Upconverting nanovesicles for the activation of ruthenium anti-cancer prodrugs with red light

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CHAPTER 1

Introduction I: Ruthenium polypyridyl complexes as potential anticancer prodrugs in photoactivated chemotherapy

Light-sensitive ruthenium(II) polypyridyl complexes are classical tools in photochemistry that have recently been proposed as prodrugs for photoactivated chemotherapy (PACT). The use of light allows for excellent spatial and temporal control over prodrug activation so that the harmful systemic side-effects of chemotherapy are prevented. In this chapter, the topics of ruthenium anti-cancer drugs and photoactivated chemotherapy are introduced. Special attention is given to the mechanism of photosubstitution in ruthenium complexes, and examples are highlighted of ruthenium photosubstitution that are activated with visible light. Finally, it is addressed how the activation wavelength of PACT prodrugs can be shifted to the phototherapeutic window, in order to achieve a better therapeutic efficacy.

1.1 Anticancer transition metal complexes

After the serendipitous discovery of cisplatin in 1969 and its clinical introduction in 1978 as the world's first platinum anticancer drug, the scientific community was convinced that heavy metal coordination compounds, in particular based on platinum, could have potent anti-tumor activity.[1] The excitement of a new form of cancer therapy soon motivated researchers to look for similar compounds with enhanced toxicity against tumors and reduced side-effects.[2] The era of platinum-based chemotherapy began, in which thousands of cisplatin-analogues were tested *in vitro* and in animals, and nearly 40 of them were tested in clinical trials.[3] Although the exact mechanism of action of cisplatin remains elusive even today, it is generally accepted that the cytotoxic activity of platinum compounds is due to binding with the DNA, which prevents cell replication.[4] Despite the tremendous research efforts only two other compounds were ultimately approved for use in clinics worldwide: carboplatin and oxaliplatin (Figure 1.1). It seemed that the discovery of cisplatin had been indeed a lucky shot, and it was realized that it had been a misconception that only compounds analogous to cisplatin are promising clinical anticancer candidates.[3] To break free from this "cisplatin-paradigm", significant efforts have been undertaken towards the development of non-classical platinum complexes. Promising routes are targeted delivery, prodrug activation by light, intracellular prodrug reduction, among others.[4b, 5]

Initially, research on coordination compounds with transition metals such as Ru, Au, Co, Fe, and Ni could not compete with the enormous enthusiasm for platinum chemistry. However, they have received increasing recognition as potent anticancer complexes in the last three decades.[2-3, 5] The leap from platinum to other metals is appealing: a wide variety in coordination geometry, binding preferences, oxidation states, redox activity, and ligand exchange kinetics may lead to a controlled mechanism of cytotoxicity that was previously unattainable with platinum compounds.[3] Of these metals, ruthenium appears to be one of the most promising.[2] As the research described in this thesis concerns the development of ruthenium-based anticancer drugs, this metal will be the focus of this introduction chapter.

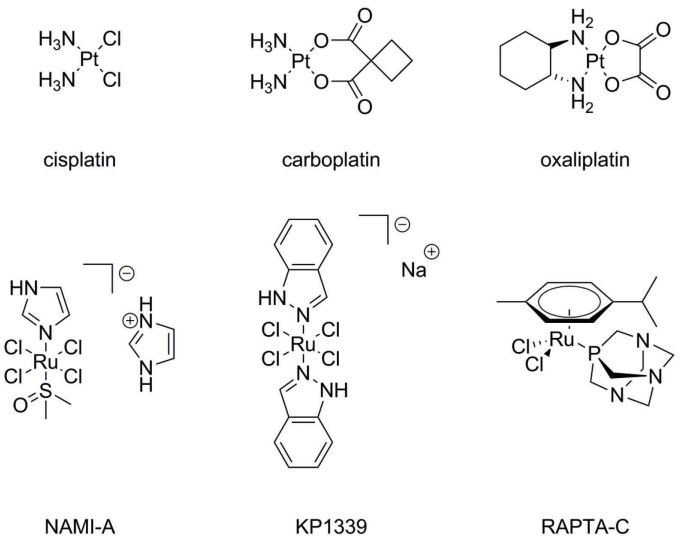

Figure 1.1. Chemical structures of cisplatin, carboplatin, oxaliplatin, NAMI-A, KP1339, and RAPTA-C.

