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Ru(II) Polypyridyl Complexes as Photocages for Bioactive Compounds Containing Nitriles and Aromatic Heterocycles

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Abstract

Photocaging allows for precise spatiotemporal control over the release of biologically active compounds with light. Most photocaged molecules employ organic photolabile protecting groups; however, biologically active compounds often contain functionalities such as nitriles and aromatic heterocycles that can not be caged with organic groups. Despite their prevalence, only a few studies have reported successful caging of nitriles and aromatic heterocycles. Recently, Ru(II)-based photocaging has emerged as a powerful method for the release of bioactive molecules containing these functional groups, in many cases providing high levels of spatial and temporal control over biological activity. This Feature Article discusses recent developments in applying Ru(II)-based photocaging towards biological problems. Our groups designed and synthesized Ru(II)-based platforms for the photoinduced delivery of cysteine protease and cytochrome P450 inhibitors in order to achieve selective control over enzyme inhibition. We also reported Ru(II) photocaging groups derived from higher-denticity ancillary ligands that possess photophysical and photochemical properties distinct from more traditional Ru(II)-based caging groups. In addition, for the first time we are able to rapidly synthesize and screen Ru(II) polypyridyl complexes that elicit desired properties by solid-phase synthesis. Finally, our work also defined steric and orbital mixing effects that are important factors in controlling photoinduced ligand exchange.

Graphical abstract

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Conflicts of interest

There are no conflicts to declare.

1 Introduction

Photocaging is a highly attractive method for providing precise spatial and temporal control over biological activity.^{1–5} Since photocaging manipulates biological systems in a non-invasive manner,⁶ this method has been employed frequently in basic research. In addition, this technique is currently being explored as a new platform for many exciting applications in photoactivated drug delivery and photochemotherapy.^{7–11}

Traditionally, photocaging has been applied to functional groups such as carboxylic acids, alcohols and amines found in bioactive molecules.^{12–13} While many of organic-based photocaged compounds are commercially available, several disadvantages exist in their application. With a few notable exceptions,^{14–16} most photoremovable organic protecting groups require UV light for uncaging, which adversely causes uncontrollable photodamage to biological systems.^{17–19} Furthermore, functional groups such as nitriles and aromatic heterocycles, which are important “warheads” that interact directly with thiolates and hemes in the active sites of protein targets,^{20–30} cannot be protected by organic groups.

Transition metal fragments are attractive for caging applications, especially complexes that bind to functional groups that cannot be protected with organic compounds. Thus, metal coordination provides an orthogonal approach to photocaging bioactive molecules. Because transition metal complexes often exhibit strong charge transfer absorption in visible range, and the bond between a metal and its ancillary ligand is almost always weaker than an organic σ -bond, metal-based photocaging allows for the facile release of bioactive molecules upon irradiation with low energy light.^{2, 31–45} By manipulating ancillary ligands, one is able to tune the photochemistry for achieving drug release over a broad spectral range in the visible range to near infrared wavelengths.^{9–10} Metal-caged molecules can be selectively liberated with spatiotemporal control, making them promising chemical agents for biological research applications.^{2, 5, 46–49}

In addition to basic research applications, metal-based photocaging is actively being applied towards novel light-activated therapeutics. Photodynamic therapy (PDT) is a clinically validated method for achieving spatiotemporal control over cancer treatment by generating the toxic species $^1\text{O}_2$ at the irradiated area.^{50–53} Although PDT is highly selective and can be localized to specific areas, it relies on the presence of molecular oxygen at the irradiated site.^{50–51} The fact that many tumors are hypoxic in nature limits the efficacy of this treatment,

which makes it imperative to discover new agents that are not only oxygen-independent but capable of generating anticancer effects upon light irradiation.

Ruthenium(II) polypyridyl complexes have been successfully developed as photocages.^{8–10, 46, 49, 54–56} The octahedral geometry of Ru(II) complexes has the ability to undergo excited state ligand dissociation, a feature that is not accessible in square planar Pt(II) complexes such as cisplatin.⁵⁷ In general, Ru(II) polypyridyl complexes are thermally stable in aqueous solutions and able to absorb in the visible range. Due to the property of mimicking iron binding, some Ru(II) complexes accumulate at tumor cells, which is an attractive property for drug delivery.^{58–60} In many Ru(II)-based polypyridyl complexes, ligand dissociation is achieved through the thermal population of ³d-d* states from a triplet metal-to-ligand charge transfer (³MLCT) state after light absorption.^{55, 57, 61}

