Photoinitiated Treatment of *Mycobacterium* using Ru (II) Isoniazid
Complexes

Robert N. Garner,^{*a} Christopher G. Pierce,^b Carly R. Reed,^c and William W. Brennessel^d

^a*Department of Chemistry and Biochemistry, University of the Incarnate Word, San
Antonio, TX 78209, USA*

^b*Department of Biology, University of the Incarnate Word, San Antonio, TX 78209, USA*

^c*Department of Chemistry and Biochemistry, College at Brockport, Brockport, NY 14420*

^d*Department of Chemistry, University of Rochester, Rochester, NY 14627*

*Corresponding author. E-mail address: rgarner@uiwtx.edu (R. N. Garner)

Abstract: New photoactive ruthenium(II) complexes of the type $[\text{Ru}(\text{tpy})(\text{LL})(\text{INH})]^{2+}$, where tpy = 2,2';6',2''-terpyridine, INH = isoniazid, and LL = 2,2'-bipyridine (bpy, **1**) and 6,6'-dimethyl-2,2'-bipyridine (Me₂bpy, **2**) were synthesized. Upon irradiation with visible light, INH substituted with solvent water molecule to produce $[\text{Ru}(\text{tpy})(\text{LL})(\text{H}_2\text{O})]^{2+}$ (LL = bpy, Me₂bpy) with quantum yields, Φ_{450} , of 0.0004(2) for **1** and 0.14(2) for **2**. The increase in quantum yield of **2** compared to **1** is due to the introduction of steric bulk in the bidentate ligand, however, this causes **2** to be unstable in the dark over long periods of time ($t_{1/2} = 7.8 \times 10^{-6} \text{ s}^{-1}$). When **1** is incubated with *M. kansasii* the complex demonstrated a minimum inhibitory concentration of >112 μM in the dark and 14 μM when irradiated with visible light.

Keywords: Ruthenium photochemistry, isoniazid, photorelease antimicrobial therapy (PRAMT)

1. Introduction

Isoniazid (INH) is a first-line antibacterial agent which has been used to treat *Mycobacterium tuberculosis* (MTB) for over 60 years [1]. INH, with a minimal inhibitory concentration (MIC) of 0.02-0.08 mg/ml, is very effective toward MTB [1]. Furthermore, several nontuberculous mycobacteria (NTM), including *M. kansasii*, are susceptible to INH with MICs ranging from 1.0 to 4.0 mg/ml [2]. The mode of action of INH is not definitively known; however, it is generally accepted that INH is activated by the mycobacterial KatG enzyme, leading to the formation of INH-nicotinamide adenine dineducleotide (INH-NAD), which is able to inhibit the InhA enzyme involved in the synthesis of mycolic acids, a unique and important mycobacterial cell wall lipid [1]. Despite the effectiveness of INH and other drugs toward MTB and NTM infections, multidrug-resistant and extensively drug-resistant strains have emerged [3].

Metal complexes which incorporate INH, such as $[\text{Fe}(\text{CN})_5(\text{INH})]^{3-}$, have shown *in vitro* and *in vivo* growth inhibition towards INH-sensitive and INH-resistant strains of TB [3]. However, $[\text{Ru}(\text{CN})_5(\text{INH})]^{3-}$ and $[\text{Ru}(\text{NH}_3)_5(\text{INH})]^{2+}$ were not able to inhibit the InhA enzyme due to differences in electrochemical potentials [3,4]. A recent study has shown $[\text{Ru}(\text{NH}_3)_5(\text{INH})]^{2+}$ to be slightly active toward certain INH-resistant strains [5]. Complexation of INH to a photoactive Ru complex can serve as a delivery mechanism whereby an inactive Ru-INH complex is delivered to the infected area and low energy light is used to release INH. Sadler and co-workers recently showed that $[\text{Ru}(\text{bpy})_2(\text{INH})_2]^{2+}$, where bpy = 2,2'-bipyridine, is able to release INH when irradiated with blue light and effectively treat *Mycobacterium smegmatis* [6]. The targeted delivery

of caged antimicrobials using light, photorelease antimicrobial therapy (PRAMT), can potentially combat bacterial resistance by releasing the molecule directly into infected areas, allowing for more effective dosing.

