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ORIGINAL PAPER

Development of a Functional Ruthenium(II) Complex that Can Act as a Photoluminescent and Electrochemiluminescent Dual-signaling Probe for Hypochlorous Acid

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Abstract A functional ruthenium(II) complex that can act as a probe for response to hypochlorous acid (HOCl) in aqueous media with photoluminescence (PL) and electrochemiluminescence (ECL) dual-signals, $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ [bpy: 2,2′-bipyridine; DB-phen: 5-(2,4-dimethoxybenzylamino)-1,10-phenanthroline)], has been designed and synthesized. The complex is highly luminescent both under the light excitation and the electrochemical induction. It can specifically react with HOCl in physiological pH aqueous media to afford its chlorinated derivative, $[Ru(bpy)_2(DBCA\text{-}phen)](PF_6)_2$ [DBCA-phen: 5-(2,4-dimethoxybenzyl-chloroamino)- 1,10-phenanthroline], accompanied by remarkable decreases in its PL and ECL intensities. The PL and ECL abatements of $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ show good linear correlation to the concentration of HOCl with detection limits at low micromolar concentration level, and the PL and ECL responses of the complex to HOCl are highly specific without interferences of other reactive oxygen/nitrogen species. These features enabled $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ to be used as a probe for the highly selective and sensitive detection of HOCl in aqueous media with PL and ECL dual-modes.

Keywords Ruthenium complex · Luminescence probe · Photoluminescence · Electrochemiluminescence · Hypochlorous acid

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Introduction

As one of the most important reactive oxygen species (ROS), hypochlorous acid (HOCl) is widely employed as a bleaching agent in our daily life [1]. In biosystems, HOCl can be produced from H_2O_2 and chloride by secreted myeloperoxidase (MPO) in vivo in response to inflammatory stimuli [2, 3]. In a certain concentration range, HOCl serves as a powerful microbicidal agent in the innate immune system due to its strong oxidizability [4]. However, the excess production of HOCl has been linked to a variety of human diseases, such as cystic fibrosis [5], hepatic ischemiareperfusion injury [6], kidney disease [7], atherosclerosis [8], lung injury [9], rheumatoid arthritis [10], inflammatory disease [11], and certain cancers [12, 13]. Due to the important significance of HOCl in chemistry and biochemistry, a number of detection methods for HOCl have been developed in recent years [14–18]. Among them, photoluminescence (PL) detection technique based on HOCl-responsive probes is considered to be an advantageous method because of its high sensitivity, selectivity, experimental feasibility, and availability for the in vivo detection [19].

In the last few years, luminescent transition metal complexes with d^6, d^8 and d^{10} electronic configurations have been developed as useful luminophores for the developments of various luminescent probes [20–23]. Among these complexes, Ru(II) complexes with diimine ligands (2,2′-bipyridine, 1,10-phenanthroline, and their derivatives) showed important applicability to contribute to molecular recognition and sensing systems for metal cations, anions and bioactive molecules [24–27], owing to their abundant photophysical properties and ligand-dependent luminescent properties. The emission properties of Ru(II) complexes, such as emission wavelength, quantum yield and lifetime, could be modulated by the structure change of a diimine ligand, which provides a convenient approach for the design of Ru(II)

complex-based PL probes. In addition to the application in PL detection technique, Ru(II) complexes showed also good applicability in electrochemiluminescent (ECL) detection technique [28]. Using Ru(II)-bipyridine complex as a signaling moiety, we recently developed several PL and ECL dual-signaling probes for nitric oxide [29] and biothiols [30].

In this work, a Ru(II) complex bearing two 2,2′-bipyridine (bpy) ligands and one 1,10-phenanthroline derivative ligands, $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ [DB-phen: 5-(2,4-dimethoxybenzylamino)-1,10-phenanthroline)], was designed and synthesized as a new PL and ECL dual-signaling probe for the detection of HOCl in aqueous media. The complex itself displayed high luminescence both under the light excitation and the electrochemical induction. After the complex was reacted with HOCl in physiological pH aqueous media to afford its chlorinated derivative, $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$ [DBCA-phen: 5-(2,4-dimethoxybenzyl- chloroamino)-1,10-phenanthroline], the PL and ECL intensities of complex were remarkably decreased, and the dose-dependent PL and ECL abatements exhibited good linear relationships against the HOCl concentration with the detection limits of 0.35 and 0.26 μM for PL and ECL detections, respectively. The PL and ECL responses of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ to HOCl are highly specific without interferences of other reactive oxygen/nitrogen species (ROS/RNS). On the basis of these features, $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ was successfully used as a probe for the detection of HOCl in aqueous media both with PL and ECL dual-modes. Scheme 1 shows the structure of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ and its reaction with HOCl.