Since Etchenique and coworkers first reported the development of the Ru(bpy)₂ (bpy = 2,2'-bipyridine) fragment for caging 4-aminopyridine (4-AP),⁹ Ru(II) photocaging groups have been applied towards a wide variety of bioactive molecules, including neurotransmitters, enzyme inhibitors and recently peptides (Fig. 1).^{8–9, 46, 62–74} Ru(bpy)₂ is the most widely used fragment for photocaging these bioactive molecules, which the Etchenique group used to cage γ -aminobutyric acid (GABA)⁴⁶ and nicotine.⁶² In addition, the Turro group showed that both Ru(bpy)₂ and Ru(tpy) (tpy = 2,2':6',2''-terpyridine) groups can be used to cage 5-cyanouracil (5-CNU), a cytotoxic agent that inhibits pyrimidine catabolism *in vivo*.^{54, 63} In order to reduce antibiotic resistance, Sadler and coworkers used Ru(bpy)₂ to cage isoniazid (INH), an anti-tuberculosis compound that is selectively released upon light irradiation to inhibit mycobacterial infections.⁶⁶ The Glazer group caged metyrapone, a potent CYP11B1 inhibitor, with Ru(bpy)₂, where light triggers the release of metyrapone for effective CYP11B1 inhibition.⁶⁸ Vázquez and coworkers also used Ru(bpy)₂ to cage a histidine-containing tripeptide, Arg-Gly-His (RGH), for controlling Ni(II)-dependent nuclease activity *in vitro*.⁶⁷ Apart from Ru(bpy)₂, the Bonnet group photoreleased *N*-acetylmethionine and biotin bound to Ru(tpy)(bpy), where the caged thioethers form stronger bonds than Ru-Cl in aqueous solutions in the dark and can be cleaved upon light irradiation.^{36, 65, 75–77} Recently, they also caged a cytotoxic nicotinamide phosphoribosyltransferase (NAMPT) inhibitor with Ru(tpy)(biq), which exhibits great photoactivated enzyme inhibition.⁷⁸ Finally, the Renfrew group used Ru(phen)₂ (phen = 1,10-phenanthroline) to photorelease econazole, demonstrating that the Ru(II)-caged complex not only selectively releases econazole ligands upon light irradiation but also exhibits strong luminescence for live cell imaging.⁶⁴

In this Feature Article, we present recent developments from our groups on caging bioactive molecules with Ru(II) compounds. Major areas of interest include the caging of bioactive nitriles and heterocycles, as well as the development of Ru(II) caging groups derived from higher-denticity ligands. The later aim was considered to be important and underexplored area ripe for investigation and development, because Ru(II) photocaging groups shown in Figure 1 are all based on planar heteroaromatic ligands, with denticities of two or three. Collectively our studies confirm that Ru(II) photocaging is widely applicable towards bioactive enzyme inhibitors, and that Ru(II) complexes derived from higher denticity ligands are attractive platforms for achieving selective drug release. In many cases our compounds

are stable in the dark, non-toxic and can be used to achieve bioactive compound release in living cells.

2 Ru(II) caging nitriles

To date, bioactive molecules that contain nitrile warheads have not been protected by organic-based photocaging. However, the ability to undergo an efficient photoinduced ligand exchange makes nitriles and nitrile-containing bioactive molecules suitable for metal-based photocaging.^{54, 79} The Ru(bpy)₂ fragment has been successfully employed in caging nitriles to form stable complexes such as [Ru(bpy)₂(MeCN)₂]²⁺ and [Ru(bpy)₂(5-CNU)₂]²⁺, where two equivalents of nitriles are coordinated to Ru(II) and released selectively upon light irradiation.^{54, 79} Although nitriles are biologically robust and commonly found in many bioactive molecules,^{80–81} only a few studies have reported the photocaging of nitriles and nitrile-containing bioactive molecules through Ru(II) coordination.

2.1 The Ru(bpy)₂ fragment as a photocaging group for cysteine protease inhibitors

Cysteine proteases play a critical role in cancer development.^{24–27} Inhibition of cysteine proteases prevents enzyme-substrate interactions and achieves anticancer effects, mainly by inhibiting invasion and metastasis.^{24–25} Nitrile-containing molecules represent a group of inhibitors that inactivate cysteine protease activity by reacting with the active site thiolate of this class of enzymes, where the nitrile serves at the “warhead” and forms a covalent bond with the target protein.^{20–23} For example, Odanacatib, a known nitrile-based inhibitor of cathepsin K that advanced to Phase III clinical trial for the treatment of osteoporosis, binds to the active site of the target enzyme and reduces bone resorption.^{82–83} Because nitriles can be stably coordinated to Ru(II), we hypothesized that cysteine protease inhibition would only occur upon light irradiation. Therefore, in order to control cysteine protease inhibition spatially and temporally, we used Ru(II)-based protecting groups to cage known nitrile-based cysteine protease inhibitors (Fig. 2),^{21, 84} with the caged inhibitors showing the ability to be released upon exposure to light.^{56, 85}

We developed a series of Ru(bpy)₂-caged cysteine protease inhibitors with the general formula [Ru(bpy)₂(L)₂]²⁺, where L = Ac-Phe-NHCH₂CN (**4**), Cbz-Leu-NHCH₂CN (**5**) and Cbz-Leu-Ser(OBn)-CN (**6**) (Fig. 3). Each inhibitor contains an electrophilic nitrile group available to react with the active site thiolate of cysteine proteases for enzyme inhibition. Generally, their Ru(II)-caged complexes share some common photochemical properties: first, they are stable in the dark in aqueous solutions; second, they efficiently release the caged inhibitors upon irradiation with visible light, and in most cases both of the two equivalents of the caged inhibitors are released (Fig. 3); third, all caged complexes show significant enhancement of cysteine protease inhibition in the presence of light, and each of the light-activated complex exhibits greater potency on cysteine protease inhibition than inhibitor alone (Table 1); fourth, the quantum yields of the first ligand exchange process of the caged complexes are lower than that of [Ru(bpy)₂(MeCN)₂]²⁺ ($\Phi_{400} = 0.21$),⁷⁹ which may be due to the larger size and reduced aqueous solubility of the nitrile-based inhibitors relative to MeCN.^{56, 85}

Irradiation of $[\text{Ru}(\text{bpy})_2(\mathbf{1})_2]^{2+}$ (**4**) results in the ~32-fold enhancement of the inhibition of cysteine protease papain compared to that in the dark. Control experiments performed with $[\text{Ru}(\text{bpy})_2(\text{MeCN})_2]^{2+}$ on papain rule out the possibility of enzyme inhibition that may be caused by other photolysis products, which implies that it is the inhibitor **1** released upon light irradiation that leads to enzyme inhibition. The inhibition of purified human cathepsin B, L and K by **4** is also stronger in the presence of light (Table 1). Finally, inhibition on human cell lysates, DU145 and hBMSC cells, that exhibit significant cathepsin B activity^{86–88} further confirms that higher levels of cysteine protease inhibition are acquired when light triggers the release of the caged inhibitors (Table 1).

