Cancer Therapy

DOI: 10.1002/anie.201204933

Designing Red-Light-Activated Multifunctional Agents for the Photodynamic Therapy**

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antitumor agents · drug design · metal complexes · photodynamic therapy · supramolecular chemistry

The quest to find a cure for cancer has led to the development of a variety of therapies. At issue is the need to selectively destroy cancer cells while not killing so many normal cells that it results in patient death. Typical treatments for cancer include surgery, chemotherapy, and radiation therapy. One means to overcome the limitations of systemic treatment with chemotherapy is the use of photodynamic therapy (PDT).[1] In PDT, an inactive form of the drug is delivered to the patient and light energy is used to generate the active drug only at the site of the tumor.[2,3] PDT allows localized versus systemic delivery and therefore application of highly toxic drugs. Ideal PDT agents need to absorb light in the therapeutic window, 600–1000 nm, where biological tissue does not absorb, be nontoxic in the inactive form, be highly toxic in the active form, and ideally function in tumor tissues that are often oxygen-depleted.

An early-generation PDT agent still in use is Photofrin (**1**; Scheme 1), which is a mixture of substituted porphyrin oligomers.[4] The state diagram (Figure 1) displays the light-activated dynamics observed for typical type II oxygen-dependent PDT agents where excitation is followed by energy transfer to molecular oxygen in tissue, which generates singlet oxygen, $^1O_2$, a reactive oxygen species (ROS). The ROS are highly damaging to a variety of biomolecules, have very short diffusion distances, and lead to cell death.

The development of metal complexes as potential PDT agents is an exciting field where the organic framework as well as the metal incorporated can be varied to tune properties often providing multifunctional activity. Multifunctional activity describes systems that have two modes of action with the biological target, for example binding to and photocleaving of DNA. Metal complexes often allow for the direct optical population of triplet states because of enhanced spin–orbit coupling. The requirement to absorb lower-energy light in the therapeutic window often leads to metal complexes with shortened excited-state lifetimes as predicted by the energy gap law. This has made the development of systems that provide for red-light excitation elusive.

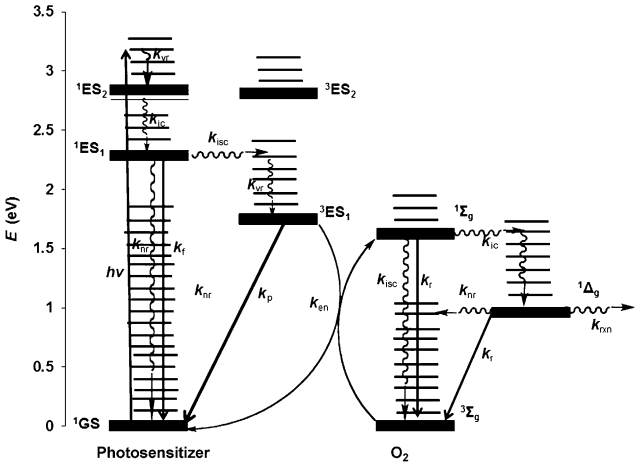

Figure 1. State diagram illustrating the mechanisms of action for drugs displaying oxygen-dependent photodynamic action with *k*'s (rate constants) for f (fluorescence), p (phosphorescence), r (radiative), nr (nonradiative), ic (internal conversion), isc (intersystem crossing), en (energy transfer), and rxn (reaction). The electronic state $^1\Delta_g$ is observed at 1270 nm or 0.97 V, $^1\Sigma_g$ is estimated at 1.6–1.8 V but is short-lived.

Recent approaches to provide for new potential drugs have been successful, including enhanced red-light absorptivity by spin–orbit coupling which allows direct singlet-to-triplet excitation, implementation of targeting agents to deliver the drugs, use of atypical excited states, and the development of type II O_2 independent systems. However, the development of totally new drug motifs through initial in vitro methods can lead to unexpected issues in vivo such as metal toxicity if a toxic level of metal ions is released from the ligand set.

