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Light-activated Ru(II) complexes for caging cytochrome P450 inhibitors

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Abstract

New Ru(II)-caged abiraterone complexes were synthesized that exhibit strong absorption in the visible region and release the steroidal CYP17A1 inhibitor abiraterone upon exposure to low energy visible light in buffer and prostate cancer cells. Photoinduced release results in abiraterone binding to its CYP17A1 target in an inhibitory mode.

Graphical abstract

Visible light triggers release of the prostate cancer drug abiraterone in cancer cells and achieves photoactivated cytochrome P450 binding.

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[†]Electronic Supplementary Information (ESI) available: Experimental procedure for the synthesis of 1 and 2, characterization data for 1 and 2, and experimental procedures for photochemical and biological studies. See DOI: 10.1039/x0xx00000x

Cytochrome P450 enzymes (CYPs) are a ubiquitous class of heme-containing proteins that catalyze many key biological transformations, including xenobiotic detoxification and hormone metabolism.¹ CYP activity is tightly regulated to specific cell types and tissues, and deregulation can contribute to human disease.² Due to their prevalence, potent and selective CYP inhibitors have been aggressively pursued as chemical tools, pharmaceuticals and more recently insecticides.³ Although many CYP inhibitors have been developed, those that can be delivered at a desired time with spatial control over CYP binding are not yet available.⁴ This represents an unmet need for basic research applications, and may also provide therapeutic value because off-target inhibition of CYPs in undesired tissues leads to negative side effects in the clinic (*vide infra*).

Photocaging allows researchers to control biological activity spatiotemporally,^{5–6} and can be used to trigger drug release in selected tissues *in vivo* through photoactivated chemotherapy.^{7–11} Ru(II) complexes are a special class of photocages, orthogonal to more traditional organic types, that have been used to cage amines,¹² thioethers,¹³ nitriles,^{14–15} and aromatic heterocycles.^{16–19} Herein we report for the first time a Ru(II)-caged CYP inhibitor that demonstrates ideal properties as a potent and selective chemical tool for controlling CYP binding with visible light in a biological setting. The complex is non-toxic and configurationally stable in the dark, including under cell culture conditions, but efficiently releases the prostate cancer drug abiraterone upon irradiation with visible light to participate in photoactivated binding to its CYP target.

The multifunctional enzyme cytochrome P450 17A1 (CYP17A1) belongs to the CYP superfamily and plays a critical role in steroidogenesis, a process that is upregulated in prostate cancer.²⁰ Inhibition of CYP17A1 prevents androgen synthesis effectively,²¹ making CYP17A1 a new therapeutic target for the treatment of prostate cancer.²² Abiraterone (ABI) is the active form of the prodrug abiraterone acetate that has recently been approved by the US Food and Drug Administration for metastatic prostate cancer,²³ and binds to the CYP17A1 active site with high affinity to prevent androgen production in a specific and irreversible manner.^{24–25} The inhibition of CYP17A1 by ABI suppresses both normal and intratumoral androgen biosynthesis, leading to an overall improvement in patient survival.²³ However, the anti-androgenic action of ABI is not restricted to the tumor, and CYP17A1 inhibition in benign tissue can produce clinically significant negative side effects.²³ Moreover, the growing number of off-target interactions of abiraterone and its metabolites highlights the complexity of this compound's pharmacology.^{26–27}

In an effort to control abiraterone's biological activity spatially with light, we synthesized two caged complexes, [Ru(tpy)(Me₂bpy)(ABI)]Cl₂ (**1**, tpy = 2,2':2',6''-terpyridine, Me₂bpy = 6,6'-dimethyl-2,2'-bipyridine) and [Ru(tpy)(biq)(ABI)]Cl₂ (**2**, biq = 2,2'-biquinoline). The treatment of [Ru(tpy)(Me₂bpy)Cl]Cl¹³ with 2 equiv of ABI in 1:1 EtOH/H₂O at 80°C, followed by chromatography over alumina affords **1** as a brown solid (Fig. 1). Similarly, treating [Ru(tpy)(biq)Cl](PF₆)²⁸ under the same conditions, followed by chromatography over alumina, then precipitation with NH₄PF₆ gives the intermediate [Ru(tpy)(biq)(ABI)](PF₆)₂, which was converted to the final complex [Ru(tpy)(biq)(ABI)]Cl₂ (**2**) by ion exchange with Bu₄NCl. Complexes **1** and **2** were characterized by electronic absorption, ¹H NMR, COSY and IR spectroscopies, electrospray ionization mass spectrometry (EIMS), and

