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Development of a Ruthenium(II) Complex-Based Luminescent Probe for Hypochlorous Acid in Living Cells

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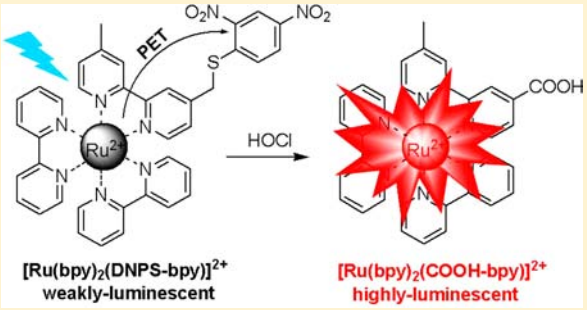
Supporting Information

ABSTRACT: A novel Ru(II) complex, $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ (bpy: 2,2′-bipyridine, DNPS-bpy: 4-(2,4-dinitrophenylthio)-methylene-4′-methyl-2,2′-bipyridine), has been designed and synthesized as a highly sensitive and selective luminescence probe for the recognition and detection of hypochlorous acid (HOCl) in living cells by exploiting a "signaling moiety-recognition linker-quencher" sandwich approach. The complex possesses large stokes shift (170 nm), long emission wavelength (626 nm), and low cytotoxicity. Owing to the effective photoinduced electron transfer (PET) from Ru(II) center to the electron acceptor, 2,4-dinitrophenyl (DNP), the red-emission of bipyridine-Ru(II) complex was completely withheld. In aqueous media, HOCl can trigger an oxidation reaction to cleave the DNP moiety from the Ru(II) complex, which results in the formation of a highly luminescent bipyridine-Ru(II) complex derivative, $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$ (COOH-bpy: 4′-methyl-2,2′-bipyridyl-4-carboxylic acid), accompanied by a 190-fold luminescence enhancement. Cell imaging experimental results demonstrated that $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ is membrane permeable, and can be applied for capturing and visualizing the exogenous/endogenous HOCl molecules in living cell samples. The development of this Ru(II) complex probe not only provides a useful tool for monitoring HOCl in living systems, but also strengthens the application of transition metal complex-based luminescent probes for bioimaging.



INTRODUCTION

The transition metal complexes with d^6, d^8, and d^{10} electronic configuration have recently attracted a wealth of interest in the field of luminescent chemosensors and cellular probes, due to their desirable photophysical properties,[1] such as visible-light absorption and emission; large Stokes shifts; high photo-, thermal, and chemical stabilities; and low cytotoxicity.[2] One important group of transition metal luminescent complexes is Ru(II) complexes coordinated by three diimine ligands, such as 2,2′-bipyridine (bpy), 1,10-phenanthroline (phen), and/or bathophenanthroline derivatives, in an octahedral disposition. Due to different chemical structures and triplet state energy levels of the ligands, Ru(II) complexes have several excited states.[2b] At room temperature, the luminescence of Ru(II) complexes mainly arises from a metal-to-ligand charge-transfer (MLCT) from Ru(II) center to a polypyridyl-centered antibonding orbital, with typical excitation and emission wavelengths around 450 and 610 nm, respectively. Since the MLCT emission is provided by the orbital combination of three diimine ligands, the emission properties of Ru(II) complexes, such as emission wavelength, intensity, and lifetime, would be changed by the structure change of a diimine ligand, which provides a convenient strategy for the design of various luminescent probes. In recent years, by modulating the ligand structure and local environment,[1,2a−c,3,4] a variety of Ru(II) complex-based luminescent probes for bioactive molecules,[3,5] metal cations,[4d,6] and anions,[4a−c,7] have been developed.

In previous works, we have also reported the developments of several Ru(II) complex-based luminescent probes based on the intramolecular photoinduced electron transfer (PET) mechanism.[5e,8] When one bipyridine ligand of tris(bipyridine)-Ru(II)-type complexes was modified with a strong electron acceptor, the luminescence of the Ru(II) complex could be turned-off through efficient PET from the Ru(II) center (a potent electron donor) to the electron acceptor. By incorporating a 3,4-diaminophenyl group into a tris-(bipyridine)Ru(II) complex, a luminescent probe, $[Ru(bpy)_2(dabpy)](PF_6)_2$, was synthesized for the luminescence imaging detections of exogenous and endogenous NO in the living cells. Recently we have been interested in the development of Ru(II) complex-based luminescent probes for other reactive oxygen species, such as hypochlorous acid (HOCl).