Experimental

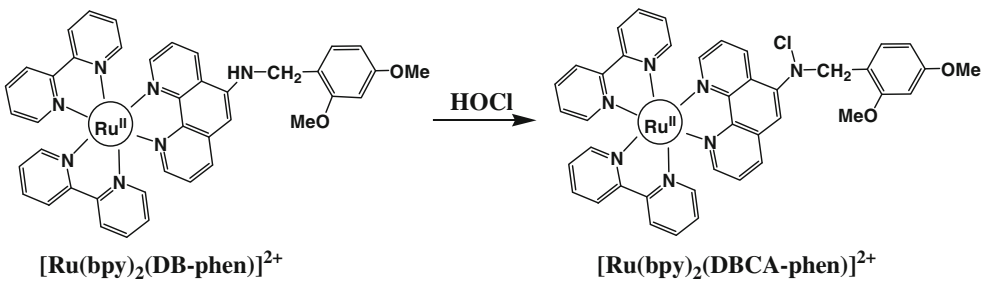
Materials and Physical Measurements

5-Amino-1,10-phenanthroline [31] and $Ru(bpy)_2Cl_2$ [32] were synthesized by using the literature methods. 2,4-Dimethoxy-benzaldehyde was purchased from Sigma-Aldrich. A stock solution of HOCl was prepared by dilution of the commercial sodium hypochlorite solution, and stored according to the literature method [33]. The HOCl concentration (total concentration of HOCl + ClO^-) was determined by using its molar extinction coefficient of 391 M^{-1} cm^{-1} at 292 nm before use [33]. Unless otherwise stated, all chemical materials were purchased from commercial sources and used without further purification.

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer (400 MHz for ^1H, and 100 MHz for ^{13}C). ESI-mass spectra were measured on a HP1100LC/MSD MS spectrometer. Absorption spectra were measured on a Perkin-Elmer Lambda 35 UV-vis spectrometer. Elemental analysis was carried out on a Vario-EL CHN analyser. PL spectra were measured on a Perkin-Elmer LS 50B luminescence spectrometer with the conditions of excitation wavelength, 455 nm; emission wavelength, 608 nm; excitation slit, 10 nm; and emission slit, 10 nm. All the ECL measurements were carried out on an ECL instrument system (MPI-A, Remex Electronics Instrument Ltd. Co.), using a small quartz ECL cell at room temperature. The glassy carbon (3.0 mm in diameter) electrode and KCl saturated Ag/AgCl electrode were used as working electrode and reference electrode, respectively, and a platinum wire (0.3 mm in diameter) was used as the auxiliary electrode. Before measurements, the glassy carbon working electrode was soaked in 10 % HNO_3 in an ultrasonic water bath for 5 min, polished by an Al_2O_3 slurry, and thoroughly rinsed with deionized water for 5 min. The voltage of the photomultiplier tube was set at 900 V in the detection process while collecting the ECL signals.

Synthesis of 5-(2,4-dimethoxy-benzylamino) -1,10-phenanthroline

A mixture of 5-amino-1,10-phenanthroline (1.85 g, 9.5 mmol), 2,4-dimethoxy-benzaldehyde (1.57 g, 9.5 mmol) and 6 mL glacial acetic acid in 150 mL anhydrous methanol was refluxed for 24 h with stirring. After cooling, $NaBH_4$ (1.78 g, 47.5 mmol) was added, and the mixture was stirred in an ice-water bath for 1 h and further refluxed for 3 h. The solvent was evaporated, and the residue was dissolved in 150 mL chloroform. The solution was washed once with saturated Na_2CO_3 solution (100 mL) and 3 times with distilled water (100 mL). After organic phase was dried with Na_2SO_4 and evaporated, the product was obtained as a red solid (2.53 g, 77 % yield). ^1H NMR (400 MHz, $CDCl_3$): δ=3.80



Scheme 1 Structure of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ and its reaction with HOCl.

