Characterization and Biological Activity of a Hydrogen Sulfide-Releasing Red Light-Activated Ruthenium(II) Complex

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ABSTRACT: Hydrogen sulfide (H2S) is a biological gasotransmitter that has been employed for the treatment of ischemia-reperfusion injury. Despite its therapeutic value, the implementation of this gaseous molecule for this purpose has required H2S-releasing prodrugs for effective intracellular delivery. The majority of these prodrugs, however, spontaneously release H2S via uncontrolled hydrolysis. Here, we describe a Ru(II)-based H2S-releasing agent that can be activated selectively by red light irradiation. This compound operates in living cells, increasing intracellular H2S concentration only upon irradiation with red light. Furthermore, the red light irradiation of this compound protects H9c2 cardiomyoblasts from an in vitro model of ischemia-reperfusion injury. These results validate the use of red light-activated H2S-releasing agents as valuable tools for studying the biology and therapeutic utility of this gasotransmitter.

The biological role of the toxic gas hydrogen sulfide (H2S) as a neuromodulator was first recognized in 1996.1 Since this initial discovery, H2S has received significant attention because of its therapeutic potential for treating inflammation,2 Parkinson’s disease,3 reproductive dysfunction,4 brain injury,5–9 diabetes,10 cancer,11 and ischemia-reperfusion (I/R) injury.12,13 The therapeutic implementation of H2S in its gaseous state, however, is challenged by difficulties in administering biologically relevant and beneficial concentrations while avoiding problems associated with its known cytotoxicity, volatility, and flammability.14–16 To circumvent this challenge, researchers have developed synthetic donors that release H2S in response to stimuli such as pH,17 external light,18–25 reactive oxygen species,26 and enzymatic activity.27–32 Among these strategies, light-activated H2S release has been recognized as a promising tool for biomedical and therapeutic applications.18,21 Light-activated prodrugs are exciting therapeutic candidates that allow for localized and noninvasive treatment of serious medical conditions, while circumventing toxic side effects that arise from traditional chemotherapy.33–37

The majority of light-activated H2S-releasing agents require UV light, which ineffectively penetrates biological tissue and can give rise to toxic effects.38 Efforts to move photoactivation wavelengths to more biologically useful regions of the visible spectrum have resulted in several systems that can be triggered with visible and near-infrared light.21–23 Except in one case,22 these systems require upconverting nanoparticles to mediate the low-energy photoactivation process.21,23 Because red light effectively penetrates biological tissue and is nontoxic, the development of small-molecule red light-activated H2S-releasing agents would be particularly valuable for both studying the biological roles of H2S and leveraging the therapeutic effects of this gasotransmitter.39–41 In this report, we describe the first prototype of this class of molecules and its implementation to protect against in vitro ischemia-reperfusion injury.

Our strategy to develop such a red light-activated H2S-releasing molecule invoked a combination of the established H2S-releasing compound morpholin-4-ium 4-methoxyphenyl(phosphinodithioate (GYY4137, Chart 1)40,41 and a photolabile ruthenium(II) scaffold [Ru(tpy)(biq)(L)]2+ (tpy = 2,2’-6’-2’-terpyridine; biq = 2,2’-biquinoline, n = 1, 2). Ru(II) compounds of this class possess low energy metal-to-ligand charge transfer (MLCT) absorption bands that extend into the red region of the visible spectrum. After population of the 1MLCT state by absorption of red light, efficient intersystem crossing to a dissociative triplet ligand field (3LF) state ejects the monodentate ligand L with high quantum yields.42–45

In aqueous or wet organic solvent, GYY4137 releases H2S over a few hours.46 Sustained H2S release from GYY4137 has been used therapeutically to treat inflammation,47 inhibit cancer cell growth,48 and prevent ischemia-reperfusion injury.49,50 The hydrolysis of GYY4137, however, occurs spontaneously in solution, limiting spatiotemporal control of H2S release from

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Supporting Information

Chart 1. GYY4137, and the Compounds [1]+ and [2]+ That Were Explored in This Study
this molecule. We hypothesized that coordination of GYY4137 to the photoactive [Ru(tpy)(biq)(L)] Cl⁺ scaffold would inhibit its spontaneous hydrolysis until it was released by irradiation with red light. Thus, the complex [Ru(tpy)(biq)(GYY4137)]Cl ([1]Cl) (Chart 1) was developed as the first red light-activated H₂S-releasing molecule.

The direct reaction between [Ru(tpy)(biq)Cl]Cl and excess GYY4137 in 50% aqueous acetone gave [1]Cl as the major product in solution. Further purification by silica gel chromatography (90/10 CH₃CN/H₂O) afforded pure [1]Cl in 24% yield. When [Ru(tpy)(biq)Cl]PF₆ was sequentially treated with AgPF₆, which removed the inner-sphere chloride as insoluble AgCl, and excess GYY4137 in refluxing methanol, we unexpectedly isolated [Ru(tpy)(biq)(GYYOMe)]Cl ([2]Cl), where GYYOMe is O-methoxy 4-methoxyphenylphosphinodithioate. Over the course of this reaction, the morpholine group of GYY4137 was replaced by the methanol solvent giving rise to the GYYOMe ligand (Chart 1).51

In addition to characterization by standard techniques, such as NMR spectroscopy, mass spectrometry, and IR spectroscopy (Figures S1−S12, Supporting Information, SI), [1]PF₆ was also characterized by single-crystal X-ray diffraction (Figure 1).