Compounds **2** and **3** represent structural modifications of **1**, and they also inhibit cysteine proteases, especially cathepsin K.^{21, 84} In addition, both inhibitors are more potent than **1** for cathepsin K inhibition.⁸⁴ Notably, the photochemical release of **2** in $[\text{Ru}(\text{bpy})_2(\mathbf{2})_2]^{2+}$ (**5**) is more efficient than that of **3** in $[\text{Ru}(\text{bpy})_2(\mathbf{3})_2]^{2+}$ (**6**) under similar conditions, which may be due to the smaller size and a better solubility of **2** compared to **3** in aqueous solutions. Inhibition of purified human cathepsin K by **5** and **6** presents a 35- and 88-fold enhancement upon light irradiation, respectively, similar to the observation in **4**. Furthermore, because cathepsin K is a critical enzyme related to osteoporosis,²¹ inhibition of cathepsin K was evaluated in a cell-based assay, where murine BMM and BMM-derived osteoclast cells were treated with complex **5** under the dark and light conditions. Results show that no cathepsin K inhibition occurs in the dark, but the enzyme is effectively inactivated upon light irradiation (Data are not shown). These results suggest that Ru(II)-based photocaging is applicable towards light-activated enzyme inhibition in cells.

2.2 The Ru(TPA) as a photocaging group for nitriles

To date, Ru(II) photocaging groups mostly focus on using bidentate or tridentate ancillary ligands, such as bpy, tpy and phen.^{8, 46, 54, 64–65} Complexes derived from these planar heteroaromatic ligands are capable of covalently binding to DNA once vacant coordination sites are opened through ligand release.^{89–90} They are also capable of undergoing DNA intercalation to cause cell death.^{91–93} Although such properties are ideal for applications in photoactivated cancer drug design, they are not desirable to be used as photochemical tools for controlling biological activity for basic research applications, where toxicity from the Ru(II) leaving groups is undesired. Notably, Ru(II) polypyridyl complexes that have ancillary ligands with denticity of four or above are still underdeveloped. Such ligands introduce geometrical constraint to metal centers, which in turn stabilize their coordination sphere. Since higher-denticity ancillary ligands can result in Ru(II) complexes with different photophysical and photochemical properties than lower-denticity ancillary ligands, we sought to explore Ru(II) polypyridyl complexes with higher-denticity ancillary ligands.

Ru(TPA) is a class of photocaging groups based on the tertiary amine scaffold tris(2-pyridylmethyl)amine (TPA).⁹⁴ This non-planar tetradentate ligand increases the structural rigidity of Ru(II) polypyridyl complexes, which can aid in decreasing non-radiative relaxation to the ground state following absorption of a photo. Ru(TPA) as a photocaging group has only been investigated for the release of nitric oxide (NO).⁹⁴ Since TPA has the

high potential of derivatization, Ru(TPA) is attractive for the development of new metal-based photocaging groups.

In our initial work, we used Ru(TPA) as the photocaging group to cage two different nitriles, MeCN (**7**) and the nitrile-based cysteine cathepsin K inhibitor Cbz-Leu-NHCH₂CN (**8**) (Fig. 4).⁹⁵ We demonstrated that Ru(TPA), similar to Ru(bpy)₂, is an effective photocaging group for nitriles, and that the enzyme inhibition of cathepsin K can be precisely controlled with light. Both complexes show strong absorption in the UV range, consistent with their singlet metal-to-ligand charge transfer (¹MLCT) bands occurring at $\lambda < 400$ nm. In addition, **7** and **8** are highly stable in the dark, with half-lives of > 730 days in aqueous solutions. Since TPA coordinates to ruthenium by four nitrogen-donor atoms, two coordination sites are thus available for caging nitriles, where one nitrile is positioned *cis* to the basic amine nitrogen, and the other is *trans*. UV Irradiation selectively releases the *cis* nitrile, while the *trans* nitrile remains bound. The quantum yields ($\lambda_{irr} = 350$ nm) for the exchange of *cis* nitrile with solvent in **7** and **8** were determined to be 0.012(1) and 0.011(1) in H₂O, respectively. These values are smaller than that of [Ru(bpy)₂(MeCN)₂]²⁺ ($\Phi_{400} = 0.21$), a point that remains to be investigated.

Notably, complex [Ru(TPA)(**2**)₂]²⁺ (**8**) also acts as a potent photoactivated inhibitor of cathepsin K and liberates the caged inhibitor upon light irradiation. The enzyme inhibition is enhanced by a factor of ~89 exposure to light, which is nearly as potent as the free inhibitor **2** under similar experimental conditions (Table 2). In addition, the inhibition of cathepsin K is not observed when complex **7** is treated against the enzyme either under dark or light conditions, indicating that the complex and their photochemical byproducts do not contribute to cathepsin K inhibition. Taken together, these data support that Ru(TPA) is appropriate as a light-activated agent for caging nitriles.