Photoactive ruthenium complexes have been utilized to cage and deliver biologically active compounds upon irradiation with light [6-8]. Due to the relative efficiency of photoinduced ligand exchange of nitrile groups as compared to pyridine and NH_3 , the majority of previous work has focused on coordination through nitrile groups [7, 9]. However, studies have shown that the introduction of steric bulk to the coordination sphere can significantly distort the octahedral geometry, lowering the ^3LF energy thereby increasing the photoinduced ligand dissociation for pyridine based ligands [10-12]. For example, the sterically strained $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{py})]^{2+}$ ($\text{tpy} = 2,2':6',2''$ -terpyridine, $\text{Me}_2\text{bpy} = 6,6'$ -methyl-2,2'-bipyridine, and $\text{py} = \text{pyridine}$) demonstrates a >1000-fold increase in quantum yield as compared to $[\text{Ru}(\text{tpy})(\text{bpy})(\text{py})]^{2+}$ which does not contain the steric bulk [12]. Additionally, Ru(II) polypyridyl complexes containing electron-withdrawing groups, such as acetyl, on the leaving pyridine can also increase the photo-release of pyridine type ligands, such as $[\text{Ru}(\text{tpy})(\text{bpy})(4\text{-actelpyridine})]^{2+}$ as compared to $[\text{Ru}(\text{tpy})(\text{bpy})(\text{py})]^{2+}$ [13].

In the present work, $[\text{Ru}(\text{tpy})(\text{bpy})(\text{IHN})]^{2+}$ (**1**) and $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{INH})]^{2+}$ (**2**) (Fig. 1) were synthesized and their photochemistry and antimicrobial activity toward *M. kansasii* was investigated. The tpy ligand was utilized due to its increased π -conjugation and the reduced molecular symmetry of the resulting metal complex, as compared to $\text{Ru}(\text{bpy})_2(\text{L})_2$ type complexes. Both qualities red-shift the absorbance, possibly into the photodynamic therapy (PDT) window, 600 – 850 nm [7e,14]. Additionally, we added

steric bulk to the bidentate ligand in **2** to facilitate an increase in photoinitiated ligand dissociation of INH. In general, the results show that **2** is able to efficiently release INH when irradiated with light in the PDT window, but **1** exhibits a significantly greater antimicrobial activity when irradiated with visible light, as compared to the dark, which

Fig. 1. Molecular structures of **1** and **2**.

is needed for PRAMT.

2. Experimental

2.1. Materials

RuCl₃, tpy, Me₂bpy, INH, NH₄PF₆, diethyl ether, acetone-d₆, deuterium oxide were purchased from SigmaAldrich, and methanol, ethanol, acetonitrile, chloroform, and ethyl acetate were purchased from Fisher Scientific. *Mycobacterium kansasii* (ATCC® 12478™) was purchased from Fisher Scientific. All materials were used as received without further purification. Ru(tpy)Cl₃ [15], [Ru(tpy)(bpy)Cl](PF₆) [16], [Ru(tpy)(Me₂bpy)Cl]Cl [17], and [Ru(tpy)(bpy)(py)]Cl₂ [18] were synthesized according to literature methods.

2.2. Synthesis of complexes

2.2.1. $[Ru(tpy)(bpy)(INH)]^{2+}$, **1**

$[Ru(tpy)(bpy)(INH)]^{2+}$ was prepared by refluxing 100 mg (0.142 mmol) of $[Ru(tpy)(bpy)Cl](PF_6)$ and 196 mg (1.43 mmol) of isoniazid in a 40 mL mixture of 1:1 ethanol and water with stirring for 24 h. The reaction mixture volume was reduced by half and precipitated with solid NH_4PF_6 . The solid was collected by vacuum filtration and washed with 10 mL cold water and 30 mL of diethyl ether. The product was recrystallized in acetone and ether if needed. Yield: 108 mg (83%). Elem. Anal. Calc. for $[1](PF_6)_2 \cdot C_3H_6O$: C, 41.9; H, 3.31; N, 11.5%. Found: C, 42.1; H, 3.19; N, 11.3. 1H NMR (300 MHz) of $[1](PF_6)_2$ in acetone- d_6 , δ ppm (splitting, integration): 7.26 (t, 1H), 7.62 (m, 5H), 7.99 (m, 2H), 8.19 (m, 6H), 8.46 (m, 2H), 8.75 (m, 3H), 8.88 (d, 2H), 9.02 (m, 2H).

The Cl^- salt, $[1]Cl_2$, was obtained by ion exchange with an Amberlite column eluted with methanol. 1H NMR (300 MHz) of $[2]Cl_2$ in D_2O , δ ppm (splitting, integration): 7.10 (t, 1H), 7.39 (d, 1H), 7.47 (m, 4H), 7.87 (t, 2H), 7.93 (d, 2H), 8.08 (m, 4H), 8.26 (t, 1H), 8.37 (t, 1H), 8.48 (m, 3H), 8.58 (d, 2H), 8.77 (m, 2H).