It is well-known that HOCl, one of the most important reactive oxygen species, plays important roles in signal transduction for cellular proliferation, migration, apoptosis, and bactericidal activity of phagocytes in living organisms.[9]

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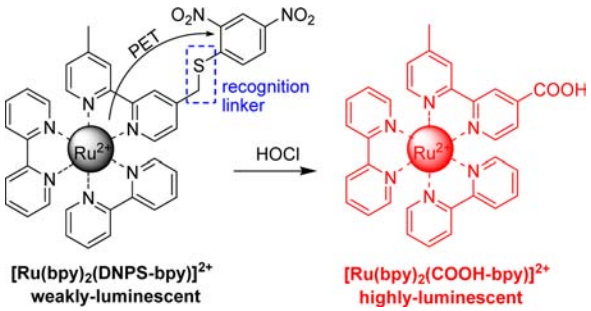
Under physiological conditions, a part of HOCl dissociates to the hypochlorite anion (ClO^-), an extensively used disinfectant and bleaching agent.[9a,10] Although hypochlorite has strong antibacterial properties, excessive or misplaced production of hypochlorite in living systems can also have a detrimental effect on host tissues by the same mechanism used to destroy invading microorganisms.[11] Thus, the development of sensitive and selective methods for HOCl detection is urgently required for the further investigation of the action mechanism of HOCl in living systems and human diseases.[12]

Toward this end, we proposed a novel Ru(II) complex-based luminescent probe specific for HOCl in living cells in this work. Scheme 1 illustrates the structure of the Ru(II) complex, $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$, and its luminescence response mechanism toward HOCl. Owing to the effective PET from the Ru(II) center to the electron acceptor (DNP), the red-emission of the bipyridine-Ru(II) complex was thoroughly quenched. However, the weakly luminescent probe could specifically react with HOCl under physiological conditions to form a highly luminescent bipyridine-Ru(II) complex derivative, $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$, accompanied by the remarkable luminescence enhancement. The internalization of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ into living cells was investigated, and the intracellular exogenous/endogenous HOCl was monitored with the luminescence microscopy imaging method.

Scheme 1. Proposed Reaction between $[Ru(bpy)_2(DNPS\text{-}bpy)]^{2+}$ and HOCl



EXPERIMENTAL SECTION

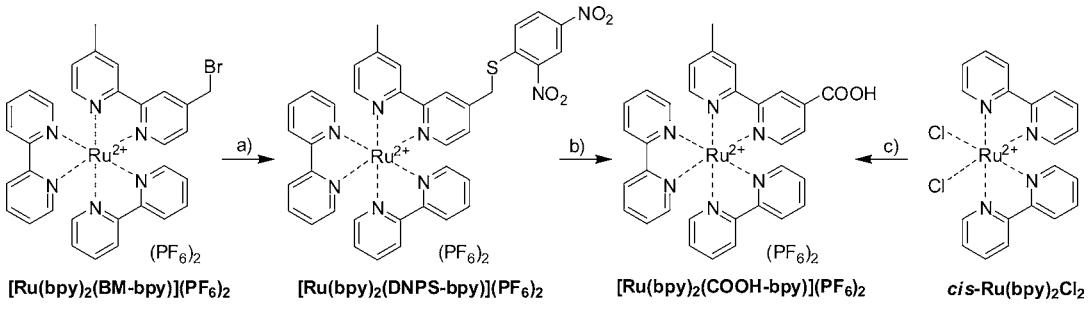
General Information. 4β-Phorbol-12-myristate-13-acetate (PMA), lipopolysaccharide (LPS), 4-aminobenzoic acid hydrazide (4-ABAH), and 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. 2,4-Dinitrobenzenethiol,[13] 4′-methyl-2,2′-bipyridyl-4-carboxylic acid,[14] *cis*-Ru(II)-$(bpy)_2Cl_2{\cdot}2H_2O$,[15] and bis(2,2′-bipyridine)(4-methyl-4′-bromomethyl-2,2′-bipyridine)Ru(II) hexafluorophosphate ($[Ru(bpy)_2(BM\text{-}bpy)](PF_6)_2$)[16] were synthesized using literature methods. A stock solution of HOCl was prepared by dilution of the commercial sodium hypochlorite solution and stored according to the literature method.[17] The HOCl concentration was determined using its molar extinction coefficient of 391 $M^{-1}cm^{-1}$ at 292 nm before use.[17] Unless otherwise stated, all chemical materials were purchased from commercial sources and used without further purification. Deionized, distilled water was used throughout.

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR). ESI-MS spectra were measured on HP1100LC/MSD MS and LC/Q-TOF MS spectrometers. Absorption spectra were measured on a Perkin-Elmer Lambda 35 UV−vis spectrometer. Elemental analysis was carried out on a Vario-EL analyzer. Luminescence spectra were measured on a Perkin-Elmer LS 50B luminescence spectrometer with the conditions of excitation wavelength, 456 nm; emission wavelength, 626 nm; excitation slit, 10 nm; and emission slit, 10 nm. HPLC analysis was carried out on a SinoChrom ODS-BP 5 μm (4.6 × 250 mm) column using an HPLC system composed of two pumps (P230) and a detector (UV 230+). All bright-field imaging and luminescence imaging measurements were carried out on a Nikon TE2000-E luminescence microscope. The microscope, equipped with a 100 W mercury lamp, a Nikon B-2A filters (excitation filter, 450−490 nm; dichroic mirror, 505 nm; emission filter, >520 nm) and a color CCD camera system (RET-2000R-F-CLR-12-C, Qimaging Ltd.), was used for the luminescence imaging measurements with an exposure time of 5 s. The relative luminescence intensities of the images were analyzed by using an ImageJ software.