$[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$

(s, 3H), 3.85 (s, 3H), 4.45 (s, 2H), 4.84 (s, 1H), 6.45~6.47 (m, 1H), 6.52 (s, 1H), 6.74 (s, 1H), 7.26 (t, J=4.0Hz, 1H), 7.42~7.57 (m, 1H), 7.95~7.97 (m, 1H), 8.23~8.25 (m, 1H), 8.86~8.87 (m, 1H), 9.13 (d, J=4.0Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=43.50, 55.41, 55.44, 98.76, 100.60, 104.16, 118.44, 122.09, 122.28, 123.24, 128.70, 130.14, 130.46, 133.71, 141.22, 141.67, 146.24, 146.70, 149.76, 158.59, 160.59. ESI-MS (m/z): 346.1 $[M + H]^+$.

Synthesis of $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$

After $Ru(bpy)_2Cl_2$ (121 mg, 0.25 mmol) was dissolved in 20 mL of 1:1 ethanol-H_2O, a solution of 5-(2,4-dimethoxybenzylamino)-1,10-phenanthroline (85 mg, 0.25 mmol) in 30 mL ethanol was added. The mixture was refluxed with stirring for 24 h under an argon atmosphere. After cooling, the solvent was evaporated, and the residue was purified by silica gel column chromatography using CH_3CN-H_2O-KNO_3(sat.) (100:10:0.5, v/v/v) as eluent. The fractions containing the target product were collected, and the solvent was evaporated. The solid was dissolved in 20 mL anhydrous CH_3CN to remove the excess KNO_3 by filtration. After evaporation, the product was dissolved in a small amount of 1:1 CH_3CN-H_2O, and then a saturated solution of NH_4PF_6 was added to give red precipitate. The product was filtered and washed with small amount of water. $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ was obtained (331 mg, 78 % yield). ^{1}H NMR (400 MHz, CD_3CN): δ=3.78 (s, 3H), 3.89 (s, 3H), 4.54 (d, J=4.0Hz, 2H), 6.22~6.24 (m, 1H), 6.47~6.49 (m, 1H), 6.60 (d, J=4.0Hz, 1H), 6.96 (s, 1H), 7.22~7.25 (m, 2H), 7.30 (d, J=8.0Hz, 1H), 7.41~7.47 (m, 3H), 7.53 (s, 1H), 7.57 (d, J=4.0Hz, 1H), 7.62~7.63 (m, 1H), 7.67~7.69 (m, 1H), 7.80~7.84 (m, 2H), 7.96~8.08 (m, 5H), 8.15~8.18 (m, 1H), 8.46~8.52 (m, 4H), 8.64~8.66 (m, 1H). ^{13}C NMR (100 MHz, CD_3CN): δ=42.15, 55.05, 55.33, 98.47, 99.17, 104.44, 124.62, 124.08, 124.11, 124.14, 124.20, 124.33, 124.79, 125.93, 127.35, 127.46, 127.50, 129.79, 130.86, 133.14, 133.79, 137.57, 137.69, 137.71, 141.63, 143.82, 147.00, 148.34, 151.78, 151.84, 151.95, 152.01, 157.00, 157.02, 157.24, 158.84, 160.72. Elemental analysis calcd.(%) for $C_{41}H_{35}F_{12}N_7O_2P_2Ru\cdot H_2O$: C 46.16, H 3.50, N 9.19; found(%): C 46.50, H 3.41, N 8.91. ESI-MS (m/z): 904.1 $[M\text{-}PF_6]^+$, 379.7 $[M\text{-}2PF_6]^{2+}$.

PL and ECL Responses of $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ Towards HOCl

The PL and ECL responses of $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ towards HOCl were measured at room temperature in aerated solutions of 0.1 M borate buffer of pH 7.4 and 0.1 M borate buffer of pH 7.4 containing 10 mM of tri-*n*-propylamine (TPrA), respectively. Different concentrations of HOCl were added into the solution of $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ (10 μM). The solutions were stirred for 30 min, and then subjected to the PL and ECL measurements on the Perkin-Elmer LS 50B luminescence spectrometer and the MPI-A ECL instrument, respectively.