elemental analysis (See details in Supporting Information). ^1H NMR spectroscopic data are consistent with **1** and **2** being obtained as a mixture of diastereomeric atropo isomers (*vide infra*). The electronic absorption spectra of complexes **1** and **2** exhibit maxima at 475 nm ($\epsilon = 7040 \text{ M}^{-1}\text{cm}^{-1}$) and 535 nm ($\epsilon = 9850 \text{ M}^{-1}\text{cm}^{-1}$) in DMSO, with strong shoulders in the visible region at ~ 420 nm and ~ 450 nm, respectively. These data are consistent with the coordination of the pyridine of ABI to the Ru center, based on the spectral similarity to the corresponding control complexes $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{py})](\text{PF}_6)_2$ (**3**; py = pyridine) and $[\text{Ru}(\text{tpy})(\text{biq})(\text{py})](\text{PF}_6)_2$ (**4**).²⁹ The EIMS spectra of **1** and **2** show ion clusters with major peaks at m/z values of 434.02 and 469.99, consistent with the cations $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{ABI})]^{2+}$ and $[\text{Ru}(\text{tpy})(\text{biq})(\text{ABI})]^{2+}$, respectively.

Exposure of complex **1** or **2** to visible light ($\lambda_{\text{irr}} = 500$ nm) promotes the release of ABI with short irradiation times. The photoactivated ligand exchange of ABI from each complex with solvent was investigated in CH_3CN and H_2O , which generated the corresponding products $[\text{Ru}(\text{tpy})(\text{NN})(\text{L})]^{2+}$ ($\text{L} = \text{CH}_3\text{CN}, \text{H}_2\text{O}$). Neither complex exhibits ligand dissociation in the dark, but spectra changes are observed upon light irradiation. Photolysis of **1** results in a blue shift in the $^1\text{MLCT}$ (metal-to-ligand charge transfer) from 475 nm to 455 nm in CH_3CN and a red shift from 475 nm to 485 nm in H_2O , consistent with the formation of the corresponding solvated complex $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{L})]^{2+}$ ($\text{L} = \text{CH}_3\text{CN}, \text{H}_2\text{O}$).³⁰ The release of ABI from **1** is complete within ~ 1 min in both cases under the present irradiation conditions (Fig. 2A and Fig. S4A). The bulky Me_2bpy ligand facilitates ligand photodissociation, as previously shown for the efficient pyridine exchange in the control complex **3**.³⁰ Similar trends are observed for **2**, as shown in Fig. 2B and Fig. S4B, with a blue shift in the maximum from 535 nm to 515 nm in CH_3CN and a red shift from 535 nm to 550 nm in H_2O , also consistent with the formation of $[\text{Ru}(\text{tpy})(\text{biq})(\text{L})]^{2+}$, where $\text{L} = \text{CH}_3\text{CN}$ and H_2O , respectively. However, the release of ABI from **2** requires irradiation times > 2 min in both cases, which is slower than that observed for **1** under similar experimental conditions. The quantum yields (Φ_{500}) for the exchange of ABI with solvent in **1** were determined to be 0.049(5) and 0.018(1) in CH_3CN and H_2O , respectively. These values are lower than that measured for **3**, 0.16(1) in CH_3CN ($\lambda_{\text{irr}} = 500$ nm).²⁹ The difference can be explained by the slower escape of ABI relative to py from the solvent cage following dissociation from the metal which results in recombination to regenerate the starting material, attributed to slower diffusion of the larger ABI molecule, together with differences in solubility between free ABI and py.³¹ Correspondingly, the quantum yields (Φ_{500}) for the exchange of ABI with solvent in **2** takes place with $\Phi_{500} = 0.018(1)$ in CH_3CN and 0.0043(2) H_2O , respectively. These values are also lower than that of **4** ($\Phi_{500} = 0.033(1)$ in CH_3CN).²⁹ However, the greater values of Φ_{500} for **1** as compared to **2** are consistent with more efficient release of ABI from **1** relative to **2**, as was also observed for the corresponding pyridine complexes.³⁰