Syntheses of the Ru(II) Complexes. The synthesis procedure of two Ru(II) complexes, $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ and $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$, are shown in Scheme 2. The details of the experiments are described as follows.

Synthesis of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$. Under an argon atmosphere, a mixture of 2,4-dinitrobenzenethiol (50.0 mg, 0.25 mmol) and NaH (10 mg, 60% in purity, 0.25 mmol) in 30 mL anhydrous acetonitrile was stirred at room temperature for 1 h. A solution of bis(2,2′-bipyridine)(4-bromomethyl-4′-methyl-2,2′-bipydine)Ru(II) hexafluorophosphate (96.6 mg, 0.1 mmol) in 5 mL anhydrous acetonitrile was added, and the reaction mixture was further stirred for 2 h. The solvent was evaporated, and the residue was purified by silica gel column chromatography using $CH_3CN-H_2O-KNO_3$ (sat.) (100:7:1, v/v/v) as eluent. The fractions containing the target product were collected, and the solvent was evaporated. The resulting solid was dissolved in a small amount of CH_3CN-H_2O (1:1), and a saturated solution of NH_4PF_6 was added to give a red precipitate. The product was filtered and washed with small amount of water. $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ was obtained as a red powder (34.7 mg, 32% yield). ^1H NMR (400 MHz, CD_3CN): δ = 2.53 (s, 3H), 4.54 (s, 2H), 7.25 (d, $J_{(H,H)}$ = 5.6 Hz, 1H), 7.38 (m, 4H), 7.43 (d, $J_{(H,H)}$ = 5.6 Hz, 1H), 7.53 (d, $J_{(H,H)}$ = 5.2 Hz, 1H), 7.65 (d, $J_{(H,H)}$ = 4.8

Scheme 2. Synthesis Procedure of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ and $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$.[a]



[a](a) 2,4-dinitrobenzenethiol, NaH, CH_3CN, RT, 3 h, 32% yield; (b) NaOCl, RT, CH_3CN-borate buffer, 1.5 h, 61% yield; and (c) 4′-methyl-2,2′-bipyridine-4-carboxylic acid, EtOH, reflux, 6 h, 65% yield.

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Hz, 1H), 7.72 (m, 5H), 8.04 (m, 4H), 8.36 (m, 2H), 8.48 (s, 4H), 8.53 (s, 1H), 8.91 (s, 1H). ^{13}C NMR (100 MHz, CD_3CN): δ = 20.26, 35.45, 121.42, 124.23, 125.30, 127.55, 128.47, 128.58, 137.73, 143.97, 144.77, 145.37, 146.82, 150.59, 150.83, 151.65, 156.06, 156.95. 156.99, 157.08. ESI-MS (m/z): 941.1 ($[M-PF_6]^+$), 398.1 ($[M-2PF_6]^{2+}$). Elemental analysis (%) calcd. for $C_{38}H_{30}F_{12}N_8O_4P_2RuS$: C 42.04, H 2.78, N 10.32; found: C 41.89, H 2.88, N, 10.21.

Synthesis of $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$. *Method 1.* Sodium hypochlorite solution (0.5 mmol) was added dropwise to a solution of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ (108.6 mg, 0.1 mmol) in CH_3CN (2 mL) and phosphate buffer of pH 7.4 (0.1 M, 50 mL). After the reaction mixture was stirred at room temperature for 1.5 h, the solvent was evaporated, and the residue was purified by silica gel column chromatography using $CH_3CN-H_2O-KNO_3$ (sat.) (100:10:1, v/v/v) as eluent. A fraction containing the target product was collected, and the solvent was evaporated. The resulting solid was dissolved in a small amount of distilled water, and a solution of HPF_6 (1 mM) was added dropwise to give a red precipitate. The product was filtered and washed with small amount of water. $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$ was obtained as a red powder (57.1 mg, 61% yield).