Reactions of $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ with Different ROS/RNS

All the reactions were carried out in aerated solutions of 0.1 M borate buffer of pH 7.4 (for PL detection) or 0.1 M borate buffer of pH 7.4 containing 10 mM TPrA (for ECL detection) with the same $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ concentration (10 μM) and HOCl (80 μM) or other ROS/RNS (100 μM) for 0.5 h at room temperature. Hydrogen peroxide (H_2O_2) was diluted immediately from a stabilized 30 % solution and was assayed by using its molar absorption coefficient of 43.6 m^{-1} cm^{-1} at 240 nm [34]. Hydroxyl radicals (OH) were generated in the Fenton system from ferrous ammonium sulfate and hydrogen peroxide [17]. 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} [35]. Superoxide solution ($O_2^{\cdot-}$) was prepared by dissolving solid KO_2 in dry dimethyl sulfoxide (DMSO) and the mixture was stirred vigorously for 10 min before use. Freshly prepared aqueous solutions of $NaNO_2$ and $NaNO_3$ were used as nitrite (NO_2^-) and nitrate (NO_3^-) sources, respectively.

Synthesis of the $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$ Solution

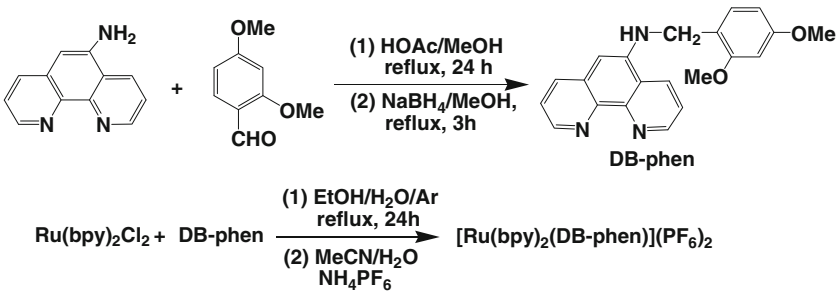
To an aerated solution of 10 μM $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ in 2.76 mL of 0.1 M borate buffer of pH 7.4 was added 240 μL aqueous solution of 1.0 mM HOCl with stirring. After the solution was incubated for 0.5 h at room temperature, the reaction was monitored by fluorometry to check the complete conversion of $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ to $[Ru(bpy)_2(DBCA\text{-}phen)](PF_6)_2$. The reaction product was confirmed by ESI-MS. ESI-MS (m/z): 938.0 $[M\text{-}PF_6]^+$, 397.3 $[M\text{-}2PF_6]^{2+}$ (the ion peak of $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$). The above solution was used for the luminescence property characterizations of $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$.

Results and Discussion

Synthesis of the Ru(II) Complex Probe for HOCl

In a previous work, we have demonstrated that a Ru(II) complex bearing two 2,2′-bipyridine ligands and one 5,6-diamino-1,10-phenanthroline (DA-phen) ligand, $[Ru(bpy)_2(DA\text{-}$

Scheme 2 Reaction pathway for the synthesis of $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$.



phen)]$^{2+}$, can sever well as a PL and ECL dual-signaling probe for NO [29]. This probe can specifically react with NO under aerobic conditions to form its triazole derivative $[Ru(bpy)_2(TA\text{-}phen)]^{2+}$, to induce the remarkable changes in the PL and ECL intensities. In this work, for developing a PL and ECL dual-signaling probe for HOCl, we used a 2,4-dimethoxybenzyl-amino group as a HOCl-reactive group to couple with 1,10-phenanthroline for the synthesis of the Ru(II) complex. It was anticipated that the reaction between the 2,4-dimethoxybenzyl-amino group and HOCl could lead to the changes of PL and ECL signals of the Ru(II) complex for the quantitative detection of HOCl.

Based on the above opinion, the complex $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ was synthesized according to the procedure as shown in Scheme 2. At first, the ligand DB-phen was synthesized by reacting 5-amino-1,10-phenanthroline with 2,4-dimethoxy-benzaldehyde in a HOAc-MeOH mixture, and followed by reducing the product with $NaBH_4$. After reacting $Ru(bpy)_2Cl_2$ with DB-phen in a 1:1 mixture of ethanol-H_2O, and followed by precipitating the pruduct with NH_4PF_6 in a 1:1 mixture of CH_3CN-H_2O, the target complex $[Ru(bpy)_2(DB\text{-}phen)](PF_6)_2$ was obtained with a good yield. The new complex was well characterized by NMR, ESI-MS and elemental analyses.