2.3 Solid-phase synthesis of Ru(II)-MeCN caged complexes

Although Ru(TPA) functions as an effective photocaging group for nitriles, UV light is still required for uncaging. In efforts to tune the spectral properties of Ru(II) polypyridyl complexes into the visible range and optimize properties of photocaging groups, we next developed a method to rapidly synthesize and screen Ru(II) polypyridyl complexes capable of selectively releasing nitriles with low-energy irradiation.⁹⁶ A library of TPA derivatives were synthesized on polystyrene resin. Accordingly, Ru(II)-MeCN complexes were generated from these resin-bound ligands, followed by cleavage from the resin for photochemical analysis.⁹⁶ This is the first time applying solid-phase synthesis to the discovery of new metal-based caging groups.

Ru(II)-MeCN complexes generated by solid phase display a wide range of spectral tuning and reactivity with visible light. All of the complexes absorb strongly in the UV range and the derivatives that possess the ancillary ligands DPAbpy (DPAbpy = *N,N*-bis(2-pyridinylmethyl)-2,2'-bipyridine-6-methanamine), TQA (TQA = tris(1-isoquinolylmethyl)amine) and N4Py (N4Py = 1,1-di(pyridin-2-yl)-*N,N*-bis(pyridin-2-yl)methyl)methanamine) (Fig. 5) also absorb in the visible range. Thus, these Ru(II)-MeCN derivatives were chosen for further evaluation of their photochemical properties in solution phase.

Our results indicate that although $[\text{Ru}(\text{DPAbpy})(\text{MeCN})]^{2+}$ (**9**) absorbs strongly in the visible range, it does not undergo photoinduced ligand exchange upon irradiation with visible light, consistent with the observation in solid phase where no bathochromic shift for ligand substitution is observed upon irradiation with visible light. Complex $[\text{Ru}(\text{TQA})(\text{MeCN})_2]^{2+}$ (**10**), however, not only absorbs strongly in the visible range but also exhibits strong reactivity with visible light. Similar to $[\text{Ru}(\text{TPA})(\text{MeCN})_2]^{2+}$ (**7**), the *cis* MeCN in **10** is selectively released upon 400 nm irradiation with a quantum yield of 0.027(1) in H_2O , a value that is > 2-fold greater than that measured for **7** ($\Phi_{400} = 0.012(1)$). An interesting phenomenon was observed for $[\text{Ru}(\text{N4Py})(\text{MeCN})]^{2+}$ (**11**): a bathochromic shift of substitution of H_2O for MeCN does not occur when upon irradiation of the N4Py complexes derived from solid or solution phase. Instead, based on the photolysis monitored by ^1H NMR spectroscopy, one of the coordinated pyridyl substituents of the N4Py ligand undergoes dissociation with H_2O trapping the vacant coordination site. To conclude, these data indicate that the photochemical behavior of the Ru(II) polypyridyl complexes synthesized through solution phase are in good agreement with those generated by solid phase, validating that solid-phase synthesis is applicable towards the rapid preparation and identification of Ru(II) photocaging groups for nitriles. However, spectral tuning to achieve absorption in the visible range does not guarantee the photorelease of the caged nitriles. Therefore, further investigation was required to obtain a clearer understanding of factors that control photoinduced ligand exchange in Ru(II) polypyridyl complexes.

2.4 Orbital mixing enhances photoinduced ligand exchange

Generally, in order to achieve photoinduced ligand exchange, the complex must absorb in the desired wavelength of the light source. Once the Ru(II) polypyridyl complex absorbs a photon, the complex is excited to a $^1\text{MLCT}$ state, which intersystem crosses to a $^3\text{MLCT}$ state with ~100 % efficiency. At this stage, it is generally believed that the thermal population of a $^3\text{dd}^*$, metal-centered (^3MC) state through internal conversion accomplishes ligand dissociation.⁵⁷ Density functional theory (DFT) calculations performed in collaboration with the Schlegel group helped to explain the reasons for differences in the photoreactivity of several Ru(II) polypyridyl complexes.⁶¹ By computing the energies and geometries of the $^3\text{MLCT}$ states and the corresponding dissociative ^3MC states, they concluded that orbital mixing is essential for efficient photoinduced ligand exchange, where the octahedral geometry of the Ru(II) complex is distorted to achieve favorable orbital overlap for efficient electron transfer.⁶¹ This accounts for the efficient photorelease of the *cis* MeCN in **10**: since the bond between the N of the *cis* MeCN and the ruthenium is perpendicular to the two isoquinolyl rings of TQA, efficient mixing between the π^* orbitals of the two isoquinolyl rings of TQA and the Ru-N (*cis* MeCN) $\text{d}\sigma^*$ orbital occurs. In contrast, orbital mixing is not possible between the *trans* MeCN and TQA, so the *trans* MeCN is not photolabile. Similarly, the only MeCN in $[\text{Ru}(\text{DPAbpy})(\text{MeCN})]^{2+}$ (**9**) is coplanar with the bpy moiety of DPAbpy, making the Ru-N($\text{d}\sigma^*$) orbital and π^* orbital of bpy orthogonal. Such an orbital orientation disfavors photodissociation since large energy barrier is likely to arise in order to achieve a favorable geometry for ligand dissociation. Therefore, in these complexes, photoinduced ligand exchange occurs most favorably when orbital mixing in photoexcited states is geometrically allowed.