Method 2. A mixture of 4′-methyl-2,2′-bipyridine-4-carboxylic acid (42.8 mg, 0.2 mmol), *cis*-$Ru(II)(bpy)_2Cl_2\cdot 2H_2O$ (104.1 mg, 0.2 mmol), and 30 mL ethanol was refluxed for 6 h. After the solvent was evaporated, the residue was purified by silica gel column chromatography using $CH_3CN-H_2O-KNO_3$ (sat.) (100:10:1, v/v/v) as eluent. A fraction containing the target product was collected, and the solvent was evaporated. The resulting solid was dissolved in a small amount of distilled water, and a solution of HPF_6 (1 mM) was added dropwise to give a red precipitate. The product was filtered and washed with small amount of water. $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$ was obtained as a red powder (121.7 mg, 65% yield). ^{1}H NMR (400 MHz, CD_3CN): δ = 2.55 (s, 3H), 7.29 (s, 1H), 7.41 (s, 4H), 7.56 (s, 1H), 7.71 (s, 4H), 7.78 (s, 1H), 7.90 (s, 1H), 8.08 (m, 4H), 8.51 (m, 5H), 8.90 (s, 1H). ^{13}C NMR (100 MHz, CD_3CN): δ = 20.14, 123.10, 124.30, 125.73, 126.19, 127.65, 128.83, 137.94, 138.43, 150.79, 151.58, 152.78, 155.78, 156.73, 156.90, 158.42, 164.12. ESI-MS (m/z): 794.5 ($[M-H + Na\text{-}PF_6]^+$), 772.5 ($[M-PF_6]^+$), 627.1 ($[M-H-2PF_6]^+$), 324.8 ($[M-H + Na\text{-}2PF_6]^{2+}$), 313.8 ($[M-2PF_6]^{2+}$). Elemental analysis (%) calcd. for $C_{32}H_{26}F_{12}N_6O_2P_2Ru\cdot H_2O$: C 41.08, H 3.02, N 8.98; found (%): C 40.79, H 3.01, N, 8.93.

Luminescence Response of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ toward HOCl. The reaction of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ with HOCl was performed in 0.1 M phosphate buffer of pH 7.4 at room temperature. Typically, the solutions of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ (10 μM) containing different concentrations of HOCl were stirred at room temperature for 60 min, and then the excitation and emission spectra were measured with Perkin-Elmer LS 50B luminescence spectrometer. The calibration curve was derived from luminescence intensities against HOCl concentrations. To determine the detection limit, the luminescence intensity of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ in the absence of HOCl was measured 20 times, and then the standard deviation of the blank measurement was calculated. The detection limit was calculated according to the reported method defined by IUPAC.[18]

Reactions of $[Ru(bpy)_2(DPNS\text{-}bpy)](PF_6)_2$ with Different ROS/RNS. All of the reactions were carried out in 0.1 M phosphate buffer of pH 7.4 (except for singlet oxygen) with the same $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ concentration (10 μM) for 1 h at room temperature. Hydrogen peroxide (H_2O_2) was diluted immediately from a stabilized 30% solution, and was assayed using its molar absorption coefficient of 43.6 $M^{-1}cm^{-1}$ at 240 nm.[19] Hydroxyl radical (•OH) was generated in the Fenton system from ferrous ammonium sulfate and hydrogen peroxide.[20] Peroxynitrite was synthesized from sodium nitrite (0.6 M) and H_2O_2 (0.65 M) in a quenched-flow reactor (excess H_2O_2 was used to minimize nitrite contamination). After the reaction, the solution was treated with MnO_2 to eliminate the excess H_2O_2. The concentration of the $ONOO^-$ stock solution was determined by measuring the absorbance at 302 nm with a molar extinction coefficient of 1670 $M^{-1}cm^{-1}$.[21] Singlet oxygen was chemically generated from the $MoO_4^{2-}-H_2O_2$ system in 0.1 M carbonate buffer of pH 10.5.[22] Superoxide solution ($O_2^-\bullet$) was prepared by dissolving solid KO_2 in dry dimethyl sulfoxide (DMSO), and the mixture was stirred vigorously for 10 min before use. NO was generated using NOC-13 (1-hydroxy-2-oxo-3-(3-aminopropyl)-3-methyl-1-triazene) as a NO donor.[23] The stock solution of hypochlorite was used throughout.

Luminescence Imaging of HOCl in Living Cells. Two kinds of cultured cells, HeLa cells and mouse RAW 264.7 macrophage cells, were used for the luminescent imaging measurements. The experimental details are as follows.

HeLa Cells. HeLa cells were cultured in RPMI-1640 medium (Sigma-Aldrich, Inc.), supplemented with 10% fetal bovine serum (Corning Incorporated), 1% penicillin, and 1% streptomycin (Gibco), at 37 °C in a 5% CO_2–95% air incubator. The concentrated stock solution of $[Ru(bpy)(DNPS\text{-}bpy)_2](PF_6)_2$ (50 mM) was prepared by dissolving $[Ru(bpy)(DNPS\text{-}bpy)_2](PF_6)_2$ in DMSO. Before cell loading, the solution was 1000-fold diluted with the cell culture medium (final concentration of the complex: 50 μM). The cultured HeLa cells in a 25 cm^2 glass culture bottle were washed with the culture medium, and then incubated with 5 mL of the above Ru(II) complex solution. After incubation for 2 h at 37 °C in a 5% CO_2–95% air incubator, the cells were washed five times with Krebs-Ringer phosphate buffer (KRP buffer: 114 mM NaCl, 4.6 mM KCl, 2.4 mM $MgSO_4$, 1.0 mM $CaCl_2$, 15 mM Na_2HPO_4/NaH_2PO_4, pH 7.4), and then further incubated with the KRP buffer containing 15 μM of HOCl for 1 h. The cells were rinsed three times with KRP buffer, and then subjected to luminescence microscopy imaging measurement.

