non-toxic organic fragment. In vitro this reaction results in a 5-fold enhancement of cytotoxicity (EC$_{50}$, dark $> 100$ µM, EC$_{50}$, light $= 17$ µM). This enhancement in cytotoxicity was attributed to the released metal fragment, as cytotoxicity studies of only the metal fragment resulted in an EC$_{50}$, dark of 16 µM.
ion to a platinum(II) species may occur upon irradiation with UV light or high-energy visible light. Apart from photolysis, this photoreaction typically results in a change from an octahedral platinum(IV) complex to a square-planar platinum(II) complex. This concept was first reported by Nagle. Photoactivation of trans,cis-[Pt(Cl)(I)(en)] (λirr = 410 nm, en = 1,2-diaminocyclobutane) resulted in [Pt(I)(en)] (Scheme 1.3). However, when SK-MEL-24 (melanoma cancer) or TCCSUP (bladder cancer) cells were treated with the platinum(IV) complex no significant enhanced cytotoxicity was observed when trans,cis-Pt(Cl)(I)(en)] was light activated in vitro compared to the dark control.

Coordination of azides to a platinum(IV) metal center enables the photoactivation of platinum(IV) complexes. Irradiation of these PtIV-azide compounds may lead to reduction to a PtII complex and the release of nitrogen. This concept was introduced by Sadler with cis,trans-[PtIV(en)(N3)(OH)2] shown in Figure 1.10.

Scheme 1.2 Photochemical reaction of the PACT compound [Ru(dppz)2(Cpp-DMNPB)](PF6)2.

1.5.4 PACT based on platinum complexes

PACT is not limited to ruthenium complexes. In fact, many transition metal complexes based on rhodium, iridium, or platinum have been developed as PACT agents. For platinum(IV) complexes photoreduction to a platinum(II) species may occur upon irradiation with UV light or high-energy visible light. Apart from photolysis, this photoreaction typically results in a change from an octahedral platinum(IV) complex to a square-planar platinum(II) complex. This concept was first reported by Nagle. Photoactivation of trans,cis-[Pt(Cl)(I)(en)] (λirr = 410 nm, en = 1,2-diaminocyclobutane) resulted in [Pt(I)(en)] (Scheme 1.3). However, when SK-MEL-24 (melanoma cancer) or TCCSUP (bladder cancer) cells were treated with the platinum(IV) complex no significant enhanced cytotoxicity was observed when trans,cis-Pt(Cl)(I)(en)] was light activated in vitro compared to the dark control.

Scheme 1.3 Photochemical reaction of the PACT compound trans,cis-[Pt(Cl)(I)(en)].
Treatment of 5637-bladder cancer cells with cis,trans-\([\text{Pt}^{\text{IV}}(\text{en})(\text{N}_3)_2(\text{OH})_2]\) resulted in a 7-fold higher cytotoxicity (EC\(_{50}\), dark = 357 \(\mu\)M, EC\(_{50}\), light = 49 \(\mu\)M) and a more than 3-fold increase for the 5637-cisplatin resistant cell line (EC\(_{50}\), dark > 200 \(\mu\)M, EC\(_{50}\), light = 67 \(\mu\)M) after activation at \(\lambda_{\text{irr}} = 366\) nm.\(^2\) In this assay the cytotoxicity of the [Pt\(_{\text{II}}\)(en)Cl\(_2\)] was found to be much lower (EC\(_{50}\) = 2.3-14 \(\mu\)M) for both cell lines without change in activity after light activation. Further development by Sadler of this type of complexes resulted in trans,trans,trans-\([\text{Pt}^{\text{IV}}(\text{N}_3)_2(\text{OH})_2(\text{NH}_3)(\text{py})]\) shown in Figure 1.10,\(^73\) that after photoactivation (\(\lambda_{\text{irr}} = 365\) nm) showed EC\(_{50}\) values of 1.9 \(\mu\)M compared to 244 \(\mu\)M in the dark on A2780 cancer cells, which corresponds to a 129-fold increase in cytotoxicity. This work has recently led to the development of trans,trans,trans-\([\text{Pt}^{\text{IV}}(\text{N}_3)_2(\text{OH})_2(\text{py})_2]\) (Figure 1.10) that after photoactivation (\(\lambda_{\text{irr}} = 365\) nm) showed an EC\(_{50}\) value of 1.4 \(\mu\)M compared to > 212 \(\mu\)M in the dark on A2780 cancer cell lines.\(^74\) Although these platinum(IV) complexes have poor absorbance in the visible spectrum, activation with blue light (\(\lambda_{\text{irr}} = 420-450\) nm) was also possible in cell tests.