2.2.2. $[Ru(tpy)(Me_2bpy)(INH)]^{2+}$, **2**

$[Ru(tpy)(Me_2bpy)(INH)]^{2+}$ was prepared using the procedure as **1** substituting $[Ru(tpy)(Me_2bpy)Cl]Cl$ (100 mg, 0.170 mmol) for $[Ru(tpy)(bpy)Cl](PF_6)$. Yield: 127 mg (79%). Elem. Anal. Calc. for $[2](PF_6)_2 \cdot H_2O$: C, 41.1; H, 3.35; N, 11.6%. Found: C, 41.1;

H, 3.32; N, 11.3%. ^1H NMR (300 MHz) of $[\mathbf{2}](\text{PF}_6)_2$ in acetone- d_6 , δ ppm (splitting, integration): 8.84 (m, 3H), 8.77 (d, 2H), 8.59 (d, 1H), 8.51 (d, 2H), 8.39 (t, 2H), 8.27 (td, 2H), 8.07 (m, 2H), 7.87 (m, 2H), 7.74 (t, 2H), 7.57 (m, 1H), 7.45 (m, 1H), 7.13 (d, 1H), 2.91 (s, 3H), 1.64 (s, 3H).

The Cl^- salt, $[\mathbf{2}]\text{Cl}_2$, was obtained by ion exchange with an Amberlite column eluted with methanol. ^1H NMR (300 MHz) of $[\mathbf{2}]\text{Cl}_2$ in D_2O , δ ppm (splitting, integration): 8.62 (d, 1H), 8.56 (d, 2H), 8.50, (d, 2H), 8.35, (d, 1H), 8.18 (m, 6H), 7.89 (d, 2H), 7.72 (m, 2H), 7.56 (t, 2H), 7.31 (d, 2H), 6.96 (d, 1H), 2.06 (s, 3H), 1.52 (s, 3H).

2.3. Instrumentation and physical measurements

^1H NMR spectra were obtained with a Bruker Advance III 300 MHz spectrometer. Electronic absorption spectroscopy for rate measurements was performed on a Beckman Coulter DU-800, and all other electronic absorption spectroscopy was performed with an Agilent Cary 8454 UV-Vis diode array spectrometer. Photolysis experiments and some susceptibility experiments were conducted with a 75 W Xe arc lamp (USHIO) in an OBB PowerArc lamp housing equipped with a universal power supply. Wavelengths for irradiation were selected with a long-pass, bandpass (Newport), and neutral density filters (Thor). Other susceptibility experiments were conducted on LuzChem photoreactor (LZC-4V) equipped with six cool white fluorescent tubes (7 W). Microtiter plates were read with an Infinite[®] M200 PRO (Tecan).

^1H NMR were performed in acetone- d_6 and D_2O and chemical shifts were referenced to the residual acetone (2.05 ppm) and H_2O (4.79 ppm) peaks. Electronic absorption was measured in a 1 x 1 quartz cuvette. Ligand exchange quantum yields were

performed by modification to a reported procedure [19], where $[\text{Ru}(\text{bpy})_2(\text{CH}_3\text{CN})_2]\text{Cl}_2$ was used as the chemical actinometer ($\Phi_{450} = 0.22$) to determine the photon flux of the lamp [20]. Solutions with a volume of ~2 mL were absorbance matched at 450 nm. The change in mol of the reactions were determined at early times by the decrease in $^1\text{MLCT}$ absorption maximum and molar absorptivity of the respective complexes.

Crystallization Methods. Compound **2**, $[\text{Ru}(\text{tpy})(6,6'\text{-Me}_2\text{-bpy})(\text{INH})][\text{PF}_6]_2$, was dissolved in a mixture of methanol, ethanol and acetonitrile. Chloroform, ethyl acetate, and diethyl ether were added to this solution. The solution was slowly evaporated at -25°C until small red crystals began to form. The solution was then placed at room temperature to evaporate further, resulting in dark red crystals. Compound **2** co-crystallized with chloroform to give the structure: $[\text{Ru}(\text{tpy})(6,6'\text{-Me}_2\text{-bpy})(\text{INH})][\text{PF}_6]_2 \cdot \text{CHCl}_3$. The structure of **2** is shown in Figure S3 with hydrogen atoms omitted for clarity.