Spectral Properties of the Ru(II) Complexes

The UV-vis absorption spectra of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ and $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$ were measured in 0.1 M borate buffer of pH 7.4. As shown in Fig. 1, the two complexes showed typical UV-vis absorption spectra of the Ru(II)-polypyridyl complexes. The absorption at 285 nm is attributed to the π–π^* transition of the ligands, while the absorptions at 456 and 450 nm are attributed to the metal-to-ligand charge transfer (MLCT) transitions for $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ and $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$, respectively. After reaction with HOCl to form $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$, the broad absorption between 320 and 400 nm caused by the absorption of 2,4-dimethoxy-benzylamino group in $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ disappeared, which reveals that the 2,4-dimethoxy-benzylamino group could be a functional group to trap HOCl in the solution [36].

To compare the PL behaviors of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ and $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$, the excitation and emission spectra of the two complexes (10 μM) in 0.1 M borate buffer of pH 7.4 at room temperature were recorded (Fig. 2), and their luminescence properties were summarized in Table 1. The two complexes showed the similar excitation and emission spectrum pattern with the maximum excitation and

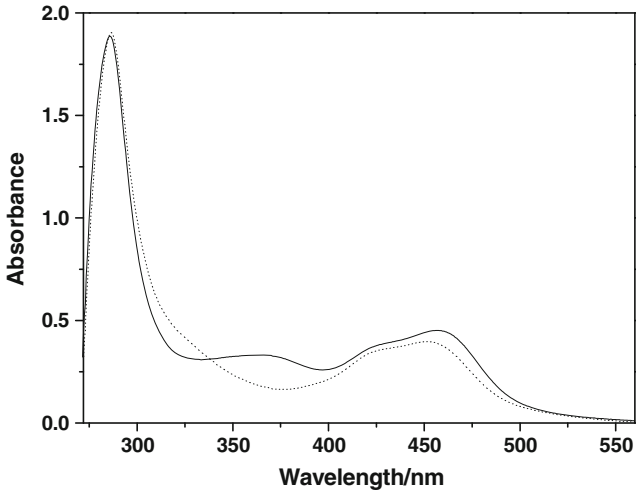


Fig. 1 UV-vis absorption spectra of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ (10 μM, *solid line*) and $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$ (10 μM, *dash line*) in 0.1 M borate buffer of pH 7.4

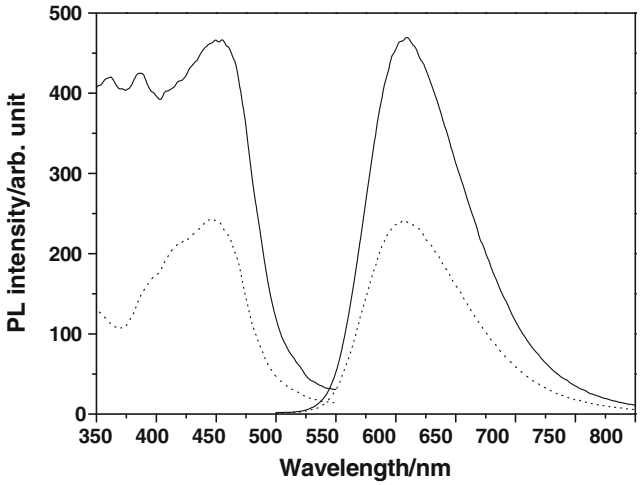


Fig. 2 Excitation (350–550 nm) and emission (500–800 nm) spectra of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ (10 μM, *solid lines*) and $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$ (10 μM, *dash lines*) in 0.1 M borate buffer of pH 7.4

Table 1 Luminescence properties of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ and $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$ in 0.1 M borate buffer of pH 7.4 at room temperature

Complex	$\lambda_{ex,\ max}$ (nm)	$\varepsilon_{455\ nm}$ ($M^{-1}\cdot cm^{-1}$)	$\lambda_{em,\ max}$ (nm)	ϕ (%)[a]
$[Ru(bpy)_2(DB\text{-}phen)_3]^{2+}$	455	1.50×10^4	608	1.85
$[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$	455	1.31×10^4	608	0.93