\[
\begin{align*}
\text{cis,trans-}\ [\text{Pt}^{\text{IV}}(\text{en})(\text{N}_3)_2(\text{OH})_2] & \quad \text{trans,trans,trans-}\ [\text{Pt}^{\text{IV}}(\text{N}_3)_2(\text{OH})_2(\text{NH}_3)(\text{py})] & \quad \text{trans,trans,trans-}\ [\text{Pt}^{\text{IV}}(\text{N}_3)_2(\text{OH})_2(\text{py})_2] \\
\end{align*}
\]

\text{Figure 1.10 Chemical structures of cis,trans-}[\text{Pt}^{\text{IV}}(\text{en})(\text{N}_3)_2(\text{OH})_2], \text{trans,trans,trans-}[\text{Pt}^{\text{IV}}(\text{N}_3)_2(\text{OH})_2(\text{NH}_3)(\text{py})], \text{and trans,trans,trans-}[\text{Pt}^{\text{IV}}(\text{N}_3)_2(\text{OH})_2(\text{py})_2].

### 1.6 Conclusion

Ruthenium complexes have gained increasing attention as metal-based anticancer drug candidates, especially in the field of PACT. However, to date ruthenium complexes have never been more than drug candidates, as only four ruthenium complexes have entered clinical trials. NAMI-A and KPI019 were not further investigated after Phase II clinical trials, NKP-1339 successfully completed Phase I trials, and initiation of Phase III clinical trials for TLD1433 is pending. These investigations are to a certain extent eclipsed by the number of platinum-based compounds (at least 24) that have entered clinical trials which resulted in seven platinum-based chemotherapeutic treatments approved for clinical use.\(^1\) None of these approved platinum drugs involve light activation or a PACT strategy. To be
noted, pharmacological and chemical properties are not the only drivers when it comes to successful development of a drug.

The MoA of platinum(IV) compounds commonly relies on the reduction of a platinum complex to a (known) cisplatin analogue. Functionalization with ligands containing aromatic functional groups instead of the chloride or ammonia ligands or extension of aromaticity can increase the absorbance properties in the visible (and phototherapy relevant) domain, but would strongly alter the biochemical properties, potentially preventing a similar MoA as cisplatin. In addition, it is difficult to reach the red region of the spectrum using this strategy. The limited light absorption properties of platinum compounds in the phototherapeutic window strongly hampers development of this type of platinum(IV) compounds as PACT agents. Ruthenium(II) anticancer complexes on the other hand almost exclusively contain aromatic arene or polypyridyl ligands. Absorbance in the far red of the spectrum is feasible with these kind of ruthenium complexes, and as powerful lasers have become cheap, low absorbance is in principle sufficient to obtain photoactivation. Overall, the potential of ruthenium complexes as photosensitizers in PDT, and their robust photochemistry in PACT, make ruthenium polypyridyl complexes more promising compared to the known light-activatable platinum systems. Furthermore, development of ruthenium complexes is not constrained by a powerful paradigm like the DNA targeting MoA of cisplatin is for research into new platinum based chemotherapy.