2.4 Susceptibility testing

M. kansasii (ATCC[®] 12478[™]) was used to determine the efficacy of the compounds in the study. For routine culture, cells were propagated by streaking onto Difco[™] Middlebrook 7H10 agar (Fisher Scientific), and incubated for 7-14 days at 30°C . From these, a loopful of cells were inoculated into culture tubes containing 6 ml of Difco[™] Middlebrook 7H9 broth, supplemented with oleic acid, albumin, dextrose, and catalase (OADC) enrichment (Fisher Scientific), and incubated at 37°C until the culture reached a concentration of 1.0×10^8 CFUs/ml (0.5 McFarland Standard), typically for 72

h. The concentration of the cells was then adjusted to 10^6 CFUs/ml in OADC supplemented Middlebrook 7H9 broth.

The broth microdilution method (BMM) was performed in presterilized, polystyrene, round-bottom 96-well microtiter plates (Corning Incorporated) as described in Leite et al [21] with slight modifications. Prior to performing the BMM assays, stock solutions of complex **1**, complex **2**, $[\text{Ru}(\text{tpy})(\text{bpy})(\text{py})]^{2+}$, and INH were prepared in water at a concentration of 2 mg/ml and filter sterilized. Serial two-fold dilutions of compound **1** and **2**, along with INH, were prepared in 0.1 ml volumes using OADC supplemented Middlebrook 7H9 broth with the final concentration gradients ranging from 0.5-112 μM . Each well was then inoculated with 0.1 ml of the 1.0×10^6 CFUs/ml bacterial suspension for a final cell concentration of 0.5×10^5 CFUs/ml. Wells without antimycobacterial agents were also inoculated with cells at the same concentration to serve as a growth control. The compounds were assayed in duplicate, and one set of plates were exposed to visible light for 30 minutes in the LuzChem illuminator, while the other set remained in the dark. The plates were sealed, placed in plastic bags to eliminate evaporation, and incubated in the dark at 37°C for 7 days.

Due to the low ligand exchange quantum yield of **1** and $[\text{Ru}(\text{tpy})(\text{bpy})(\text{py})]^{2+}$, the broth microdilution method above was modified to allow for exposure of treated cells to a higher intensity light source (75 W Xenon Arc lamp) to ensure the exchange of INH and py ligands. *M. kansasii* cultures were prepared as described above and 1 ml cell suspensions of 0.5×10^5 CFUs/ml were prepared in 1.5 ml clear microcentrifuge tubes in the presence of complex **1**, INH, or $[\text{Ru}(\text{tpy})(\text{bpy})(\text{py})]^{2+}$ at concentration of 14 μM , 7 μM and 3.5 μM , as well as, a control tube containing cells in media alone (no

antimycobacterial agent). The suspensions were exposed to visible light for 30 minutes before 0.2 ml of the suspension was transferred to round-bottom 96-well microtiter plates. The plates were sealed, placed in plastic bags, and incubated in the dark at 37°C for 7 days. The compounds were assayed in triplicate.

The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration that exhibited no growth by visual reading. The results were evaluated for MICs at 7 days. The growth was also monitored at 24 h, 48 h, 72 h, and 7 d by determining the optical density (O.D.) at 550 nm in a microtiter plate reader (Infinite[®] M200 PRO, Tecan). The background (O.D. of Complex 1, Complex 2 or INH in media without cells) was subtracted from the readings to control for any changes in O.D. that might be observed because of the light absorbing properties of the complexes.

3. Results and Discussion

Fig. 2. Thermal ellipsoid plot of **2** drawn at the 50% probability level with selected numbering. Solvent, hydrogen atoms, and counterions omitted for clarity.

3.1 Synthesis and characterization

The synthesis of the caged antibacterial began with $[\text{Ru}(\text{tpy})(\text{bpy})\text{Cl}](\text{PF}_6)$ which was refluxed with 10-fold excess of INH in an ethanol/water mixture for 24 hr to generate **1**. Complex **2** was synthesized in a similar manner substituting $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})\text{Cl}]\text{Cl}$ for $[\text{Ru}(\text{tpy})(\text{bpy})\text{Cl}](\text{PF}_6)$. ^1H NMR and elemental analysis confirm the structure and purity of **1** and **2**. The crystal structure of **2** exhibits distortion from octahedral geometry about the metal center (Fig. 2) [22]. The Ru-N(6) bond length of 2.088(6) is similar to the 2.100 Ru-N(6) bond length observed in $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{py})]^{2+}$. A tilt in the INH ligand away from N(1) and towards N(2) and N(3) with N(6)–Ru–N(1–3) bond angles of $94.8(3)^\circ$, $85.6(2)^\circ$, and $88.2(3)^\circ$, respectively, is also similar to what is observed in $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{py})]^{2+}$ where the py-tpy ligand tilt angles are 94.17° , 85.45° , and 86.64° [12]. This tilt in the INH ligand is expected to weaken its σ -bonding and π -bonding with the ruthenium [12]. Additionally, the dihedral angle between the N(2)-Ru-N(6)-C(28) atoms is measured at $58.9(6)^\circ$, similar to the 56.66° observed in the respective atoms of $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{py})]^{2+}$. This angle is expected to decrease the π -back-bonding ability of the INH to the ruthenium. These distortions to the octahedral geometry may explain the photoinduced ligand dissociation efficiency of the INH ligand in **2** as compared to **1**.