Figure 1. X-ray crystal structure of [1]PF₆. Hydrogen atoms and the PF₆⁻ counterion are omitted for clarity. Thermal ellipsoids are shown at the 50% probability level.

Crystallographic parameters and relevant interatomic distances and angles are listed in Tables S1 and S2 in the SI. The Ru(II) center of [1]PF₆ attains a distorted octahedral geometry with GYY4137 coordinated trans to the biquinoline ligand. The stericly demanding tpy and biq ligands perturb the Ru-ligand bond angles, which range from 79.31° to 90.23°, significantly from the 90° of an ideal octahedron. This steric strain contributes to the efficient photosubstitution reactions of these complexes by lowering the energy of the dissociative 3LF state, allowing for it to be thermally populated from the photogenerated 1MLCT state.42−55

The Ru−S1 distance (2.41 Å) is similar to that found in a related organometallic Ru(II) complex bearing GYY4137 as a ligand (2.403 Å).56

Table 1. Absorption Maxima (λmax), Molar Extinction Coefficients (ε) at λmax, and Photosubstitution Quantum Yields (Φ626) of [1]Cl and [2]Cl

<table>
<thead>
<tr>
<th>Compound</th>
<th>λmax (nm)</th>
<th>ε (M⁻¹ cm⁻¹)</th>
<th>Φ626 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]Cl</td>
<td>581</td>
<td>4050 ± 140</td>
<td>0.85 ± 0.03</td>
</tr>
<tr>
<td>[2]Cl</td>
<td>570</td>
<td>4000 ± 200</td>
<td>1.02 ± 0.11</td>
</tr>
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1MLCT absorption bands at λ = 581 nm ([1]Cl) and λ = 568 nm ([2]Cl), which both tail past 650 nm. Upon irradiation of the complexes with 626 nm light (Figures S13 and S14, SI), the UV−vis spectra evolve, resulting in a blue shift of the 1MLCT band to a new maximum at 549 nm, characteristic of the expected photoproduct [Ru(tpy)(biq)(OH₂)]²⁺ (Figures 2 and

Figure 2. Top: Changes in the electronic absorption spectrum of [1]Cl in 100 mM MOPS (pH 7.4) under 626 nm light irradiation (photon flux = 2.01 × 10⁻⁸ mol s⁻¹, t irr = 15 min, T = 298 K). Bottom: Electronic absorption spectrum of [1]Cl in 100 mM MOPS in the dark after 5 days (T = 298 K).

The photophysical properties of [1]Cl and [2]Cl in buffered aqueous solution (3-morpholinopropanesulfonic acid; MOPS; pH 7.4) were investigated (Table 1). The complexes exhibit
Ru(II) compounds can bind to DNA and induce cytotoxicity. As expected, GYY4137 is nontoxic (Figures S22 and S23, SI).

Given the promising toxicity profile of [1]Cl and its photoproducts, the H₂S-releasing capabilities of this compound were measured in living cells using the cell-trappable H₂S-responsive fluorescent probe, SF7-AM. Lung cancer (A549) cells were loaded with SF7-AM and treated with the complexes, followed by irradiation with red light for 30 min prior to fluorescence microscopy imaging. Cells that were only treated with [1]Cl or exposed to red light showed no significant increase in fluorescence intensity of SF7-AM. In contrast, when both [1]Cl and red light were administered to cells, a significant increase in fluorescence intensity was observed, indicating that both components are critical for the intracellular release of H₂S (Figure 4).

Because H₂S can protect against I/R injury, we investigated the ability of [1]Cl to give rise to cytotoxic effects selectively upon red light irradiation in H9c2 cells subjected to hypoxia/reoxygenation injury. Cells were incubated in a pH 6.4 ischemia mimetic buffer (see SI for details) in hypoxic conditions, followed by treatment with [1]Cl or GYY4137 in the dark or under red light irradiation for 30 min prior to reoxygenation. Control cells were incubated in normoxic conditions for the duration of the experiment.

The viability of untreated cells, measured by the colorimetric MTT assay, subjected to this I/R injury model was decreased by approximately 80%, indicating that this model accurately captures the cytotoxic effects of this condition. When cells were treated with [1]Cl in the dark, there was no significant change in cell viability compared to untreated cells. In the presence of red light, however, cells treated with [1]Cl show a 75% increase in viability relative to the untreated control cells. In comparison, the extent of the cytotoxic effect of GYY4137 does not change appreciably when cells are treated either in the light or dark (Figure 5). These results demonstrate that [1]Cl can effectively cage GYY4137 and prevent H₂S release until selective red light activation to prevent cell death in a model of I/R injury.

In summary, we have developed a novel red light-activated H₂S-donating complex. Compared to other light-activated H₂S-
releasing systems, this compound is the first small molecule that is capable of releasing H₂S upon irradiation with red light without the need for a secondary nanoparticle system. We have shown that [1]Cl is stable in the dark and can be activated to release H₂S in biological conditions. In addition, [1]Cl is capable of preventing cell death in a model of I/R injury. This work demonstrates the relatively understudied cytoprotective properties of transition metal compounds, and suggests a broader utility for these complexes as innovative drugs for the treatment and prevention of serious medical conditions.

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b08695.

Complex characterization data, cell viability curves, crystal data tables, UV−vis spectra, NMR data of irradiation (PDF)

Data for [1]PF₆ (CIF)

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**


Figure 5. Protective effects of [1]Cl and GYY4137 (10 μM) in cells subjected to hypoxia/reoxygenation injury. Error bars are SE of three replicates with n = 6 wells. Statistical significance was determined using a two-tailed student’s t test. ***p < 0.0015.