1.2 Ruthenium anticancer drugs

Ruthenium is one of the transition metals most studied for the synthesis of anticancer coordination compounds, and is only surpassed in the number of reported studies by platinum.[6] The advantages of ruthenium complexes include:

i. The preparative coordination chemistry is well-developed, reliable, and the chemical structures are predictable.[3, 7] The usual hexacoordinate octahedral geometry implies a very different reactivity compared to square-planar platinum(II) compounds.[3]

ii. The ligand exchange rates are in the order of minutes to days and can be tuned so that the complexes are relatively kinetically inert under physiological conditions or can interact with physiological processes occurring on the same timescale.[2-3, 8]

iii. The 2+, 3+, and 4+ oxidation states are accessible under physiological conditions,[2-3, 7] which allows the possibility of *in situ* redox-activation of substitutionally inert Ru(III) complexes to active Ru(II) complexes in the highly reductive environment of tumors.[2]

iv. The photophysics and photochemistry of ruthenium complexes is well described and understood,[9] allowing for the smart design of *in situ* light-activatable anticancer compounds.

In the last decade, three major ruthenium-based anticancer drugs have been tested in (pre)clinical trials: KP1339, NAMI-A, and RAPTA-C, see Figure 1.1.[10] In preclinical studies, ruthenium chemotherapeutic drugs were shown to have better selectivity towards tumors and exhibit fewer side-effects than platinum drugs.[2] The activity of ruthenium anticancer drugs are still compared to that of cisplatin, even though they have little in common and each ruthenium compound appears to have a very distinct mode of action.[2-3, 7, 10-11] The cytotoxic effects of KP1339, NAMI-A, and RAPTA-C are mainly attributed to interactions with other biomolecules than nuclear DNA. Regardless, the binding of ruthenium anticancer drugs to DNA is a well-studied subject that is preferred over interaction studies with different biological targets.[12] Ru(II) and Ru(III) coordination compounds interact with DNA due to the relative softness of these ions, which lead to high binding affinities for nitrogen-rich DNA bases.[7] DNA intercalation, groove-bending, and other non-covalent interactions are also possible when the ruthenium compounds contain large planar aromatic ligands.[13] Upon interaction, the complex can either stay covalently or non-covalently bound and disrupt cell proliferation, or induce DNA damage.[7] However, it is important to realize that many Ru complexes do not end up in the nucleus and assert their toxicity through other interactions that are not yet well-explored.

Ruthenium anticancer drugs can be roughly divided into five categories:

i. Active Ru(II) complexes that easily hydrolyze and coordinate to their target biomolecule.
ii. Ru(III) complexes that are activated upon reduction to the Ru(II) complex.
iii. Substitutionally inert Ru(II) complexes that bind non-covalently to DNA or proteins by groove binding or intercalation.[14]
iv. Complexes targeted to unconventional biomolecular targets, *e.g.* specific enzyme inhibitors.[2]
v. Photoactivatable complexes that become toxic or have a toxic effect upon light irradiation

The research described in this thesis focusses on the last category of compounds. They are potential compounds for photoactivated chemotherapy (PACT) and photodynamic therapy (PDT). These topics are introduced in the following sections.