3.2. Electronic structure and photochemistry

The electronic absorption spectra of **1** and **2** are shown in Fig. 3. Both exhibit a $\pi\pi^*$ transition in the UV region at 311 nm ($\epsilon = 33\,690\text{ M}^{-1}\text{ cm}^{-1}$) and 310 nm ($\epsilon = 37\,750\text{ M}^{-1}\text{ cm}^{-1}$) for **1** and **2**, respectively. Both also exhibit a broad $^1\text{MLCT}$ transition in the visible region. In **1** the λ_{max} of the $^1\text{MLCT}$ is located at 430 nm ($\epsilon = 10\,020\text{ M}^{-1}\text{ cm}^{-1}$)

Fig. 3. Electronic absorption spectra of **1** (black solid) and **2** (blue dash) in H₂O.

with a shoulder at 470 nm ($\epsilon = 9\,070\text{ M}^{-1}\text{ cm}^{-1}$) and a low energy tail that extends past 600 nm. The shape of the peak, containing a higher energy maximum and lower energy shoulder, is comparable to $[\text{Ru}(\text{tpy})(\text{bpy})(4\text{-acetylpyridine})]^{2+}$ where the electron-withdrawing acetyl group stabilizes the pyridine π^* orbitals such that they contribute to the $^1\text{MLCT}$, which is assigned as $\text{Ru}(\text{d}\pi) \rightarrow \text{tpy/bpy/4-R-py}(\pi^*)$ transition [13]. Therefore the $^1\text{MLCT}$ of **1** is assigned as $\text{Ru}(\text{d}\pi) \rightarrow \text{tpy/bpy/INH}(\pi^*)$ transition. Complex **2** exhibits a similar $^1\text{MLCT}$ with λ_{max} at 431 nm ($\epsilon = 8\,780\text{ M}^{-1}\text{ cm}^{-1}$), shoulder at 470 nm ($\epsilon = 7\,990\text{ M}^{-1}\text{ cm}^{-1}$), and a low energy tail. This is assigned as $\text{Ru}(\text{d}\pi) \rightarrow \text{tpy/Me}_2\text{bpy/INH}(\pi^*)$ transition. The low energy tail can be useful in exciting the complex with light with wavelength greater than 600 nm, which is necessary for photodynamic therapy

The stability of the complexes in the dark as well as their photochemical reactions were monitored by electronic spectroscopy. An aqueous solution of **1** undergoes no change to its electronic absorption in 24 hr in the dark at room temperature (Fig. S4). Additionally, **1** is stable at 37 °C in media and water (Figs. S5 and S6). However, minute changes in electronic absorption are observed for the dark reaction of **2** in water at room temperature (Fig. S7). The rate of decomposition was determined for **2** in water and

Fig. 4. Changes in the electronic absorption spectrum of (a) 49.6 μM **1** in H_2O upon photolysis with $\lambda_{\text{irr}} \geq 395$ nm for $t_{\text{irr}} = 0 - 25$ min, and (b) 60.5 μM **2** in H_2O upon photolysis with $\lambda_{\text{irr}} \geq 645$ nm for $t_{\text{irr}} = 0 - 2$ hr.

media at 37 °C. Plots of $\ln A$ versus t were linear and give rate constants of 7.8×10^{-6} and $7.9 \times 10^{-6} \text{ s}^{-1}$ in media and water, respectively (Figs. S8 and S9). This activity in the dark for **2** further demonstrates the weakened Ru-N(INH) bound resulting from the methyl pyridine steric crowding/geometric distortion. Upon irradiation with visible light ($\lambda_{\text{irr}} \geq 395$ nm), changes to the electronic absorption spectrum of **1** in H_2O are observed (Fig. 4a). The peak of the $^1\text{MLCT}$ at 430 nm decreases in intensity with a concomitant increase in intensity at 476 nm. This red-shift in absorption is consistent with the exchange of INH

ligand with a solvent water molecule to form $[\text{Ru}(\text{tpy})(\text{bpy})(\text{H}_2\text{O})]^{2+}$ which has been reported to have an absorbance with a maximum at 476 nm [23]. This reaction takes place in 25 min. Under similar experimental conditions the exchange of INH for H_2O in **2** happens in 20 sec (Fig. S10). The peak at 431 nm decreases in intensity and a new peak at 485 nm is observed. $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{H}_2\text{O})]^{2+}$ has a reported λ_{max} of 485 nm [12]. A slower exchange is observed in **2** when irradiated with low energy light ($\lambda_{\text{irr}} \geq 645$ nm) (Fig. 4b). The ligand loss quantum yield at 450 nm (λ_{450}) is 0.0004(2) for **1** and 0.14(2) for **2**. It should be noted that the quantum yield measurement of **2** was determined using a short time scale (15 sec) such that any change in absorbance due to the dark reaction is negligible.