1.7 Aim and outline of this thesis

The aim of the research described in this thesis is to develop transition metal-based anticancer drugs. In Chapter 2, the potential of thiols and thioethers as photosubstitutionally labile ligands using [Ru(tpy)(bpy)(OH₂)]²⁺ as model complex are compared. Thiols have been often overlooked as photosubstitutable ligands, although they are omnipresent in biotechnology. This omission is all the more remarkable as GSH is abundant in vitro and in vivo and known to interact with anticancer metallodrugs. It is shown in Chapter 2 that once coordinated to ruthenium, thiols are very sensitive to oxidation, leading to dissociation even in the dark. This specific reactivity makes them unsuitable as protective group for metal-based PACT compounds. In Chapter 3, the use of the poorly toxic [Ru(tpy)(bpy)(OH₄)]²⁺ fragment to cage an organic thioether-containing anticancer
Aim and outline of this thesis

The aim of the research described in this thesis is to develop transition metallodrugs. It is shown in Chapter 2 that once coordinated to a metal center in an octahedral geometry it leaves two trans coordination positions for the binding of (non-toxic) sulfur-based ligands. Upon green light irradiation a 20-fold increase in cytotoxicity is demonstrated in vitro compared to the dark control. Green light activation of these compounds induces cell death via apoptosis, unlike PDT compounds that commonly induce necrosis. We also demonstrate that light-induced cell death is obtained even with insignificant \( \cdot \text{O}_2 \) generation quantum yields. In Chapter 5, the synthesis of a series of three new ruthenium polypyridyl complexes based on the \( \text{H}_2\text{biqbpy} \) ligand and the analogous \( \text{H}_2\text{bapbpy} \) (6,6'-bis[N-(pyridyl)-1-amino]-2,2'-bipyridine) is described. The interaction of these complexes with a 12-mer oligonucleotide is demonstrated to be controlled by light. For the first time, the ruthenium-oligonucleotide adducts formed under light irradiation are analyzed by high-resolution mass spectrometry and gel electrophoresis.

Investigations regarding tetrapyridyl ligands, already discussed in Chapter 4 and 5, are extended in Chapters 6, 7, and 8, to ‘regular’, i.e. non light-activatable, chemotherapeutic metallodrugs based on column 10 transition metals. In Chapter 6, the synthesis and anticancer properties of a series of three new complexes based on the \( \text{H}_2\text{bapbpy} \) ligand coordinated to nickel(II), palladium(II), and platinum(II) are presented. The \( \text{in vitro} \) cytotoxic activity of these complexes ranges from \( \text{EC}_{50} \) values in a mild micromolar range for the nickel complex, to sub-micromolar concentrations for the platinum analogue, and even spectacular nanomolar \( \text{EC}_{50} \) values for the palladium complex. We further show that the activity of the palladium complex is based on the generation of ROS. On the other hand, the
activity of the structurally similar platinum complex seems to depend on DNA interaction. A physicochemical study of the [Pd(H·bapbpy)]$^{2+}$ and [Pt(H·bapbpy)]$^{2+}$ complexes is presented in Chapter 7. In particular, deprotonation of the H·bapbpy ligand leads to a dramatic increase of the absorbance of these complexes in the visible domain; TD-DFT calculations confirm that Intra-Ligand-Charge-Transfer excited states responsible for the absorption changes are far less present in [Pd(H·bapbpy)]$^{2+}$ than in [Pd(bapbpy)]. Crystal structures of each protonation state of the palladium(II) complex ([Pd(H·bapbpy)]$^{2+}$, [Pd(Hbapbpy)]$^{+}$, and [Pd(bapbpy)]) were obtained and described. In Chapter 8, the synthesis and characterization of [Pd(Hbbpya)]$^{2+}$ and [Pt(Hbbpya)]$^{2+}$ are described (Hbbpya = N,N-bis(2,2'-bipyrid-6-y1)amine). Compared to H·bapbpy, the Hbbpya ligand is much more coordinated in a single plane to the metal center, and contains not two, but only one secondary amine. This difference causes marked changes in cytotoxicity, pKa, and uptake of [Pd(Hbbpya)]$^{2+}$ and [Pt(Hbbpya)]$^{2+}$ compared to the H·bapbpy analogues. In Chapter 9, a summary, general conclusion, and outlook are presented.
1.8 References

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CHAPTER 2
Preparation, stability, and photoreactivity of thiolato ruthenium polypyridyl complexes: can cysteine derivatives protect ruthenium-based anticancer complexes?

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