1.3 Photoactivated chemotherapy

Photoactivated chemotherapy (PACT) is a form of anticancer chemotherapy in which a non-toxic prodrug is systemically or dermally administered, and activated selectively at the tumor site by irradiation with visible light. This promising technique provides accurate spatial and temporal control over drug activation that may lead to selective tumor treatment with less side-effects.[9-10, 15] The mechanisms of photoactivated chemotherapy fall into four broad categories: (i) photosensitization or singlet oxygen generation, also known as photodynamic therapy (PDT), (ii) photothermal reaction, (iii) photoinduced redox reactions, and (iv) photosubstitution.[9] In practice, it is difficult to distinguish the different pathways in the complex confinement of a cell. The different photoactivation pathways are often in competition with each other, and depend on solvent, oxygen level, possible reactants present, and the excitation wavelength.[9] The following sections focus on PDT and photosubstitution mechanisms.

1.4 The phototherapeutic window

The organic and metal-organic molecules used in PACT often only absorb considerably in the ultraviolet and blue wavelength region. Light with these wavelengths does not penetrate the body very well due to significant absorption by biomolecules such as melanin and hemoglobin (see Figure 1.2).[16] Furthermore, spatial variations in refractive index within human tissue result in substantial scattering of light: higher energy light (blue region) is scattered more than low energy light (red region).[16] As a result of light scattering and absorption, wavelengths between 700 – 800 nm penetrate human tissue to about 1 cm, while wavelengths near 600 nm penetrate to only 0.5 cm.[17] For blue and ultraviolet light, the penetration depth is a millimeter or less. Wavelengths above 950 nm are absorbed by the molecular vibrations of water. For these reasons, the wavelength domain between about 600 and 950 nm has the optimum transmittance of light, and is therefore called the "phototherapeutic window".[7, 10, 17-18] Moreover, a high dose of blue light itself is toxic for certain tissue types,[19] while red light does not damage tissues at light intensities relevant to PACT.[I] Overall, using red to near-infrared light

[I] Superficial PDT is usually executed with light intensities of 50 – 100 $mW.cm^{-2}$.[20] In the case of internal irradiation using diffuser-tipped light-fibers, the intensity is expressed in terms of $mW.cm^{-1}$ diffuser length. For example, the 630 nm light dose for photodynamic therapy with the clinically approved drug "photofrin" is prescribed as 270 $mW.cm^{-1}$ for < 15 minutes.[21]

would lead to the simultaneous irradiation of a greater tumor volume, while not harming the healthy tissue around it.

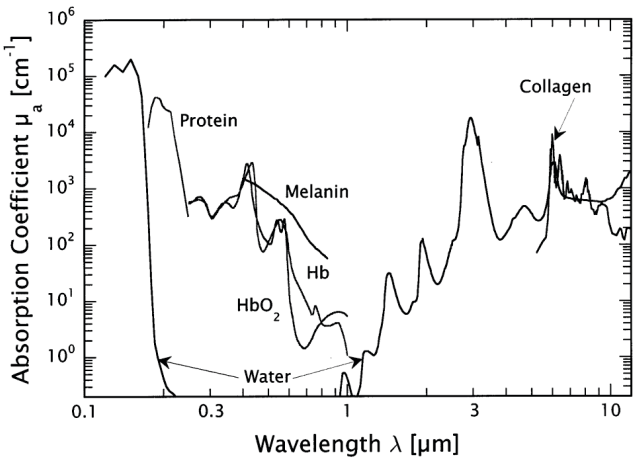

Figure 1.2: Optical absorption coefficients of the major human body chromophores. The phototherapeutic window in which light penetrates the body the deepest, lies between 600 and 950 nm. Reprinted with permission from Vogel et al. [16] © (2003) American Chemical Society.