[a] Luminescence quantum yield was measured by using $[Ru(bpy)_3]Cl_2$ (ϕ=2.8 %) [37] as a standard

emission wavelengths at ~455 and 608 nm, respectively. They are attributed to the characteristic MLCT-based phosphorescence typically observed in the spectra of Ru(II)-diimine complexes. However, compared to highly luminescent $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$, $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$ exhibited considerably weak luminescence (~50 % decrease in PL intensity), which can be considered to be due to the electron-withdrawing effect of chlorine atom in $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$, since the electron transferred from Ru(II) to the orbital of diimine ligands will be partly diverted to the chlorine atom, to result in the luminescence quenching of the Ru(II) complex.

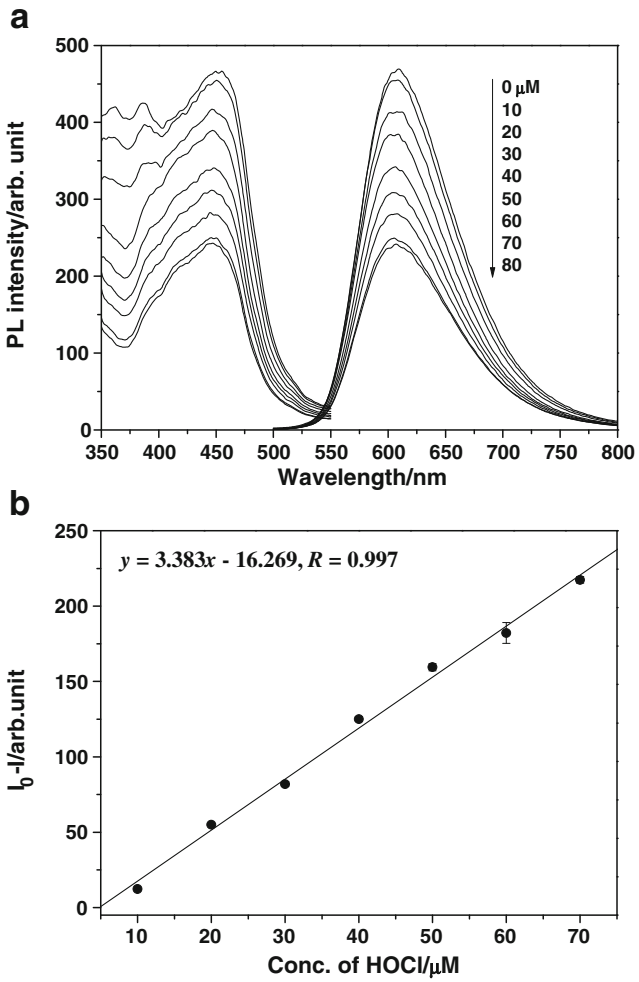


Fig. 3 **a** Excitation and emission spectra of the products of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ (10 μM) reacted with different concentrations of HOCl (0–80 μM) in 0.1 M borate buffer of pH 7.4. **b** Correlation between PL intensity changes at 608 nm (I_0-I) of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ and the HOCl concentration

PL Response of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ Towards HOCl

To quantitatively evaluate the PL response of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ to HOCl in aqueous media, the excitation and emission spectra of the products of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ (10 μM) reacted with different concentrations of HOCl in 0.1 M borate buffer of pH 7.4 were recorded. As shown in Fig. 3a, upon reaction with different concentrations of HOCl, the PL intensity of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ was gradually decreased with the increase of HOCl concentration. The PL intensity changes at 608 nm, I_0-I, displayed a linear response to the HOCl concentration with a dynamic range of 0–70 μM (Fig. 3b). The detection limit for HOCl, calculated as the concentration corresponding to triple standard deviations of the background signal, is 0.35 μM, which indicates that $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ can be used as a PL probe for the quantitative detection of HOCl at low micromolar concentration level.