Photolysis reaction of **1** and **2** were also followed by ^1H NMR in D_2O . The ^1H NMR spectrum of **1** (2.5 mM) demonstrates a clear decrease in peaks at 7.10, 7.48, and 8.77 ppm corresponding to **1** with a simultaneous increase of new peaks at 6.97, 7.73, and 9.58 ppm over a 2.5 hr irradiation time ($\lambda_{\text{irr}} \geq 395$ nm). The new peaks correspond to shifts previously reported for $[\text{Ru}(\text{tpy})(\text{bpy})(\text{D}_2\text{O})]^{2+}$ [23]. Overlapping of peaks preclude further assignment. There was no change observed in the ^1H NMR of **1** in the dark under similar conditions. When irradiated under similar conditions the reaction of **2** (2.4 mM) reaches completion in 3 mins (Fig. 5). The aliphatic peaks of the Me_2bpy ligand at 1.52 and 2.06 ppm decrease and new peaks appear at 1.57 and 2.91 ppm. Additionally, in the aromatic peaks of **2** at 6.96, 7.31, and 8.35 ppm decrease in intensity. The final spectrum consist of peaks at 6.83, 7.40, 7.53, 7.80, 8.00, 8.06, 8.17, 8.42, and 8.50 that agree with previous reports of $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{D}_2\text{O})]^{2+}$ and peaks at 7.67 and 8.67 ppm that are consistent with free INH (Fig. S11) [24]. No change for **2** was observed in the dark for

the same time period; however, after 1 hr in the dark minor changes were observed consistent with formation of $[\text{Ru}(\text{tpy})(\text{Me}_2\text{bpy})(\text{D}_2\text{O})]^{2+}$.

Fig. 5. Changes to the ^1H NMR spectrum of 2.4 mM of **2** in D_2O upon photolysis with $\lambda_{\text{irr}} \geq 395$ nm for $t_{\text{irr}} = 0 - 3$ min.

3.3 Bacteria testing

Table 1. MICs of INH, Complex **1** and Complex **2** for *M. kansasii* determined by BMM.

	MIC (μM)	
	Light	Dark
INH	7	7
1	14	>112
2	14	14

Considering that *M. kansasii* is the second most common cause of NTB

pulmonary disease, and these infections most closely parallel the clinical course of MTB

[25], we assessed the susceptibility of *M. kansasii* to our complexes. The activity of

complex **1** and **2** upon exposure to visible light was determined using the broth

microdilution method (BMM) [21]. Briefly, *M. kansasii* was grown in the presence of complex **1** or complex **2** at concentrations ranging from 0.5 μM to 112 μM . Prior to incubation at 37 $^{\circ}\text{C}$ for 7 d, the suspension of cells and compounds were exposed to visible light for 30 minutes to release the caged INH. In parallel, the susceptibility of *M. kansasii* to complex **1** and **2** in the dark, as well as to INH, were also performed to serve as controls. The minimum inhibitory concentrations (MICs), which represent a measure of potency, are presented in table 1. *M. kansasii* is susceptible to complex **1** with exposure to visible light, with a MIC of 14 μM . The MIC of complex **1** is similar to the MIC of INH alone (7 μM) found in our study and previously reported by others [2]. Furthermore, complex **1** in the dark had no effect on *M. kansasii* growth with a resulting MIC of $>112 \mu\text{M}$, consistent with the stability test which demonstrates that complex **1**

Fig. 6. Growth curves for *M. kansasii* grown in the presence of complex **1** (solid lines) or INH (dotted lines) after exposure to visible light for 30 minutes (red) or in the dark (blue). Growth was determined by measuring the optical density (O.D.) at 550 nm after 7 d. Experiment was done in triplicate and bars represent standard deviations.

effectively cages INH (Fig. 6). The MICs of complex **2**, both in the light and dark, were 14 μM . Considering the instability of complex **2** over long time periods, the results for complex **2** were expected since the MICs are determined at 7 d post inoculation. Control experiments with $[\text{Ru}(\text{tpy})(\text{bpy})(\text{py})]^{2+}$ give MICs of $>112\mu\text{M}$ in the dark and light demonstrating that it is the release of INH and not the metal fragment responsible for *M. kansasii* susceptibility.