1.5 Photodynamic therapy

Photodynamic therapy (PDT) was developed as early as the 1900s, but was popularized in clinical therapy by Dougherty in the late 1970s and early 1980s.[18c] Two types of PDT are known that both include strongly absorbing photosensitizer molecules such as porphyrins and phthalocyanins.[18c] In PDT type 1, the photosensitizer absorbs light and then reacts with biomolecules by means of an electron-transfer mechanism.[7] In PDT type 2, which is by far the most common, the photosensitizer is used to generate reactive oxygen species (ROS). Figure 1.3 schematically shows the most important photophysical pathways that are involved in this mechanism. Upon absorption of light the photosensitizer molecule reaches an excited singlet state, which is immediately followed by intersystem crossing (ISC) to a triplet state. Upon collision with ground-state molecular oxygen ($^3\Sigma_g$ state), which is also a triplet state, triplet-triplet annihilation (TTA) may occur. TTA causes the photosensitizer to relax to the singlet ground state, while dioxygen is promoted to a higher-energy singlet state ($^1\Sigma_g$). After internal conversion to the $^1\Delta_g$ state, singlet dioxygen may either chemically react with other molecules, or relax back to the ground state non-radiatively or by emission of a 1270 nm photon. Reaction of singlet oxygen with cell constituents leads to the irreversible oxidation of DNA, lipids, amino-acids, cofactors, and proteins.

This damage triggers pathways towards programmed cell death (apoptosis), or cause instant cell death (necrosis).

Although PDT is a promising and increasingly accepted therapy, a few issues need to be addressed.[18a] Firstly, many photosensitizers do not absorb strongly in the phototherapeutic window and rely on blue to green light for activation, leading to poor therapeutic efficiency. Furthermore, many photosensitizers suffer from photobleaching during treatment and poor water solubility, and are often retained in tissues which causes prolonged light-sensitivity for the patient. Most importantly, many tumor tissues are poorly oxygenated ("hypoxic") because of lack of angiogenesis, while the functioning of PDT relies on the presence of dioxygen.[22] It would therefore be beneficial to use PACT drugs that are activated by light but are toxic *via* an oxygen-independent mechanism.

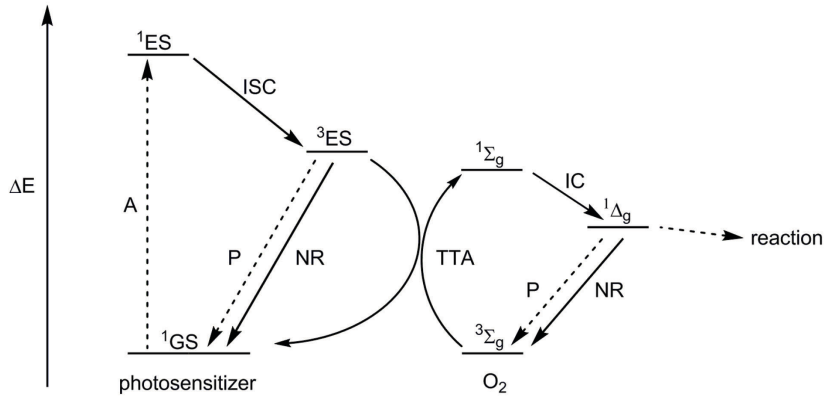

Figure 1.3. Jablonski diagram of the foremost photophysical pathways in photodynamic therapy (type 2), involving a photosensitizer drug and molecular oxygen. Dashed arrows represent transitions in which photons are involved. Abbreviations: GS (ground state), A (Absorption), ES (excited state), ISC (intersystem crossing), P (phosphorescence), NR (non-radiative decay), TTA (triplet-triplet annihilation), IC (internal conversion).

1.6 Photosubstitution

A different PACT mechanism is based on photosubstitution, which relies on caging of a drug with a light-cleavable protective ligand. Upon light activation, the protective ligand dissociates and the active compound is released. Such a strategy does not rely on the presence of dioxygen and is therefore appealing for treatment of hypoxic tumors. Especially ruthenium complexes with heterocyclic N-donor ligands have been widely recognized as particularly attractive candidates for PACT, because of the near-unity intersystem crossing

efficiency to the triplet Metal-to-Ligand Charge Transfer state (^{3}MLCT) state, long-lived excited states, highly tunable photochemical properties, and intensively studied properties in general.[7, 18a] The desirable features for such ruthenium photosubstitution anticancer drugs include: (i) solubility and stability in aqueous biological media, (ii) high cell uptake, (iii) negligible cytotoxicity in the dark and acute anticancer activity when irradiated, (iv) high quantum yield for photosubstitution, and (v) low influence of oxygen on the photophysical and photochemical properties.[9]