RAW 264.7 Macrophage Cells. RAW 264.7 macrophage cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich), supplemented with 10% fetal bovine serum (Corning Incorporated), 1% penicillin, and 1% streptomycin (Gibco), at 37 °C in a 5% CO_2–95% air incubator. Before cell loading, the concentrated stock solution of $[Ru(bpy)(DNPS\text{-}bpy)_2](PF_6)_2$ (50 mM) in DMSO was 1000-fold diluted with the cell culture medium (final concentration of the complex: 50 μM). The cultured RAW 264.7 macrophage cells in a 25 cm^2 glass culture bottle were treated with LPS (1 μg/mL) for 24 h, rinsed 3 times with culture medium, and then further incubated with the above Ru(II) complex solution and PMA (1 μg/mL) for 2 h. The cells were rinsed three times with KRP buffer, and then their luminescence images were recorded. For the control experiment, 4-ABAH (20 μM) was added during the stimulation of the cells with PMA, while the other procedures were the same as above.

For the quantification of luminescence intensities in the images, the randomly recorded images from different areas of the cells were analyzed by using the ImageJ software.[24] The net luminescence intensity of the interest area of the selected single cells was obtained by subtracting the background luminescence of the same selected area. Mean values for the positive and control groups were represented by relative luminescence intensities.

Cytotoxicity of $[Ru(bpy)(DNPS\text{-}bpy)_2](PF_6)_2$. The cytotoxicity of the probe $[Ru(bpy)(DNPS\text{-}bpy)_2](PF_6)_2$ was measured by the MTT test using the previously described method.[25] Briefly, RAW 264.7 cells grown in 96-well cell culture plates at a density of 1×10^4 cells/well were incubated with different concentrations of $[Ru(bpy)(DNPS\text{-}bpy)_2](PF_6)_2$ in fresh medium at 37 °C in a 5% CO_2–95% air incubator for 24 h. After MTT was added at 250 μg/mL to each well, the cells were further incubated for 4 h. The culture medium was removed, and the cell layer was dissolved in DMSO (100 μL). The absorbance of each well at 540 nm was measured in a 96-well multiwell-plate reader (Bio-Rad iMark).

RESULTS AND DISCUSSION

Design, Synthesis and Photophysical Properties of the Ru(II) Complex Probe. Recently, phosphorescent Ru(II) complexes have provided great contributions to the development of chemosensors and cellular imaging probes, due to their abundant photochemical, photophysical, and electrochemical properties.[1,2a−c,3a,d,4,26] It is well-known that the ^{3}MLCT-transition-based red emission of these complexes can be finely

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tuned by ramification of ligand structures or by introducing coligands in complexes.[5c−e,8,26] In this work, the design strategy for $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ is illustrated in Scheme 1. This complex consists of three parts: a bipyridine-Ru(II) complex core as the signaling moiety, a strong electron acceptor (DNP) as the luminescence quencher, and a benzylthioether linker as the HOCl-recognition unit. In this "signaling moiety-recognition linker-quencher" sandwich approach, the emission of the Ru(II) complex is turned-off due to the PET process from the Ru(II) center to the DNP moiety, while it can be turned-on after the HOCl-induced cleavage of DNP moiety from $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$. On the basis of this opinion, $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ was synthesized by a one-step reaction starting from $[Ru(bpy)_2(BM\text{-}bpy)](PF_6)_2$ (BM-bpy: 4-methyl-4′-bromomethyl-2,2′-bipyridine), and was well-characterized by NMR, ESI-MS, and elemental analyses (Scheme 2, Figures S1−S3 of the Supporting Information, SI).

The absorption and luminescence properties of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ were investigated in 0.1 M phosphate buffer of pH 7.4 at room temperature. As shown in Figure 1, $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ exhibited two strong absorption bands at 290 and 456 nm, which can be attributed to the spin-allowed ligand $\pi\rightarrow\pi^*$ transition and the MLCT transition of the Ru(II) complex, respectively. As anticipated, under the excitation of 456 nm light, the emission of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ is rather weak (Table S1 of the SI), indicating that the DNP moiety in the complex can indeed quench the Ru(II) complex luminescence via an efficient intramolecular PET process.

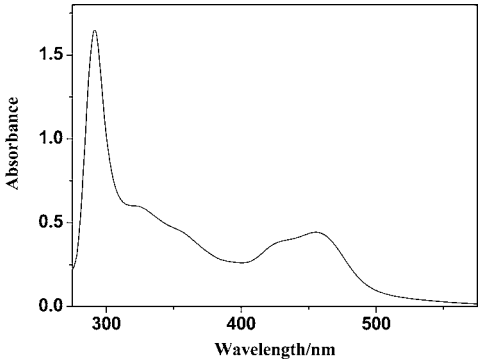

Figure 1. Absorption spectra of $[Ru(bpy)_2(DNPS\text{-}bpy)]^{2+}$ (30 μM) in 0.1 M phosphate buffer of pH 7.4.