4. Conclusions

The work presented here demonstrates that **1** effectively cages INH, and upon photolysis with visible light, releases an active INH molecule which can treat mycobacteria infection. Complex **2** is able to efficiently release INH with visible and low energy light; however, **2** is unstable in the dark over long time periods. The photorelease of an antibacterial agent from a metal center has the potential to be useful in the treatment of resistant infections. Future work will be directed toward synthesizing compounds that release a caged antibacterial more efficiently than **1**, but has greater stability in the dark than **2**.

Acknowledgments

The authors would like to thank The Welch Foundation (BN-0032), University of the Incarnate Word, and the College of Brockport, SUNY for partial support of this research. We gratefully acknowledge the X-ray Crystallographic Facility of the Department of Chemistry at the University of Rochester for XRD data. Instrument upgrade and local outreach was made possible through NSF grant CHE-0342508.

Supporting Information. ^1H NMR spectra of **1** and **2**, crystal data, dark control and rate plots in H_2O and media, photolysis of **2** with $\lambda_{\text{irr}} \geq 395\text{nm}$, and ^1H NMR spectrum of INH.

-
- 1 V. Bernardes-Genisson, C. Daraeve, A. Chollet, J. Bernadou, and G. Pratviel, *Curr. Med. Chem.*, 2013, **20**, 4370-4385.
 - 2 R. J. Wallace Jr., D. Dunbar, B. A. Brown, G. Onyi, R. Dunlap, C. H. Ahn, D. T. Murphy, *Clin. Infect. Dis.*, 1994, **18**, 736-743
 - 3 L. Viganor, C. Skerry, M. McCann, and M. Devereuz, *Curr. Med. Chem.*, 2015, **22**, 2199-2224.
 - 4 E. Sousa, L. Basso, D. Santos, I. Diogenes, E. Longhinotti, L. Lopes, I. Moreira, *J. Biol. Inorg. Chem.*, **2012**, 17, 275-283.
 - 5 I. de Aguiar, A. Tavares, A. Roveda, A. de Silva, L. Marino, E. Lopes, F. Pavan, L. Lopes, D. Franco, *Eur. J. Pharm. Sci.*, 2015, **70**, 45-54.
 - 6 N. A. Smith, P. Zhang, S. E. Greenough, M. D. Horbury, G. J. Clarkson, D. McFeely, A. Habtemariam, L. Salassa, V. G. Stavros, C. G. Dowson, P. J. Sadler, *Chem.Sci.*, 2017, 8, 395-404.
 - 7 (a) M. K. Herroon, R. Sharma, E. Rajagurubandara, C. Turro, J. J. Kodanko, I. Podgorski, *Biol.Chem.*, 2016, **397**, 571-582. (b) S. D. Ramalho, R. Sharma, J. K. White, N. Aggarwal, A. Chalasani, M. Sameni, K. Moin, P. C. Vieira, C. Turro, J. J. Kodanko, B. F. Sloane, *PLoS ONE*, 2015, **10**, 1-17. (c) T. Respondek, R. Sharma, M. K. Herroon, R. N. Garner, J. D. Knoll, E. Cueny, C. Turro, I. Podgorski, J. J. Kodanko, *ChemMedChem*, 2014, **9**, 1306-1315. (d) R. Sharma, J. D. Knoll, P. D. Martin, I. Podgorski, C. Turro, J. J. Kodanko, *Inorg. Chem.*, 2014, **53**, 3272-3274. (e) M. A. Sgambellone, A. David, R. N. Garner, K. R. Dunbar, C. Turro, *J. Am. Chem. Soc.*, 2013, **135**, 11274-11282. (f) T. Respondek, R. N. Garner, M. K. Herroon, I. Podgorski, C. Turro, J. J. Kodanko, *J. Am. Chem. Soc.*, 2011, **133**, 17164-17167. (g) R. N. Garner, J. C. Gallucci, K. R. Dunbar, C. Turro, *Inorg. Chem.*, 2011, **50**, 9213-9215.
 - 8 (a) O. Filevich, M. Salierno, R. Etchenique, *J.Inorg. Biochem.*, 2010, **104**, 1248-1251. (b) V. Nikolenko, R. Yuste, L. Zayat, L. M. Baraldo, R. Etchenique, *Chem. Commun.*, 2005, 1752-1754. (c) L. Zayat, C. Calero, P. Albores, L. Baraldo, R. Etchenique, *J. Am. Chem.Soc.*, 2003, **125**, 882-883. (d) L. Zayat, M. Salierno, R. Etchenique, *Inorg. Chem.*, 2006, **45**, 1728-1731.
 - 9 J. K. White, R. H. Schmehl, C. Turro, *Inorg. Chim. Acta*, 2016, in press.
 - 10 (a) S. Bonnet, J. -. Collin, M. Koizumi, P. Mobian, J. -. Sauvage, *Adv. Mater.*, 2006, **18**, 1239-1250. (b) F. Barigelletti, B. Ventura, J. Collin, R. Kayhanian, P. Gaviña, J. Sauvage, *Eur. J. Inorg. Chem.*, 2000, **2000**, 113-119. (c) S. Bonnet, J. Collin, J. Sauvage, E. Schofield, *Inorg. Chem.*, 2004, **43**, 8346-8354.
 - 11 (a) A. N. Hidayatullah, E. Wachter, D. K. Heidary, S. Parkin, E. C. Glazer, *Inorg. Chem.*, 2014, **53**, 10030-10032. (b) B. S. Howerton, D. K. Heidary, E. C. Glazer, *J.*