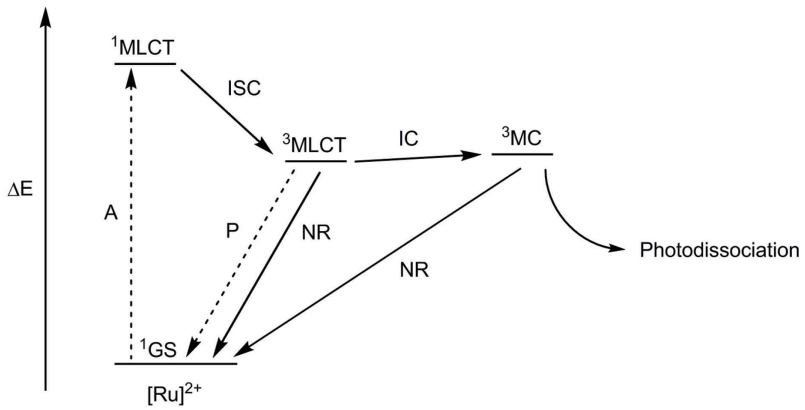

Figure 1.4. Jablonski diagram of the foremost photophysical pathways of a typical photosubstitution Ru polypyridyl complex. Dashed arrows represent transitions in which photons are involved. Abbreviations: GS (ground state), A (Absorption), ISC (intersystem crossing), P (phosphorescence), NR (non-radiative decay), IC (internal conversion), MC (metal centered). Adapted from Göttle et al. [23]

The mechanism of photosubstitution is well understood for ruthenium bipyridine and terpyridine complexes.[23-24] Figure 1.4 illustrates the photosubstitution mechanism for a typical Ru(II) complex with a photolabile ligand. After excitation to the singlet Metal-to-Ligand Charge Transfer state (^{1}MLCT) state and intersystem crossing to the ^{3}MLCT state, a dissociative triplet metal-centered state (^{3}MC) is within reach of (thermal) internal conversion. This ^{3}MC state has dissociative character because the antibonding dσ* orbitals of the metal center become partially occupied, which weakens and elongates a metal-ligand bond. This weakening allows one of the ligands to be substituted by water, thereby giving rise to the potentially cytotoxic aqua derivative. In a biological setting, it is proposed that the aquated coordination site can be used for interactions with biomolecules.[18a, 25]