2.5 Steric effect influences photoinduced ligand exchange

In addition to orbital mixing, steric effect also plays an important role in the photochemical reactivity of Ru(II) polypyridyl complexes.^{49, 70, 97} In order to evaluate steric effects on the photodissociation of nitriles from Ru(TPA), methyl groups were introduced consecutively to the 6-position of pyridine donors of TPA, resulting in three new complexes with the general formula $[\text{Ru}(\text{L})(\text{MeCN})_2]^{2+}$, where L = MeTPA (**12**), Me₂TPA (**13**) and Me₃TPA (**14**) (Fig. 6).⁹⁸ Complex **14** is isolated as single isomers while complex **12** and **13** are obtained as 2:1 and 1.5:1 mixtures of stereoisomers, respectively. All three complexes are less stable than $[\text{Ru}(\text{TPA})(\text{MeCN})_2]^{2+}$ (**7**), implying that the steric crowding introduced by the methyl groups enhances the dissociation of the caged MeCN ligands. Photochemical studies carried out in CH₂Cl₂ with 10 mM Bu₄NCl show that complex **12** is stable for over 1 h in the dark, and the *cis* MeCN in **12** is released with a similar efficiency as that of the *cis* MeCN in $[\text{Ru}(\text{TPA})(\text{MeCN})_2]^{2+}$ (**7**). However, the *trans* MeCN in **12** is more labile than the *trans* MeCN in **7**, suggesting that the methyl group increases the photolability of the *trans* MeCN in **12**. Interestingly, complexes **13** and **14** undergo ligand exchange in the absence of light, which illustrates that a delicate balance exist between the addition of some vs. too much steric bulk to facilitate photochemical nitrile release, since the latter accelerates thermal ligand exchange in the dark.

In order to gain insight into the relationship between the geometric structures of methyl-substituted Ru(TPA) complexes and their photochemical properties, DFT calculations were performed to investigate the behaviors of these complexes. These calculations were used to gain insight into the thermal reactivity from their ground states and the energy barriers required for photochemical ligand dissociation from the excited states of these complexes.⁹⁸ Relaxed potential energy scans revealed that the estimated energy barrier for the *trans* MeCN to dissociate in **14** (12 kcal/mol) is roughly half of that for the *cis* MeCN to dissociate in **14**. This energy barrier is also nearly half of those required for the dissociation of MeCNs in **7** (20–21 kcal/mol). The sterically encumbered geometry of **14** accelerates the thermal release of the *trans* MeCN, which then relieves the steric repulsion between the two caged MeCN ligands. Similar steric effects were also observed for complexes **12** and **13** (Data are not shown). Taken together, the addition of methyl groups at the axial pyridine of TPA distort the *trans* MeCN and facilitate its release under thermal conditions and confirms that photoinduced ligand exchange is significantly influenced by steric effect.

3 Ru(II) caging aromatic heterocycles

Nitrogen-containing aromatic heterocycles are common functional groups found in many bioactive molecules.⁹⁹ In order to achieve effective spatiotemporal control over their release, coordination metal complexes can be used to protect these aromatic heterocycles.^{19, 64, 66–67, 70} Ru(bpy)₂ is a common photocaging group used for caging aromatic heterocycles, such as 4-AP, nicotine and histidine-containing peptides; however, owing to their stronger metal coordination, the quantum yield for pyridine release is generally significantly lower than that for nitriles.^{9, 62, 67} In addition, other Ru(II)-based photocages have also been developed for caging aromatic heterocycles, such as $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{py})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{econazole})_2]^{2+}$.^{64, 70} All of these Ru(II)-caged complexes are able to

effectively release the caged aromatic heterocycles upon light irradiation. However, most of the ongoing research still largely relies on the use of Ru(bpy)₂ or Ru(phen)₂ for photocaging.

3.1 [Ru(TPA)]²⁺ as a photocaging group for aromatic heterocycles

Because aromatic heterocycles are considerably stronger σ -donors than nitriles, we continued the development of Ru(TPA) for caging aromatic heterocycles. As an initial study, we used Ru(TPA) to cage pyridines (py, **15**), nicotinamides (NA, **16**), imidazoles (Im, **17**) (Fig. 7). Similar to the nitrile-caged complex **7**, two equivalents of aromatic heterocycles are bound to ruthenium center.¹⁰⁰ However, in contrast to those nitrile-caged Ru(II) complexes that require UV light for uncaging, Ru(TPA)-caged aromatic heterocycles show strong absorption in the visible range and can be selectively released upon irradiation with visible light. The absorption of these complexes in the visible range is attributed to several overlapping ¹MLCT transitions that occur between the Ru(II) $d\pi$ orbitals and the π^* orbital of the pyridines in TPA, according to DFT calculations. As expected, the *cis* aromatic heterocycles are rapidly released upon irradiation with visible light. The quantum yields (Φ_{400}) for the release of the *cis* aromatic heterocycles were determined to be $9.7(8) \times 10^{-3}$, $9.1(8) \times 10^{-3}$ and $5.3(3) \times 10^{-4}$ in H₂O, respectively. As expected, these values are lower than that measured for [Ru(TPA)(MeCN)₂]²⁺ (**7**), $\Phi_{350} = 0.012(1)$, consistent with the binding characters of pyridines and imidazoles that form stronger interactions with ruthenium center compared to nitriles. It is worthy to note that in **15** – **17** longer irradiation times also achieve the substitution of the *trans* aromatic heterocycles by H₂O, although extra irradiation energy is required. All Ru(TPA)-caged aromatic heterocycles are extremely stable in the dark in aqueous solutions, including in cell growth media at 37 °C over the course of 24 h. Cell viability measured against human prostate carcinoma PC-3 cells indicate that the complexes and their metal-containing photoproducts are non-toxic either under dark or light conditions. Therefore, Ru(TPA) is not only an effective photocaging group for nitriles and aromatic heterocycles but also has ideal properties as chemical tools. The different photophysical and photochemical properties of Ru(TPA) complexes support further development of Ru(II) photocaging groups with higher-denticity ancillary ligands.