The PL response specificity of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ to HOCl was also evaluated in 0.1 M borate buffer of pH 7.4. After reaction with different ROS and RNS, respectively, the PL intensity changes of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ (10 μM) at 608 nm were recorded. As shown in Fig. 4, the PL intensity of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ did not show significant responses to

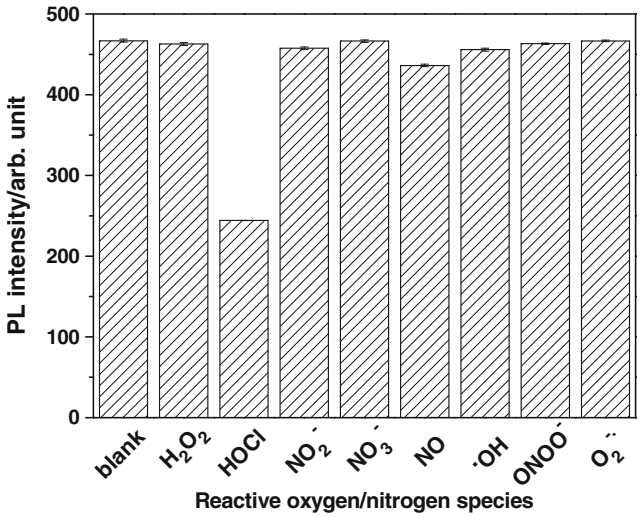


Fig. 4 PL intensity changes of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ (10 μM) upon reaction with different ROS and RNS in 0.1 M borate buffer of pH 7.4. HOCl: 80 μM; H_2O_2: 100 μM; OH: 100 μM H_2O_2 + 100 μM $(NH_4)_2Fe(SO_4)_2$; NO_2^-: 100 μM $NaNO_2$; NO_3^-: 100 μM $NaNO_3$; NO: 100 μM; $ONOO^-$: 100 μM NaONOO; $O_2^{\cdot-}$: 100 μM KO_2

H_2O_2, OH, NO, NO_2^-, NO_3^-, $ONOO^-$ and $O_2^{\cdot-}$, whereas it was remarkably decreased after reaction with HOCl. These results reveal that the PL response of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ to HOCl is highly specific without interferences of other ROS/RNS in physiological pH aqueous media.

ECL Response of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ Towards HOCl

To confirm the quantitative ECL response of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ to HOCl, the ECL intensities of the products of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ (10 μM) reacted with different concentrations of HOCl in 0.1 M borate buffer at pH 7.4 containing 10 mM of TPrA were determined. As shown in Fig. 5a, two ECL peaks appeared when the cyclic potential was scanned from 0.2 to 1.8 V and then backed from 1.8 to 0.2 V, respectively. The first main peak during the oxidation process of the Ru(II) complex with a maximum at ~1.1 V is a typical ECL emission from the excited state of the Ru(II) complex, $[RuL_3]^{2+*}$, that produced by the reduction of $[RuL_3]^{3+}$ with the TPrA radicals ($[RuL_3]^{3+}$ + TPrA → $[RuL_3]^{2+*}$) [38, 39], while the second side peak appeared during the reduction process of the Ru(II) complex can be attributed to the emission from the excited state of the Ru(I) complex, $[RuL_3]^{+*}$, that produced by the reduction of $[RuL_3]^{2+}$ with the TPrA radicals ($[RuL_3]^{2+}$ + TPrA → $[RuL_3]^{+*}$) [39, 40]. The ECL intensity of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ itself is strong, but remarkably decreases upon reaction with HOCl, which is identical to the PL behavior of the complex. By plotting the ECL intensity change (I_0-I) against the HOCl concentration, a good linear calibration curve with a dynamic range of 0–80 μM and a detection limit of 0.26 μM for the ECL detection of HOCl was obtained (Fig. 5b). These results indicate that $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ can be used as a ECL probe for the highly sensitive detection of HOCl in aqueous media.

The ECL response specificity of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ to HOCl was further examined in 0.1 M borate buffer of pH 7.4 containing 10 mM of TPrA. After reaction with different ROS and RNS, respectively, the ECL intensity changes of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ (10 μM) were recorded. As shown in Fig. 6, the ECL intensity of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ was almost unchanged upon reaction with different ROS/RNS except for HOCl. These results are similar to the PL response results, demonstrating that $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ cannot react with other ROS and RNS even under the electrochemical conditions, and the ECL response of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ to HOCl is also highly specific without interferences of other ROS/RNS.