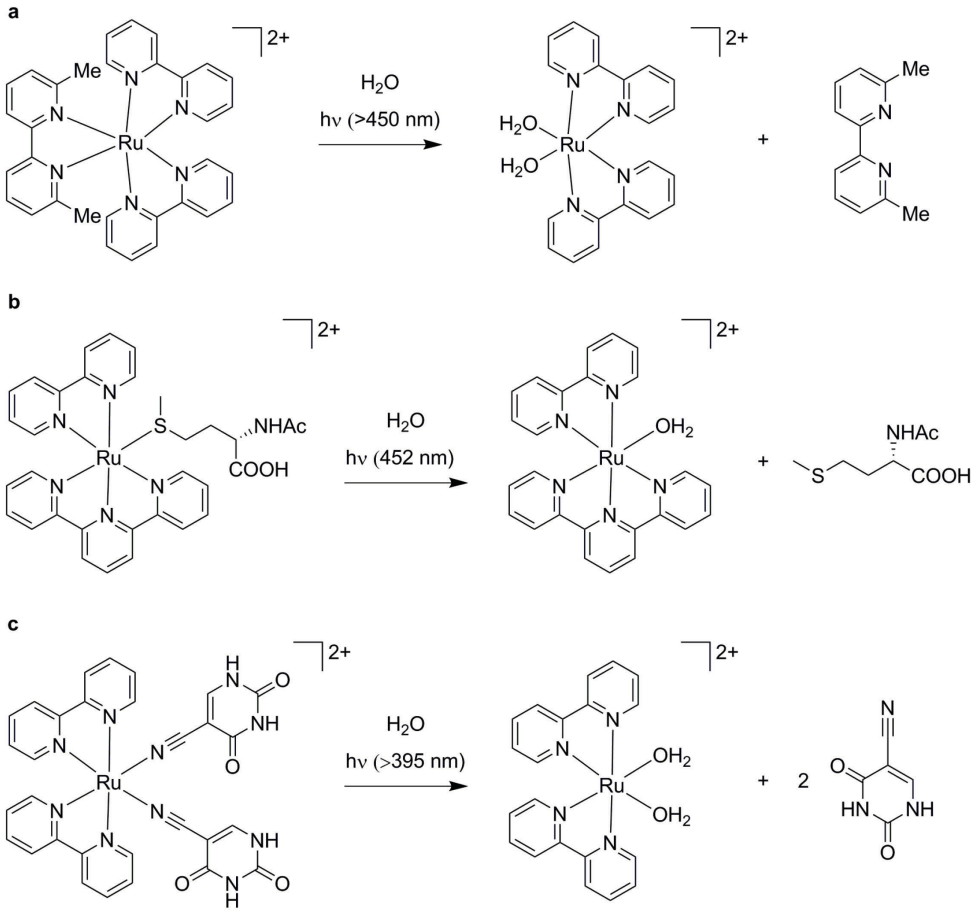

Figure 1.5. Representative examples of photosubstitution ruthenium polypyridyl complexes from the groups of Glazer (a),[26] Bonnet (b),[25d] and Turro (d).[27]

Most Ru(II) polypyridyl complexes are actually quite photostable and the photosubstitution pathway is in competition with other processes such as phosphorescence and non-radiative relaxation.[9, 23a] The mechanism of photosubstitution is strongly dependent on the energy, shape, and position of the potential energy surfaces of the ^{3}MLCT and ^{3}MC states, which determine the accessibility of the ^{3}MC state and hence the dissociation rate.[23a] Meanwhile, the energy gap between ground state and the ^{1}MLCT state determines the maximum absorption wavelength. This means that, ideally, the ^{1}MLCT and ^{3}MC states are both low in energy, so that the complex absorbs in the phototherapeutic window and the photosubstitution takes place efficiently.[23a, 28] In practice, a good trade-off between these two parameters is difficult to achieve.