3.2 Ru(tpy)(diimine) fragments as photocaging groups for enzyme inhibitors

[Ru(tpy)(diimine)(L)]²⁺ (L = monodentate ligand) complexes represent another common class of Ru(II)-based protecting groups for caging bioactive molecules.^{36, 49, 65, 70, 75, 77–78} Previous work has successfully demonstrate that the molecules caged by [Ru(tpy)(diimine)]²⁺, such as thioethers and pyridines, are efficiently released upon light irradiation.^{49, 65, 70, 101} The introduction of the steric bulk on the bpy framework, such as bpy, Me₂bpy (6,6'-dimethyl-2,2'-bipyridine) and biq (2,2'-biquinoline), distorts the pseudo octahedral geometry around ruthenium center, which weakens the Ru-L (L = caged molecules) bond and then affords an enhancement of the quantum efficiency of ligand dissociation.^{70, 102–103} In addition, [Ru(tpy)(diimine)]²⁺ only has one coordination site available for caging. Recently, our groups applied [Ru(tpy)(diimine)]²⁺ to caging larger bioactive molecules that contain aromatic heterocycles, including epoxysuccinyl-based inhibitors of cysteine cathepsin L¹⁰⁴ and inhibitors of cytochrome P450 (CYPs)¹⁰⁵, for the purpose of achieving high spatial and temporal control over enzyme activity.

3.2.1 [Ru(tpy)(diimine)]²⁺ for caging cysteine cathepsin L inhibitors—Following the work based on using Ru(bpy)₂ to cage nitrile-based cathepsin inhibitors, we continued to probe cysteine protease inhibition by caging cathepsin inhibitors with the [Ru(tpy)(Me₂bpy)] group. CLIK-148 and CLIK-181 (Fig. 8A) are known epoxysuccinyl-based inhibitors that effectively inhibit cathepsin L activity in a biological environment, where a covalent σ-bond is generated through the irreversible nucleophilic attack from the active thiolate of cathepsin L to the epoxide ring within these inhibitors.^{106–108} However, since epoxysuccinyl-based inhibitors normally lack of functional groups that are capable of metal chelating, two modified CLIK inhibitors, **18** and **19** (Fig. 8A), that have similar potency to CLIK-148 and CLIK-181 were designed, synthesized and caged in [Ru(tpy)(Me₂bpy)(L)]²⁺ (L = **18** and **19**) to generate **20** and **21** (Fig. 8B), respectively.¹⁰⁴ Each modified CLIK inhibitors possesses a pyridyl moiety that binds stably to ruthenium center.

Interestingly, although complex **20** is stable in the dark, cathepsin L activity is inactivated in the dark once the enzyme is treated with the complex. The inhibition introduced by complex **20** under dark conditions is comparable to that caused by the free inhibitor **18** alone, suggesting that the caged **18** can bind to the active site of cathepsin L without breaking the Ru-N (py-R) bond. Complex **21**, on the other hand, is not as stable in the dark, with 30% of cathepsin L activity consumed after 1 h of incubation with the enzyme. However, a > 10-fold stronger cathepsin L inhibition was observed when **21** was exposed to visible light. This suggests that the inhibition of cathepsin L by **21** largely depends on light irradiation, where the caged inhibitor is unleashed, leading to enzyme inhibition. Therefore, these [Ru(tpy)(Me₂bpy)]²⁺-caged epoxysuccinyl inhibitors, **20** and **21**, exhibit different inhibition behaviors in inactivating cathepsin L.

Since complex **20** inhibits cathepsin L in the dark, the binding behavior of **20** was further evaluated by using papain. Data measured by LCMS revealed that **20** binds to the active site thiolate of cathepsin L in the dark, and that [Ru(tpy)(Me₂bpy)(OH₂)]²⁺ is released from the papain inhibitor-conjugate upon exposure to light (Fig. 9). This experiment demonstrates that Ru(II)-caged enzyme inhibitors can inhibit enzymes in a different manner, where the Ru(II) photocaging groups are released upon light irradiation after the caged inhibitor binds to its target.

3.2.2 [Ru(tpy)(diimine)]²⁺ for caging cytochrome P450 inhibitors—Cytochrome P450 enzymes (CYPs) are heme-containing monooxygenases that catalyzes steroid biosynthesis and drug metabolism in humans.¹⁰⁹ Inhibition of CYPs regulates adverse drug-substrate interactions and prevents steroid production, making them attractive targets for the treatments of common diseases.^{110–113} However, since CYP activity is sometimes upregulated in diseased tissues but also plays a crucial role in other areas of the body, traditional CYP inhibition lacks selectivity and usually causes severe side effects.¹¹⁴ Metal-based photocaging provides an attractive approach for gaining spatiotemporal control over CYP inhibition *in vivo*. Recently, the Glazer group reported Ru(bpy)₂-caged CYP11B1 inhibitors as prodrugs for controlling CYP11B1 inhibition, where the caged inhibitors were released upon light irradiation, resulting in CYP11B1 inhibition.⁶⁸ However, such a method still relies on Ru(bpy)₂ as photocages. In addition, they stated that the aqua complex

$[\text{Ru}(\text{bpy})_2(\text{H}_2\text{O})_2]^{2+}$ generated after the photorelease of the caged inhibitors directly targets DNA to induce DNA damage, which offers a dual function except CYP11B1 inhibition.⁶⁸ However, according to the recent study of the Bonnet group on the determination of cytotoxicity of Ru(II) polypyridyl complexes and their photoproducts, $[\text{Ru}(\text{bpy})_2(\text{H}_2\text{O})_2]^{2+}$ is in fact hydrophilic and thus unable to cross cell membranes to cause damage to DNA.¹¹⁵ In an effort to develop photochemical agents for controlling CYP activity with low-energy light, our group further applied the Ru(tpy)(diimine) group for caging CYP inhibitors.