The Fe^{III} complex **2** (Scheme 1) is unique in many aspects.[5] The use of Fe in PDT is not typical, but may lead to lower metal toxicity as the complex is cleared from the system following treatment. The low stability of bidentate coordinated complexes in vivo often limits their application. Complex **2** absorbs light in the red using an unusual ligand-to-metal charge transfer (LMCT) excitation shifted into the red at 805 nm by the catechol ligand with a molar extinction coefficient, ε, of 2400 $M^{-1}cm^{-1}$. The catechol ligand provides for higher-energy donor orbitals moving the excitation into

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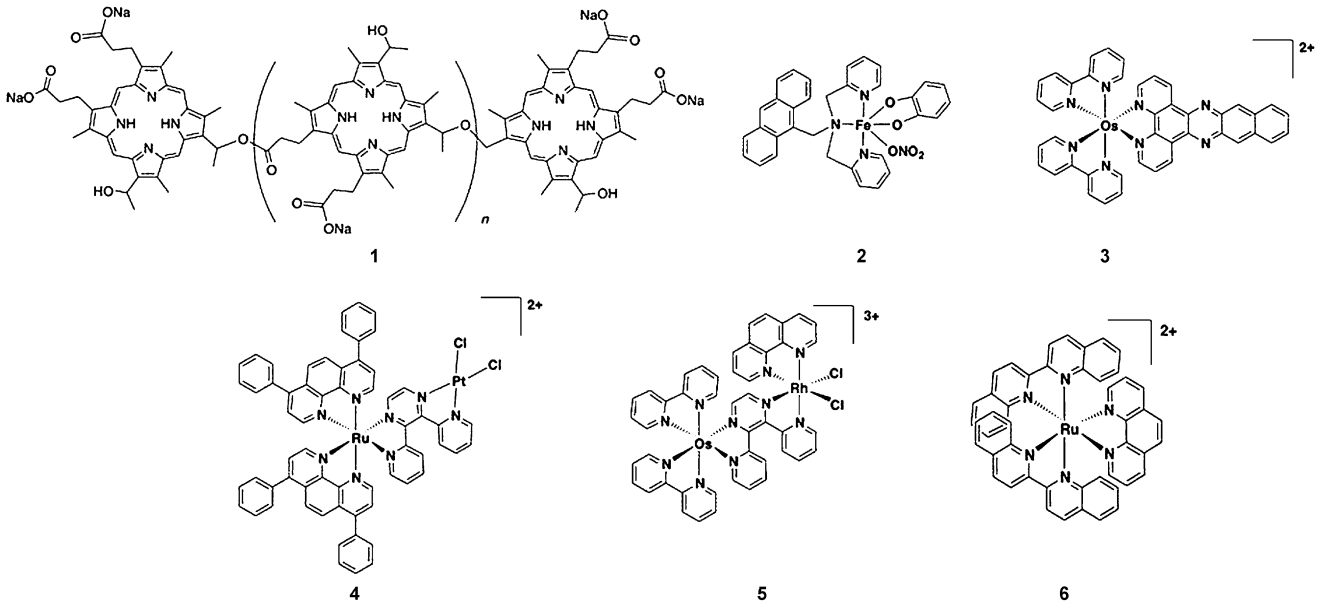
[**] Partial support of our research in photodynamic therapy is provided by Theralase.



Scheme 1. Chemical structures of molecules for photodynamic action. *n* for **1**: 0–6.

the therapeutic window. This is comparable to the 620–650 nm absorption of photofrin with a typical ε value of $3500\,\text{M}^{-1}\,\text{cm}^{-1}$. Metal-based transitions are often broad providing for larger integrated intensities for red-light excitation than expected by simple single-wavelength ε values. The appended anthracene unit provides for DNA targeting of the drug as well as an emissive reporter to show the presence of the complex in the nucleus of cells in culture.

Interaction of **2** with plasmid DNA suggested photocleavage occurred through an electron-transfer mechanism that produces hydroxyl radicals. Proposed is the delivery of the drug to the DNA in the nucleus, which subsequently is excited with red light leading to ROS and DNA cleavage. Intercalation could provide a means to target this drug to the DNA and control the mechanism of cell death. The design of the multifunctional agent **2** coupling a DNA cleavage agent, a possible DNA intercalator to deliver the drug, and an emissive probe to follow the drug in vivo provides for a promising new approach for PDT drug development. The anthracene unit is shown to provide evidence of localization of the drug in the nucleus and indication of DNA targeting by **2**.