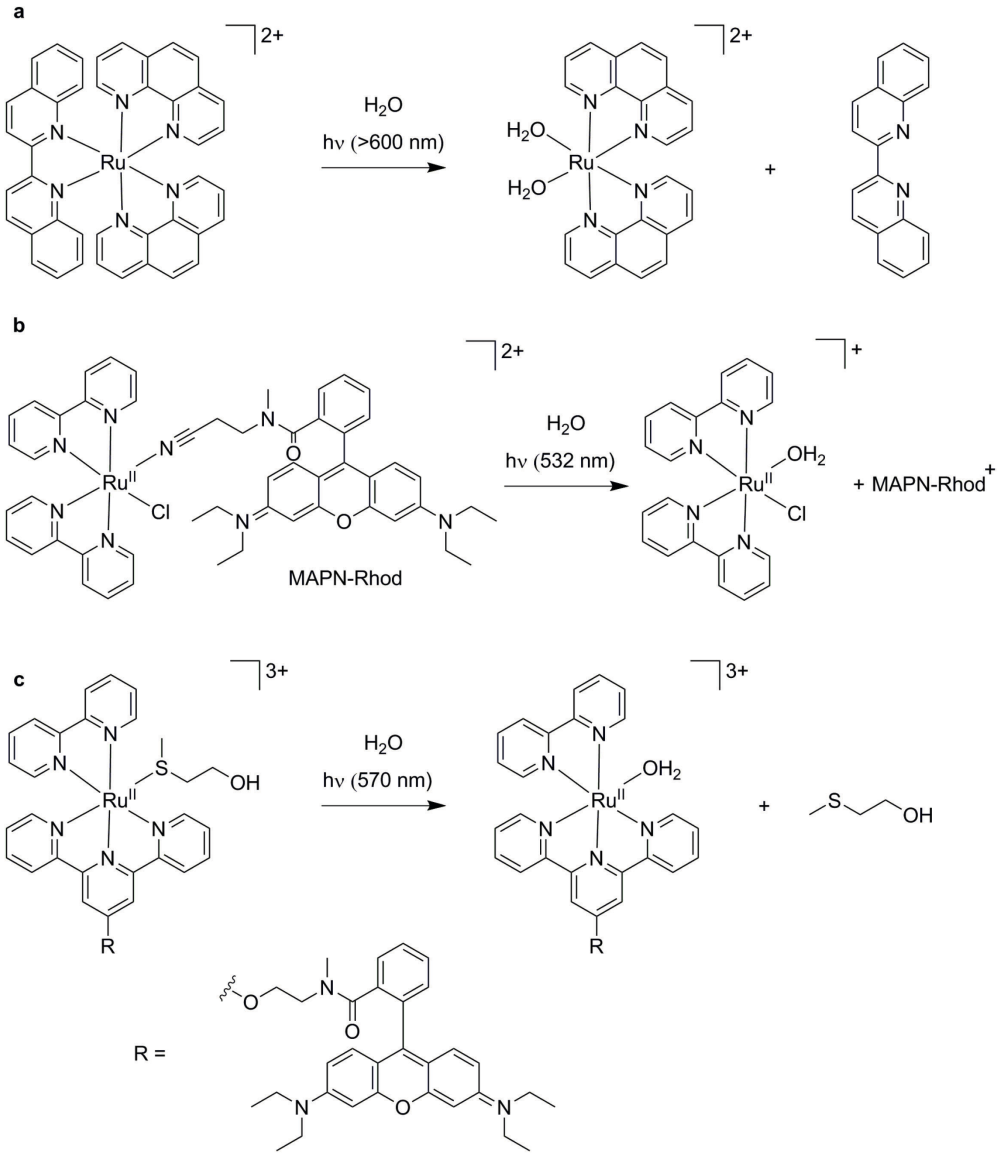

Figure 1.6. Representative examples of photosubstitutionally active ruthenium polypyridyl complexes that are activated with red, green, or yellow light from the groups of Glazer (a),[18a] Etchenique (b),[28] and Bonnet (c), respectively.[29]

A viable strategy to optimize photochemical access to the ^{3}MC state is to induce distortion of the octahedral symmetry around the Ru(II) center. Such distortion leads to smaller overlap between the nitrogen lone pairs and the orbitals of the ruthenium center, and consequently to a smaller ligand field splitting and lower energy of the dissociative triplet Metal-Centered state (^{3}MC) state.[18a, 23b, 26] This makes the ^{3}MC state more accessible from the

photochemically generated ^{3}MLCT state. Popular strategies to induce such distortion include the use of bulky polypyridyl ligands that induce steric hindrance or use of the terpyridine ligand, which coordinates in a strained manner. However, lowering the ^{3}MC state too much is known to cause complex instability in the dark, which is highly undesirable for phototherapeutic purposes.

Some noteworthy examples of blue-light responsive photosubstitutionally active Ru(II) polypyridyl complexes as PACT compounds are given in Figure 1.5. The group of Glazer reported that the strained complex $[Ru(bpy)_2(dmbpy)]^{2+}$ (bpy = bipyridine, dmbpy = 6,6'-dimethylbipyridine) ejects the dmbpy ligand upon >450 nm irradiation which causes a 2 order of magnitude increase in cytotoxicity.[26] The group of Turro reported the complex *cis*-$[Ru(bpy)_2(CNU)_2]^{2+}$ (CNU = 5-cyanouracil) that ejects two equivalents of CNU upon >395 nm irradiation.[27] Both the resulting ruthenium aqua species and the CNU potentially have a biological effect, but photocytotoxicity data on this complex have not been yet published. In recent years, our own research group has mainly focused on analogues of $[Ru(tpy)(bpy)(SRR')]^{2+}$ (tpy = terpyridine, SRR' = thioether ligand) such as $[Ru(tpy)(bpy)(N\text{-acetyl-L-methionine})]^{2+}$, which selectively photoejects the thioether ligand upon 452 nm light irradiation.[23b, 25a, 25d]