CYP17A1 is an important CYP protein that catalyzes androgen synthesis in humans that contributes to proliferation of prostate cancer cells.³⁰ Abiraterone (ABI) is a known potent steroidal inhibitor of CYP17A1 and effectively prevents androgen production.^{29, 116–119} Abiraterone acetate, a prodrug form of ABI, has been approved by the US Food and Drug Administration (FDA) for the treatment of metastatic prostate cancer.¹¹⁹ In order to control CYP17A1 activity with light, our group designed and synthesized $[\text{Ru}(\text{tpy})(\text{diimine})]^{2+}$ -caged ABI complexes, which effectively release ABI upon irradiation with low-energy light.¹⁰⁵ Due to the outstanding efficacy of photoinduced ligand exchange presented by caging pyridines with Ru(tpy)(Me₂bpy) and Ru(tpy)(biq) fragments,^{49, 70} both were used to cage ABI in complexes **22** and **23** (Fig. 10), which exhibit strong absorption in visible range. However, complex **22** photodissociates ABI more efficiently than complex **23**, with quantum yields ABI exchange for solvent of 0.049(5) and 0.018(1) in CH₃CN, respectively.

In order to gain insight into the binding behavior of CYP17A1, complex **22** was chosen to evaluate the CYP17A1-ABI binding under dark and photolysis conditions. The spectral properties of the heme of CYP binding are usually utilized to monitor the CYP-substrate binding.^{28, 120} According to the structural analysis of CYP17A1 reported by the Scott group, ABI binds to CYP17A1 with a high affinity through a typical Type II binding mode.²⁸ Similar binding difference spectra were obtained with the light-exposed complex **22**, where the released ABI replaced the H₂O molecule that is bound to the heme iron for CYP17A1 inhibition (Fig. 11A).^{28, 30, 116} This binding assay resulted in an estimated K_d value of 89.6 ± 10.5 nM (Fig. 11B), similar to free ABI which binds to CYP17A1 with K_d ≈ 100 nM. In contrast, similar Type II binding difference spectra were not observed under dark conditions (Data are not shown), which implies that the Ru(II)-caged ABI complex is still intact and does not inhibit the enzyme in the absence of light.

The evaluation of **22** and **23** against DU145 human prostate carcinoma cells¹²¹ confirmed that low-energy light can be used to liberate ABI in a cellular environment, achieving photochemical control over cell death. For these experiments **22**, **23** and ABI were evaluated for effects on viability against the DU145 cell line. With **22**, cell viability drops sharply at concentrations higher than 20 μM under irradiation, similar to ABI, whereas in the dark minimal effects on viability are observed (Fig. 12). In contrast, **23** resulted in significant toxicity under both light and dark conditions (data are not shown). Taken together, these results indicate that the nature of the Ru(II) caging group plays a role in controlling cellular behavior, including toxicity. To conclude, CYP17A1 binding is controlled with light using Ru(tpy)(diimine) photocaging groups for caging. The bidentate diimine ligands increase the efficiency of photoinduced ligand exchange, making Ru(tpy)(diimine) fragments promising chemical agents for controlling enzyme inhibition with low-energy light.

Conclusions

In this Feature Article, recent developments of Ru(II) photocaging nitriles and aromatic heterocycles from our group have been discussed. Our groups designed and synthesized Ru(II) polypyridyl complexes that can selectively release caged nitriles and aromatic heterocycles as photochemical agents for controlling biological activity with light. Because most of the Ru(II)-based photocaging groups are based on lower-denticity ancillary ligands, we developed new ancillary ligands with denticity of four or above for caging. Nitriles and nitrile-containing bioactive molecules, such as cysteine protease inhibitors are successfully caged by Ru(bpy)₂ and Ru(TPA), and the caged inhibitors are effectively released upon light irradiation for enzyme inhibition. In addition, we, for the first time, introduced solid phase synthesis to rapidly screen and identify Ru(II) polypyridyl complexes that tune the photochemical properties into visible range. Further, we demonstrated that orbital mixing facilitates effective photoinduced ligand exchange, and steric effects can also be used to increase the efficacy of photodissociation. Recently, we used Ru(TPA) and Ru(tpy)(diimine) to cage aromatic heterocycles, including cysteine protease inhibitors and CYP inhibitors. The Ru(II)-caged aromatic heterocycles absorb strongly in visible range and can be released upon irradiation with low-energy light. Importantly, we validated CYP17A1 binding behavior with Ru(II)-caged ABI complexes under dark and light conditions, making it promising to design photoactivated CYP inhibitors to inactivate CYP activity with high spatiotemporal control.

Many exciting advancements have been made in the last decade by directing Ru(II)-based photocaging towards biological problems, including the development of chemical tools and prototypes for photoactivated drugs. Our groups have contributed to this field by demonstrating light-activated control over enzyme inhibition in cells and by developing high-denticity ligands for Ru(II) caging groups that provide distinctive photophysical and photochemical properties. However, most of these studies investigate Ru(II) photocaging *in vitro*. Therefore, future work will be needed in this area for *in vivo* validation, including the development of Ru(II) photocaging groups that release ligands with longer wavelengths for achieving tissue penetration into the red and near-IR regions. This can be achieved by tuning the nature of ancillary ligands of ruthenium polypyridyl complexes with extended π system or by introducing electron-donating substituents to ancillary ligands. In addition, in order to increase the biocompatibility of ruthenium polypyridyl complexes, complexes need to be more water-soluble and show slow rates of ligand exchange in solution in the dark, in addition to higher levels of turn on for readouts such as cytotoxicity upon photoexcitation. Overall, Ru(II) photocaging is still in an early stage of development, but given its potential to control where and when bioactive compounds and drugs are released, further work is certainly needed.