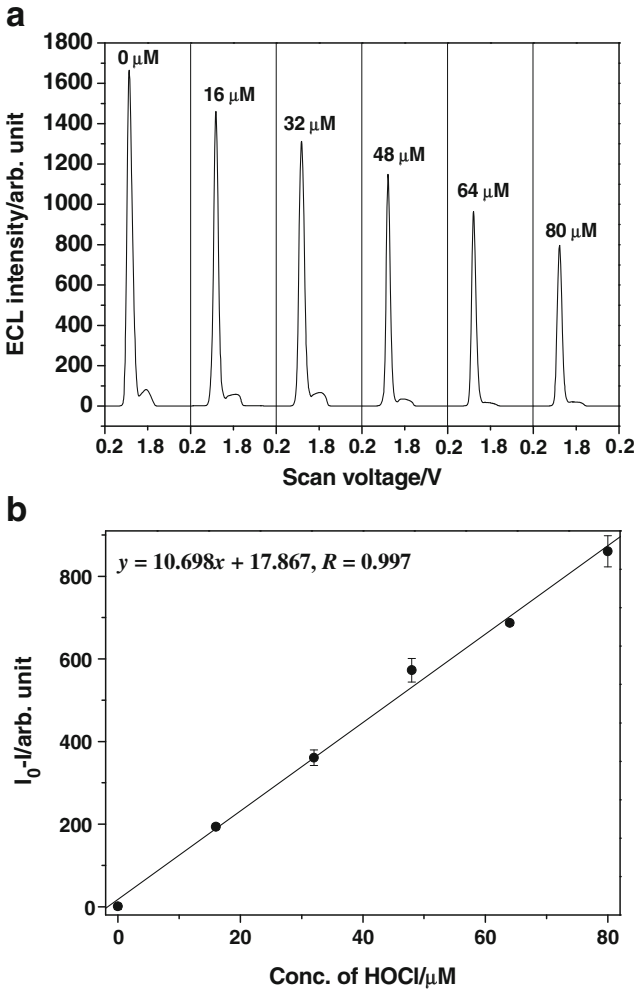


Fig. 5 **a** ECL intensity responses of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ (10 μM) to the additions of different concentrations of HOCl (0–80 μM) in 0.1 M borate buffer of pH 7.4 containing 10 mM of TPrA (the voltage of cyclic voltammetry was set up from 0.2 to 1.8 V, and scan rate of ECL was 100 mV s^{-1}). **b** Calibration curve for the ECL detection of HOCl

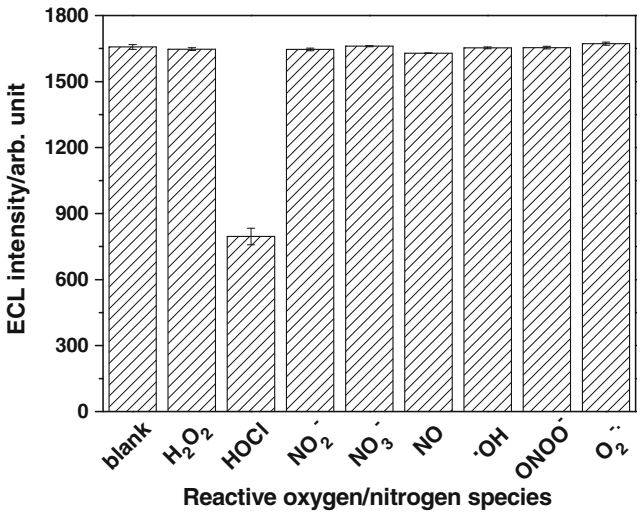


Fig. 6 ECL intensity changes of $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ (10 μM) upon reaction with different ROS and RNS in 0.1 M borate buffer of pH 7.4 containing 10 mM of TPrA (HOCl: 80 μM; the others are the same as shown in the caption of Fig. 4)

Conclusions

In summary, a functional Ru(II) complex that can act as a PL and ECL dual signaling probe for the detection of HOCl in aqueous media, $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$, has been successfully developed in this work. The complex can specifically react with HOCl to afford its chlorinated derivative, $[Ru(bpy)_2(DBCA\text{-}phen)]^{2+}$, accompanied by remarkable decreases in PL and ECL intensities with good linear dose-dependent intensity changes and low detection limits. These features enable $[Ru(bpy)_2(DB\text{-}phen)]^{2+}$ to be used both as a PL probe and as a ECL probe for the highly selective and sensitive detection of HOCl in aqueous media, which would be a useful tool for the monitoring of HOCl in various chemical, environmental and biological samples.

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