In order to further probe the identity of the photolysis products of **1** and **2**, the changes of each complex were followed in CD_3CN as a function of irradiation time ($\lambda_{\text{irr}} = 395$ nm). Before irradiation, a mixture of two stereoisomers was apparent in each case, consistent with the presence of two diastereomeric atropo isomers, generated presumably because of the steric clash between one of the methyl groups of Me_2bpy and ABI for **1** or between one of

the quinoline groups of biq and ABI for **2**, placing that methyl or quinoline substituent on either side of the plane defined by Ru-N pyridine bond and the central N atom of the tpy ligand (see Fig. S5 for details).³² After irradiation, new resonances appeared in the region of 9.0 – 5.0 ppm, consistent with those obtained from free ABI and [Ru(tpy)(Me₂bpy)(NCCD₃)]²⁺ or [Ru(tpy)(biq)(NCCD₃)]²⁺ in CD₃CN (see details in Fig. S6 and S7). These observations firmly establish the photoinduced formation of [Ru(tpy)(NN)(NCCD₃)]²⁺ and free ABI as products.

Many CYP inhibitors contain aromatic heterocycles that coordinate tightly to the heme iron, yielding a red shift in electronic absorption of the Soret band, referred to as a Type II shift.^{33–34} Ru(II)-based molecules are ideal caging groups for aromatic heterocycles because they are stable, generally non-toxic and release aromatic heterocycles with light in the visible range.¹⁸ Due to the higher efficiency of photodissociation for **1** vs. **2**, compound **1** was chosen for a spectral shift assay under dark and light conditions to probe binding to CYP17A1. CYP17A1 is isolated in the resting state with a water molecule occupying the sixth coordination position of the heme iron, and this species has a characteristic Soret absorption maximum at 417 nm. Lewis bases, such as nitrogen heterocycles, can replace the bound water molecule through direct coordination to the heme iron to form a new six-coordinate complex with the Soret band red-shifted to ~424 nm.³⁵ This spectroscopic property of cytochrome P450 enzymes has been utilized extensively to characterize ligand binding³⁵ and previously demonstrated that CYP17A1 binds ABI tightly with such a Type II shift. These results are consistent with N-Fe coordination and were confirmed through X-ray crystallographic analysis of the ABI:CYP17A1 complex.³⁶ Similarly, difference spectra of CYP17A1 titration with increasing concentrations of light-exposed complex **1** resulted in a progressive increase in the absorbance at 424 nm and a concomitant absorbance decreases at 488–496 nm (Fig. 3A). This observation indicates that light liberates caged ABI from **1** and that the resulting free ABI then interacts with CYP17A1 in the typical binding mode. In contrast, CYP17A1 titration with complex **1** kept in the dark did not yield this distinctive shift (Fig. S8). Instead the observed changes were at wavelengths consistent with the differences between the spectral features of complex **1** in the sample cuvette and the absorption spectrum of [Ru(tpy)(Me₂bpy)Cl]Cl in the reference cuvette. Plotting the difference in absorption between 424 nm and 393 nm (ΔA) as **1** is irradiated vs. ligand concentration further underscores the absence of binding for complex **1** in the dark (Fig. 3B, red triangles), in contrast to clear progressive binding of the light-exposed complex **1** (Fig. 3B, blue circles). Fitting these latter data yielded a dissociation constant (K_d) of 89.6 ± 10.5 nM. As is the case for free ABI,³⁶ this value is lower than the 100 nM CYP17A1 concentration used in the assay and the CYP17A1 concentration cannot be further reduced without significant loss of signal/noise. Thus the K_d value is only an estimate, but, as expected, photo-released ABI binds to CYP17A1 with a similarly high affinity as free ABI.