Luminescence Response of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ to HOCl. The reaction of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ with HOCl was investigated under simulative physiological conditions. In the presence of HOCl, weakly luminescent $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ exhibited an approximately 190-fold enhancement in luminescence intensity (Figure S4 of the SI). This result could be considered to be attributed to the oxidation of benzylthioether linker in the probe, which caused the cleavage of electron acceptor DNP, to result in the formation of a highly luminescent Ru(II) complex, $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$. For validating the reaction product, the reaction mixture of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ and HOCl was subjected to HPLC and ESI-MS analyses (Figures S5 and S6 of the SI). The results indicated that no major product other than $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$ was detected in the reaction mixture. After purification, the generated $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$ was further confirmed by NMR, ESI-MS and elementary analyses (Figures S7−S9 of the SI). In addition, the reaction mixture displayed identical excitation and emission spectra with the standard solution of $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$ that synthesized by two different methods (Figure S10 of the SI).

We then proceeded to evaluate the quantitative luminescence response of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF6)_2$ to HOCl in 0.1 M phosphate buffer of pH 7.4. As shown in Figure 2A, treatments of the complex with different concentrations of HOCl elicited dramatic increases in luminescence intensity at 626 nm. The dose-dependent luminescence enhancement followed a good linear relationship with HOCl concentration in a range of 2.5−50 μM (Figure 2B). The detection limit, calculated according to the reported method,[18] is 53.5 nM, which indicates that $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ can be used as a highly sensitive luminescence probe for detecting HOCl under physiological conditions. In addition, we examined the effect of pH on the luminescence intensities of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ and its reaction product with HOCl, $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$. The result of Figure 3 reveals that the two Ru(II) complexes are essentially pH-insensitive in the range of pH 4.5 to 10.5, which indicates that the luminescence of the two Ru(II) complexes is stable in a wide pH range.

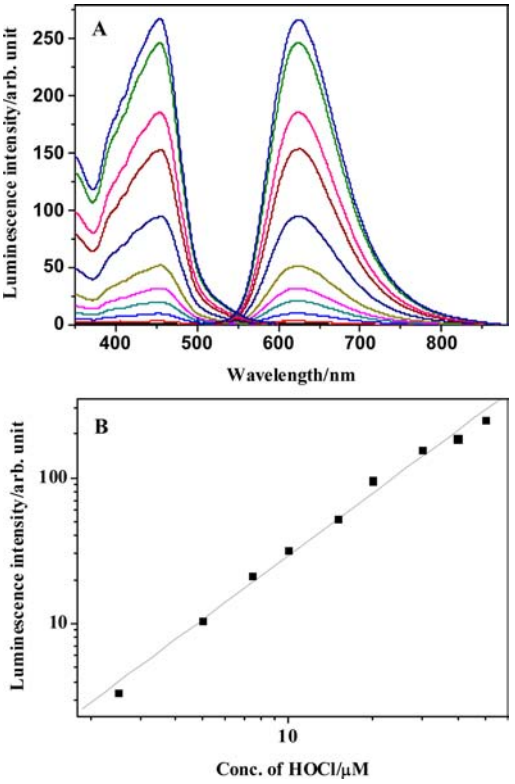

Figure 2. (A) Excitation and emission spectra of $[Ru(bpy)_2(DNPS\text{-}bpy)]^{2+}$ (10 μM) in the presence of different concentrations of HOCl. The concentrations of HOCl are 0.0, 2.5, 5.0, 7.5, 10, 15, 20, 30, 40, 50, and 60 μM, respectively. (B) Calibration curve for the luminescence detection of HOCl using $[Ru(bpy)_2(DNPS\text{-}bpy)]^{2+}$ (10 μM) as a probe.

The reaction specificity of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ with HOCl was also investigated in 0.1 M phosphate buffer of pH 7.4. First, $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ was treated with various ROS and RNS. As shown in Figure 4, Figures S12 and S13 of the SI, $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ exhibited a

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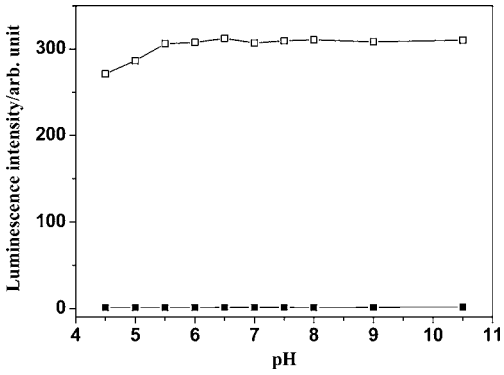

Figure 3. Effects of pH on the luminescence intensities of $[Ru(bpy)_2(COOH\text{-}bpy)]^{2+}$ (10 μM, □) and $[Ru(bpy)_2(DNPS\text{-}bpy)]^{2+}$ (10 μM, ■) in 50 mM HEPES buffers with different pHs.