Complex **2** is one of only a few red-light-activated metal complexes reported in the current literature. Complex **3** (Scheme 1), an Os^{II}-based molecule (Scheme 1), uses spin–orbit coupling to provide enhanced singlet-to-triplet excitation with red light. Spin–orbit coupling is significant in heavy-atom-containing complexes, mixing the spin–orbital angular momentum quantum numbers. This results in relaxation of the spin selection rule and allows transition between states that are formally of differing spin multiplicities or the observation of transitions that are formally spin forbidden. In these examples excitation from the ^{1}GS of the photosensitizer directly to a ^{3}ES occurs without the need to pass through the higher-energy ^{1}ES (GS: ground state; ES: excited state). System **3** uses direct population of a ^{3}MLCT (metal-to-ligand charge transfer) state from the ^{1}GS. The benzo[*i*]dipyrido[3,2-*a*:20,30-*c*]phenazine ligand provides for an extended π system that could intercalate into DNA providing multifunctional interactions. Through red excitation at $\lambda_{irr} \geq 650$ nm ($\varepsilon = 3300\,\text{M}^{-1}\,\text{cm}^{-1}$), complex **3** generates $^1O_2$ and photocleaves plasmid DNA.[6]

The Ru^{II}–Pt^{II} complex **4** (Scheme 1) is reported to use the *cis*-$PtCl_2$ moiety also found in cisplatin to deliver the Ru^{II} ROS generator at the DNA target and afford red-light-based DNA photobinding and photocleavage.[7,8] Using a ^{1}MLCT state generated through excitation at 520 nm with $\varepsilon = 11\,400\,\text{M}^{-1}\,\text{cm}^{-1}$ to bind to DNA is unique and delivers significantly enhanced absorptivity of visible light compared to the classical Laporte-forbidden ligand field excitation. Red-light excitation of **4** at 590–660 nm is possible because of spin–orbit coupling directly populating the ^{3}MLCT state with an ε value of about $2500\,\text{M}^{-1}\,\text{cm}^{-1}$.

Complex **5** (Scheme 1), an Os^{II}–Rh^{III} complex, provides for the only oxygen-independent, red-light-excited DNA-photobinding and -photocleavage agent.[9] This system uses a MMCT (metal-to-metal charge transfer) state and an Os^{II} chromophore to direct DNA photobinding through Rh to allow the system to cleave DNA in an oxygen-independent manner. The ^{3}MLCT state can be directly populated because of the large degree of spin–orbit coupling in Os^{II} complexes providing absorption at 750 nm with an ε value of about $2900\,\text{M}^{-1}\,\text{cm}^{-1}$. The ^{3}MLCT state then converts to the ^{3}MMCT state with a formally oxidized Os and reduced Rh, which results in frank DNA-single-strand cleavage. Frank cleavage is DNA scission without the need for additional DNA workup. A new conjugated porphyrin with metals added to enhance red-light absorption covalently coupled to a cerasome forming unit affords another multifunctional agent.[10] The cerasome is a partially silica-coated liposome typically employed as a transfection agent, but in this study is used for PDT drug delivery.

Complex **6** (Scheme 1) uses a sterically crowded Ru MLCT chromophore employing 2,2′-biquinoline and direct ^{1}GS-to-^{3}MLCT excitation at $\lambda > 650$ nm ($\varepsilon(650\,\text{nm}) \approx 5 \times 10^{-2}\,\text{M}^{-1}\,\text{cm}^{-1}$) to populate a lower lying ^{3}LF (ligand field) state. The ^{3}LF state promotes ligand loss providing an aqua species that can bind to DNA.[11] This Ru complex is shown to be cytotoxic to HL-60 human leukemia cells.

These recent developments provide clear design parameters to allow for the construction of future multifunctional molecules with promise to deliver new, highly reactive PDT agents that can be excited with red light, target DNA for specific cell death mechanisms, and use oxygen-independent mechanisms of action. Continued development of improved synthetic skills and purification methods allows construction of complex, multifunctional structural motifs. In addition, the dramatic enhancement in analysis techniques provides for clear investigation of these structurally complicated molecules and their functioning.

Received: June 24, 2012
Published online: October 10, 2012

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