-
- Am. Chem. Soc.*, 2012, **134**, 8324-8327. (c) E. Wachter, D. K. Heidary, B. S. Howerton, S. Parkin, E. C. Glazer, *Chem. Commun.*, 2012, **48**, 9649-9651.
- 12 (a) J. D. Knoll, B. A. Albani, C. B. Durr, C. Turro, *J. Phys. Chem. A*, 2014, **118**, 10603-10610. (b) J. D. Knoll, B.A. Albani, C. Turro, *Acc. Chem. Res.*, 2015, **48**, 2280-2287.
- 13 A. T. Vu, D. A. Santos, J. G. Hale, R. N. Garner, *Inorg. Chim. Acta*, 2016, **450**, 23-29.
- 14 R. R. Allison, C. H. Sibata, *Photodiagn. Photodyn. Ther.*, 2010, **7**, 61-75.
- 15 B. P. Sullivan, J. M. Calvert, T. J. Meyer, *Inorg. Chem.*, 1980, **19**, 1404-1407.
- 16 S. C. Rasmussen, S. E. Ronco, D. A. Mlsna, M. A. Billadeau, W. T. Pennington, J. W. Kolis, J. D. Peterson, *Inorg. Chem.*, 1995, **34**, 821-829.
- 17 A. Bahreman, B. Limburg, M. A. Siegler, E. Bouwman, S. Bonnet, *Inorg. Chem.*, 2013, **52**, 9456-9469.
- 18 Hecker, C. R.; Fanwick, P. E.; McMillin, D. R., *Inorg. Chem.*, 1991, **30**, 659-666.
- 19 M. Montalti, A. Credi, L. Prodi, M. T. Gandolfi, *Handbook of Photochemistry*; CRC press: Boca Raton, FL, USA, 2006.
- 20 Y. Liu, D. B. Turner, T. N. Singh, A. Angeles-Boza, A. Chouai, K. R. Dunbar, C. Turro, *J. Am. Chem. Soc.*, 2009, **131**, 26-27.
- 21 C. Q. F. Leite, A. L. R. Z. Beretta, I. S. Anno, M. A. S. Telles, *Mem. Inst. Oswaldo Cruz.*, 2000, **95**, 127-129.
- 22 Crystal data. $C_{34}H_{31}C_{13}F_{12}N_8OP_2Ru$, $M = 1065.03$, monoclinic, $a = 21.3512(19)$, $b = 13.5980(12)$, $c = 14.6431(13)$ Å³, $\beta = 105.520(2)^\circ$, $T = 100.0(5)$ K, space group $P2_1/c$, $Z = 4$, 65959 reflections measured, 7353 unique ($R_{int} = 0.1769$), which were used in all calculations. The final $R1 = 0.073$ ($I > 2\sigma(I)$) and $wR(F^2) = 0.207$ (all data).
- 23 J. T. Hewitt, J. J. Concepcion, N. H. Damrauer, *J. Am. Chem. Soc.*, 2013, **135**, 12500-12503.
- 24 A. Bahreman, B. Limburg, M. A. Siegler, E. Bouwman, S. Bonnet, *Inorg. Chem.*, 2013, **52**, 9456-9469.
- 25 D. E. Griffith, B. A. Brown-Elliott, R. J. Wallace, Jr., *Am. J. Respir. Crit. Care Med.*, 2001, A762.

Graphical Abstract