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Biographies

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Ac-Phe-NHCH₂CN
1

Cbz-Leu-NHCH₂CN
2

Cbz-Leu-Ser(OBn)-CN
3

Fig. 2.
Structures of nitrile-based cysteine protease inhibitors.

Fig. 3.
Schematic representation of photoinduced ligand exchange in Ru(bpy)₂-caged nitrile-based cysteine protease inhibitors.

Fig. 4.
 Structures of $[\text{Ru}(\text{TPA})(\text{RCN})_2](\text{PF}_6)_2$, where $\text{RCN} = \text{MeCN}$ (**7**) or $\text{Cbz-Leu-NHCH}_2\text{CN}$ (**8**).

Fig. 5. Structures of [Ru(L)(MeCN)_n](PF₆)₂, where L = DPAbpy (**9**), n = 1; L = TQA (**10**), n = 2; and L = N₄Py (**11**), n = 1.

A. MeTPA ($R_1 = \text{Me}$, $R_2, R_3 = \text{H}$)Me₂TPA ($R_1, R_2 = \text{Me}$, $R_3 = \text{H}$)Me₃TPA ($R_1, R_2, R_3 = \text{Me}$)**B.** $R = \text{H or Me}$ **Fig. 6.**

(A) Structures of MeTPA, Me₂TPA and Me₃TPA. (B) Model of $[\text{Ru}(\text{L})(\text{MeCN})_2]^{2+}$, where $\text{L} = \text{MeTPA}$ (**12**), Me₂TPA (**13**) or Me₃TPA (**14**), showing MeCN groups *cis* and *trans* to the basic nitrogen donor that undergo selective thermal or photochemical release.

Fig. 7.
Structures of [Ru(TPA)(L)₂](PF₆)₂, where L = py (**15**), NA (**16**), or Im (**17**).

A. **CLIK-148 (X = CH, Y = N)**
18 **CLIK-181 (X = CH)**
19**B.**

Fig. 8.
 (A) Structures of CLIK-148 and CLIK-181; Structures of CLIK-modified cathepsin L inhibitors, **18** and **19**; Structures of $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{py-R})]\text{Cl}_2$, where py-R is inhibitor **18** and **19**, respectively.

Fig. 11.
(A) Titration of CYP17A1 with increasing concentrations (0–280 nM) of light-irradiated complex **22**. (B) A plot of $\Delta A_{424-393 \text{ nm}}$ vs. ligand concentration (0 – 280 nM).

Fig. 12.

Cell viability of **22** against DU145 cell line. The cell viability was determined by MTT assay after 48 h and is reported relative to control with only the vehicle (0.5% DMSO) added. Cells were incubated in the presence of **22** (10 mM to 100 mM) at 37 °C for 30 min, followed by 10 min incubation in the dark at room temperature or irradiated with a 250 W tungsten halogen lamp ($\lambda_{irr} = 395$ nm). The cytotoxic compound thapsigargin (TPG; 10 mM) was used as a positive control. Error bars represent the standard error of the mean of quadruple wells, and data are representative of four independent experiments.

Table 1

IC₅₀ values of **1** and **4** under dark and light conditions against human cathepsin B, K and L and cathepsin B activity in human cell lysates.

	IC ₅₀ (μM)			
	1	4 (dark)	4 (light)	dark/light
Cathepsin B ^a	133	892	63	14
Cathepsin K ^a	12	176	5.4	33
Cathepsin L ^a	72	225	40	5.6
DU145 ^b	182	658	82	8.0
hBMSC ^b	183	580	88	6.6

^a Activities were determined with the fluorogenic substrates Z-Arg-Arg-AMC (cathepsin B), Z-Gly-Pro-Arg-AMC (cathepsin K), and Z-Phe-Arg-AMC (cathepsin L). Substrate concentrations were 100 μM. Reactions were conducted in the dark or after 10 min of irradiation of **4** with a tungsten halogen lamp (>395 nm and H₂O filter, 250 W). Cathepsin B conditions: 0.4 M acetate buffer, pH 6.0, 4 mM EDTA, 8 mM DTT, [cat B] = 8 nM. Cathepsin K and L conditions: 0.4 M acetate buffer, pH 5.5, 4 mM EDTA, 8 mM DTT, [cat K or L] = 20 nM. Final solutions contained 1% DMSO.

^b Activities were determined in DU145 and hBMSC lysates using the fluorogenic substrate Z-Arg-Arg-AMC. Reactions were conducted in the dark or after 10 min of irradiation of **4** with a tungsten halogen lamp (>395 nm and H₂O filter, 250 W).

Table 2

IC₅₀ values of Cbz-Leu-NHCH₂CN (**2**) and complex **8** under dark and light conditions against human cathepsin K^a.

	Cbz-Leu-NHCH ₂ CN (2)		complex 8	
IC ₅₀ (nM)	dark	light	dark	light
	34	27	5600	63

^aThe enzyme activity was determined with the fluorogenic substrate Z-Gly-Pro-Arg-AMC and is expressed as a percentage, with 100% equal to the activity in the absence of inhibitor. Data are representative of three independent experiments. Conditions: 0.4 M acetate buffer, pH 5.5, 1% DMSO, [cathepsin K] = 2 nM, [Z-Gly-Pro-Arg-AMC] = 100 μM, DTT = 8 mM, and 15 min of irradiation with a 365 nm light source (8W).