Examples of ruthenium complex activation with green or red light are much more rare. An interesting approach to achieve green light activation is demonstrated by the group of Etchenique with the complex $[Ru(bpy)_2(MAPN\text{-}Rhod)Cl]^+$ (MAPN-Rhod = N-methylaminopropionitrile-rhodamine), see Figure 1.6b.[28] The MAPN-Rhod ligand absorbs strongly around 532 nm, and is able to sensitize the GS→^{1}MLCT transition of the ruthenium complex, which normally is not very sensitive for green light, by an intramolecular FRET mechanism (Förster Resonance Energy Transfer). Formally, this was designated to be a "reverse-FRET" mechanism, because in contrast to normal FRET, the maximum emission wavelength of the energy donor is lower in energy than the maximum absorption of the energy acceptor. Photosubstitution is then achieved by the same mechanism as explained above. A similar reverse-FRET strategy was pursued within our group with the complex $[Ru(tpy\text{-}Rhod)(bpy)(2\text{-methylthioethanol})]^{3+}$, see Figure 1.6c.[29] Due to the presence of the rhodamine ligand, it was found that the complex absorbed yellow light very strongly (ε_{570nm} = 44 000 $M^{-1}.cm^{-1}$), while surprisingly, the photodissociation reaction was equally efficient with yellow

(570 nm) and blue light (452 nm). The group of Glazer prepared the strained complex $[Ru(phen)_2(biq)]^{2+}$ (phen = 1,10-phenanthroline, biq = 2,2'-biquinoline), which ejects the biq ligand after light irradiation (Figure 1.6a).[18a] Interestingly, this compound could be photoactivated with red and near-infrared light, which represents the first example of ruthenium based PACT in the phototherapeutic window. The phototoxicity index (PI, *i.e.* the EC_{50} in dark conditions divided by the EC_{50} in light conditions) with blue and infra-red light (both at a dose of 7 $J.cm^{-2}$) was determined to have values of 44 and 3, respectively, while the PI for the well-known PDT drug aminolevulinic acid was determined to be >18. The substantial absorbance up to 700 nm (ε at 650 nm = 500 $M^{-1}.cm^{-1}$) was attributed to direct ^{1}GS to ^{3}MLCT absorption.[30]

1.7 Photosubstitution in the phototherapeutic window

Although some examples exist of photosubstitution ruthenium complexes that are activated with green to near-infrared light (see section 1.6), it remains challenging to realize high photosubstitution efficiency in the phototherapeutic window. Apart from molecular design and modification, other photochemical and photophysical strategies are under development to red-shift the activation wavelength. First of all, two-photon absorption (TPA) can be used, which is the quasi-simultaneous absorption of two photons of low energy to match the ^{1}GS→^{1}MLCT transition energy. Although this technique is effective in shifting the wavelength of activation, and has been used before for ruthenium polypyridyl complexes,[31] it requires high photon density light sources, and it is technically challenging to realize the irradiation of large volumes (*e.g.* a tumor). Secondly, photon upconversion can be used to combine multiple low energy photons into one higher energy photon. The most popular techniques to achieve photon upconversion *in vitro* are by use of lanthanoid-based upconverting nanoparticles (UCNP) and triplet-triplet annihilation upconversion (TTA-UC). The focus of the research described in this thesis is using TTA-UC for the activation of Ru polypyridyl compounds, and is further introduced in Chapter 2.

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