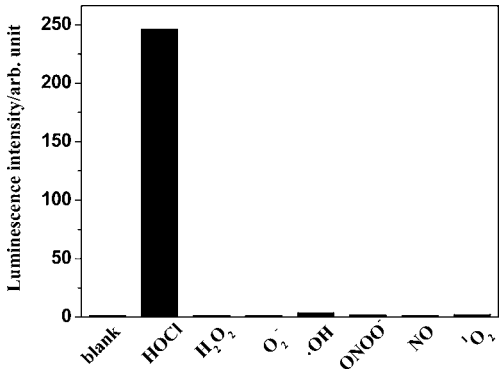

Figure 4. Luminescence intensities of the products of $[Ru(bpy)_2(DNPS\text{-}bpy)]^{2+}$ (10 μM) reacted with various ROS and RNS in 0.1 M phosphate buffer of pH 7.4. HOCl: 50 μM; H_2O_2: 100 μM; $O_2^{\cdot-}$: 100 μM KO_2; •OH: 100 μM H_2O_2 + 100 μM $(NH_4)_2Fe(SO_4)_2$; $ONOO^-$: 100 μM NaONOO; NO: 100 μM NOC-13; $^1O_2$: 200 μM H_2O_2 + 100 μM MoO_4^{2-} (in 0.1 M carbonate buffer of pH 10.5).

positive luminescence response only in the presence of HOCl, whereas no obvious luminescence signals were observed upon the additions of other ROS/RNS, such as H_2O_2, $O_2^{\cdot-}$,•OH, $ONOO^-$, NO, and $^1O_2$. The excellent response specificity of the sensor to HOCl might be attributed to the high oxidative activity of HOCl. Upon the addition of HOCl, the bond between sulfur and benzyl of DNPS-bpy in $[Ru(bpy)_2(DNPS\text{-}bpy)]^{2+}$ was broken by the oxidation of HOCl,[27] after the DPNS moiety was removed, the residual was further oxidized by HOCl to result in the highly luminescent product, $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$. We then examined the luminescence responses of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ toward common cations (Na^+, Zn^{2+}, Ag^+, K^+, Ba^{2+}, Ni^{2+}, Mn^{2+}, Li^+, Hg^{2+}, Co^{2+}, Ca^{2+}, Cu^{2+}) and amino acids (cysteine: Cys; glutathione: GSH; tyrosine: Tyr; alanine: Ala; glycine: Gly; lysine: Lys; valine: Val; proline: Pro; tryptophan: Trp; methionine: Met; histidine: His; serine: Ser; aspartic acid: Asp; threonine: Thr; leucine: Leu; arginine: Arg) under the same conditions (Figures S14 and S15 of the SI). Fortunately, none of the tested cations and amino acids elicited an observable increase in luminescence intensity. All of above results indicate that the luminescence response of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ to HOCl is highly specific without interferences of other ROS/RNS, cations and amino acids.

Luminescence Imaging of HOCl in Living Cells Using $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ as a Probe. With the above encouraging results, we examined the applicability of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ for the luminescent imaging of HOCl in living cells. For proof-of-concept, the applicability of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ for imaging exogenous HOCl in HeLa cells was initially assessed. As shown in Figure 5, negligible intracellular luminescence signals were observed after the cells were incubated with $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ for 2 h. However, the further incubation of the cells in the HOCl-containing medium resulted in the remarkable enhancement of the red intracellular luminescence. After the $[Ru(bpy)_2(DNPS\text{-}bpy)]^{2+}$-loaded cells were treated with 15 μM HOCl for 1 h, an approximately 7.9-fold increase in intracellular luminescence signal was observed (Figure 5C). Furthermore, the quantitative analysis of the luminescence intensity distribution in HeLa cells revealed that the red luminescence signals mainly localized in the cytoplasm region (Figure S16 of the SI), indicating that $[Ru(bpy)_2(DNPS\text{-}bpy)]^{2+}$ was transferred into the cells with a cytoplasm uptake profile.

It is known that macrophage and other phagocytic cells may produce endogenous HOCl when stimulated by lipopolysaccharide (LPS) and phorbol myristate acetate (PMA).[9a,b,11a,28] In the present work, we further examined the applicability of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ for imaging endogenous HOCl generated in RAW 264.7 cells under physiological stimulation. As depicted in Figure 6, almost no luminescence could be observed from the $[Ru(bpy)_2(DNPS\text{-}bpy)]^{2+}$-loaded RAW 264.7 cells (Figure 6A). However, after the cells were activated