We next sought to examine ABI release from **1** and **2** in a biological setting. To start, the stabilities of complexes **1** and **2** were monitored in phosphate-based buffer (100 mM, pH 7.4) at 23°C (Fig. S9) and in Dulbecco's modified Eagle's cell growth medium (pH 7.2) at 37°C (Fig. S11), respectively. As references, the control complexes **3** and **4** were also examined (see details in Fig. S10 and S12). No changes in the electronic absorption spectra

were observed, revealing that the complexes are exceptionally stable in the dark over the course of 24 h, with no discernible spectral changes during this time, except for background changes due to cell growth media, thus making them appropriate for experiments in a cell-based assay.

To probe for release of ABI in cells, complex **1** and **2** were evaluated against DU145 cells for effects on cell viability. The DU145 cells are an aggressive cell line derived from human prostate adenocarcinoma metastatic to the brain and are known to be sensitive to abiraterone.³⁷ These cells are androgen receptor-negative, but show significant expression of CYP17A1 both at the mRNA and protein levels.³⁸ The cytotoxic compound thapsigargin (TPG; 10 μ M) was used as a positive control. Data for **1** are shown in Fig. 4, and data for **2** and the control complexes **3** and **4** are shown in Fig. S13 and Fig. S14. DU145 cells were treated with complex **1** (Fig. 4) over a broad concentration range (10 μ M to 100 μ M) under dark and light conditions. The viability was determined after 48 h using the MTT assay. The data shown in Fig. 4 indicate that ABI is not well tolerated by DU145 cells, with cell viability decreasing sharply at [ABI] > 20 μ M. These data paralleled observations made with complex **1** under light conditions, whereas a dose-dependent response was not observed under similar experimental conditions in the dark. As expected, complex **3** did not show the same dose-dependent response upon irradiation, and had minimal effects on cell viability (Fig. S13). Because the excited state lifetimes of dissociative Ru(II) complexes such as **3** are not long enough to generate singlet oxygen,²⁹ these data are consistent with irradiated **1** eliciting cell death through release of ABI, rather than generation of reactive oxygen species. Interestingly, complexes **2** and **4** did not exhibit the same strong difference between dark and light conditions (see details in Fig. S14), with dose-dependent toxicity occurring at concentrations higher than 10 μ M, indicating that tuning the nature of the Ru(II) caging group has a significant influence in the behavior in cells. Taken together, these data provide strong support for using [Ru(tpy)(Me₂bpy)]²⁺ as a caging group for CYP inhibitors in biological assays when it is desirable to reduce off-target effects and control spatiotemporal delivery.

Conclusions

In summary, we synthesized Ru(II)-caged abiraterone complexes that selectively release a potent CYP inhibitor with visible light exposure. Complex **1** shows high stability under cell growth condition and no apparent toxicity for Ru(II) byproducts in cell culture. Photoactivated CYP binding was confirmed by electronic absorption difference spectroscopy. These data provide strong support for the development of Ru(II)-photocaged CYP inhibitors as chemical reagents for controlling spatial and kinetic aspects of CYP activity with low energy light. Further development of this approach may provide a method for tissue specific CYP inhibition *in vivo*.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Synthesis of caged abiraterone complex of the formula $[\text{Ru}(\text{tpy})(\text{NN})(\text{ABI})]\text{Cl}_2$, where (a) $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})\text{Cl}]\text{Cl}$, 1:1 EtOH/H₂O, 80°C; (b) $[\text{Ru}(\text{tpy})(\text{biq})\text{Cl}](\text{PF}_6)$, 1:1 EtOH/H₂O, 80°C, then NH_4PF_6 ; (c) then Bu_4NCl .

Fig. 2.

Changes to the electronic absorption spectra of **1** (A) and **2** (B) as a function of irradiation time (λ_{irr} = 500 nm) in CH_3CN for 0–4 min (**1**) and for 0–7 min (**2**).

Fig. 3. (A) Titration of CYP17A1 with increasing concentrations (0–280 nM) of light-irradiated complex **1**. (B) A plot of $A_{424-393 \text{ nm}}$ vs. ligand concentration (0 – 280 nM).

Fig. 4.

Cell viability of **1** 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 **1** (10 μ M to 100 μ M) 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 (λ_{irr} 395 nm). The cytotoxic compound thapsigargin (TPG; 10 μ M) 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.