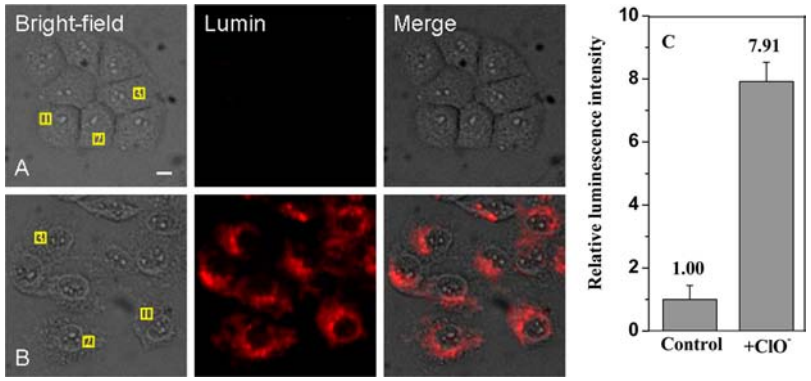

Figure 5. Bright-field, luminescence, and bright-field-luminescence merged images of the $[Ru(bpy)_2(DNPS\text{-}bpy)]^{2+}$-loaded HeLa cells in the absence (A) and presence (B) of HOCl (15 μM). (D) Relative luminescence intensities (the mean of the integrated intensities of three regions) of the $[Ru(bpy)_2(DNPS\text{-}bpy)]^{2+}$-loaded HeLa cells before and after incubation with HOCl. Scale bar: 5 μm.

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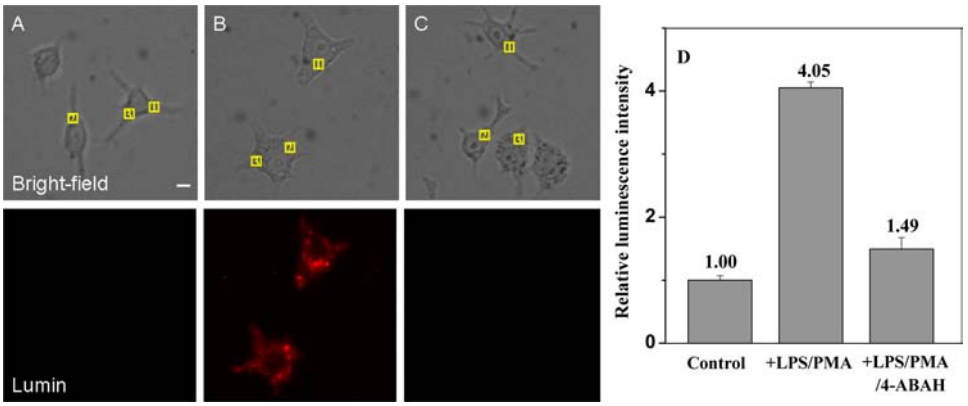

Figure 6. Bright-field and luminescence images of the HOCl generation in RAW 264.7 cells using $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ as a probe (scale bar: 5 μm). (A) The cells were incubated with $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ (50 μM) alone for 2 h. (b) The cells were pretreated with LPS (1 μg/mL) for 24 h, and then incubated with $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ (50 μM) and PMA (1 μg/mL) for 2 h. (C) The LPS-pretreated cells were incubated with $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ (50 μM), PMA (1 μg/mL) and 4-ABAH (20 μM) for 2 h. (D) Relative luminescence intensities (the mean of the integrated intensities of three regions) of the cells in images of (A), (B), and (C).

by LPS and PMA, the red intracellular luminescence was clearly observed (Figure 6B), and the luminescence intensity was 4-fold increased compared to that of the nonactivated cells (Figure 6D). When the cells were treated with 4-ABAH (an MPO inhibitor[10a,29]) during the stimulation, almost no luminescence was observed from the cells (Figure 6C). These results indicate that the red luminescence signals from the cells are indeed induced by the reaction of $[Ru(bpy)_2(DNPS\text{-}bpy)]^{2+}$ with the endogenous HOCl molecules. Similar to the result of HeLa cells, the probe molecules were also retained throughout the cytoplasm region in RAW 264.7 cells (Figure S17 of the SI).

The toxicity of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ to RAW 264.7 cells was determined by an MTT assay method. As shown in Figure S18 of the SI, after the cells were incubated with different concentrations of $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ (25, 50, 75, 100, and 150 μM) for 24 h, the cell viabilities were still greater than 85%. These results reveal that $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ is low toxic to the cultured macrophage cells.

CONCLUSIONS

In summary, a unique Ru(II) complex, $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$, has been developed as a luminescent probe for the detection of HOCl in living cells. The specifically HOCl-promoted oxidation reaction of the probe triggers the cleavage of the electron acceptor, DNP, leading to the formation of a highly luminescent complex, $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$, with a 190-fold enhancement in luminescence intensity. The turn-on luminescence response of the probe is highly specific to HOCl under the physiological pH condition. By using $[Ru(bpy)_2(DNPS\text{-}bpy)](PF_6)_2$ as a probe, luminescent imaging detections for exogenous HOCl in living HeLa cells and endogenous HOCl generation in macrophage cells were successfully performed. The findings suggest that the newly developed Ru(II) complex probe could serve as a valuable tool for intracellular HOCl detection and biomedical studies on the impact of HOCl in living cells.

ASSOCIATED CONTENT

Supporting Information

Supplementary spectra and cell imaging results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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