Photoluminescence and Electrochemiluminescence Dual-Signaling Sensors for Selective Detection of Cysteine Based on Iridium(III) Complexes

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Supporting Information

ABSTRACT: Cysteine (Cys) is important in biosynthesis, detoxification, and metabolism. The selective detection of Cys over structurally similar homocysteine (Hcy) or glutathione (GSH) remains an immense challenge. Although there are many methods for detecting Cys, photoluminescence (PL) and electrochemiluminescence (ECL) techniques are well-suited for clinical diagnostics and analytical technology because of their high sensitivities. Herein, we report PL and ECL dual-channel sensors using cyclometalated iridium(III) complexes for the discrimination of Cys from Hcy and GSH. The sensors react with cysteine preferentially because of kinetic differences in intramolecular conjugate addition/cyclization, enabling phosphorescence enhancement and ECL decrease in the blue-shifted region. Sensor 1 shows ratiometric PL turn-on and ECL turn-off for Cys. In addition, unique ECL-enhancing behavior of sensor 1 toward GSH enables discrimination between Cys and GSH. Sensor 1 was successfully applied to the detection of Cys in human serum by the ECL method. We demonstrate the first case of a Cys-selective PL and ECL dual-channel chemodosimetric sensor based on cyclometalated iridium(III) complexes and expect that the rational design of efficient PL and ECL dual-channel sensors will be useful in diagnostic technology.

INTRODUCTION

Biothiols such as cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) perform important roles in biological systems.1 Cys is related to detoxification, protein synthesis, and metabolism.2 Elevated levels of Cys are often found in patients with neurotoxicity3 and schizophrenia.4 The lack of Cys is associated with slow growth, hair depigmentation, edema, exhaustion, liver impairment, reduced muscles and fats, skin lesion, and feebleness.5 At the same time, irregular levels of Hcy are related to cardiovascular disease6 and Alzheimer’s disease.7 Because Cys and Hcy are related to different diseases, the selective detection of Cys or Hcy is of great importance in clinical aspects; however, this has remained a great challenge because of their structural similarities.

Although various detection methods for Cys have been developed including high-performance liquid chromatography, gas chromatography, electrophoresis, and electrochemical methods,8−12 a fluorescent chemodosimetric sensing method has attracted attention because of its simple operation, high selectivity, and rapid response to target analytes, thereby enabling real-time monitoring and imaging.13 One of the best known chemodosimetric approaches for detecting Cys is based on conjugate addition/cyclization using the acrylate group as the reaction site. An acrylate connected to a fluorophore effectively quenches the fluorescence of the fluorophore by photoinduced electron transfer (PET).14 However, the conjugate addition of Cys to acrylates followed by intramolecular cyclization of the resulting addition product leads to the recovery of intrinsic fluorescence. Most studies using this strategy utilize organic dyes,14−16 which suffer from limitations such as small Stokes shifts and poor photochemical/electrochemical stability.

We selected phosphorescent iridium complexes as electrochemiluminescence (ECL) luminophores because of their long emission lifetimes, large Stokes shifts, high quantum efficiencies, thermal stabilities, and electrochemical stabil-
For these reasons, a lot of iridium-complex-based sensors for biothiols have been developed.\textsuperscript{21−28} ECL is a chemiluminescence phenomenon that occurs by electron transfer reactions between radical ions generated by electrochemical oxidation–reduction reactions at the working electrode.\textsuperscript{5,34} Since ECL does not need a light source, it has advantages of high sensitivity and device miniaturization. Therefore, ECL is a powerful candidate for the point-of-care testing of diagnostic biomarkers. Recently, ECL has been successfully utilized to image single cells and their membrane proteins.\textsuperscript{35,36} We expect that the introduction of reaction sites to iridium-complex-based photoluminescence (PL) and ECL luminophores can provide effective and powerful sensing tools for specific biomarkers in clinical diagnosis. In this study, we report PL and ECL dual-channel chemodosimetric sensors using cyclometalated iridium(III) complexes for the discrimination of Cys from other biothiols such as Hcy and glutathione (GSH) (Scheme 1).

### RESULTS AND DISCUSSION

**Characterization of Sensors.** Sensors 1 and 12 were synthesized as shown in Scheme 2. All the synthesized compounds were fully characterized by \textsuperscript{1}H and \textsuperscript{13}C NMR spectroscopy and HRMS spectrometry (Figures S3−S24). The structure of sensor 1 was clearly determined using X-ray crystallography. The ORTEP view of sensor 1 is shown in Figure 1. The iridium(III) center is coordinated to two nitrogen atoms and two carbon donor atoms from two 6-phenylpyridin-3-yl acrylate ligands and two oxygen atoms from the acetylacetonato ligand, forming a distorted octahedral coordination geometry. As expected, the iridium(III) complex 1 shows a trans-\(\text{N},\text{N}\) configuration. Crystal data and details of structure refinement for sensor 1 are listed in Table S1.

**PL Properties of Sensors.** To investigate the Cys-sensing property of the sensors, we examined the PL of 1 and 12 after titration with Cys in a mixed solvent (CH\(_3\)CN/H\(_2\)O, 1:1 v/v, 10 mM HEPES, pH 7.4) (Figure 2, Figure S1). Sensor 1 containing two acrylate groups in the pyridine rings of the main ligands showed a weak yellow emission at 540 nm, which was \(~\text{20 nm} \) red-shifted compared to \((\text{ppy})_2\text{Ir(acac)}\). This was because the electron-withdrawing acrylate group stabilized its LUMO level, and thus, the acrylate carbon–carbon double bond could act as an electron acceptor from the Ir complex to decrease the phosphorescence intensity through donor-excited PET.\textsuperscript{17} The reaction of 1 with Cys increased immediately the emission intensity at 540 nm because the acrylate carbon–carbon double bond became saturated by conjugate addition, resulting in the recovery of phosphorescence (Figure 2a). Phosphorescence emission then decreased and shifted to the green region with the maximum wavelength at 516 nm over a period of 1 h, which was attributed to the conversion of acrylate moieties to OH groups by the removal of lactam formed by conjugate-addition/cyclization (Figure 2b). In the case of the reaction with Hcy, the emission intensity increased at 540 nm over a period of 1 h, then decreased, and shifted to 516 nm over a period of 9 h (Figure S2), which means that sensor 1 can effectively discriminate between Cys and Hcy within 1 h. Sensor 12, having two acrylate groups in the phenyl rings of the main ligands, exhibited different behaviors in response to Cys, as compared to sensor 1 (Figure S1). The green emission of sensor 12 with the maximum wavelength at 504 nm increased without chromic shifts even after 16 h of reaction with Cys. The maximum wavelength and spectral shape did not change when compared to those of sensor 12. However, the maximum wavelength of sensor 12 was different from that of compound 11 with phenolic OH. This suggested that Cys reacted more slowly with sensor 12 than with sensor 1. This was supported by MALDI-TOF MS analysis (Figure S3), indicating that Cys was added to only one of the two acrylate groups of sensor 12.

The PL intensity of sensor 1 (10 \textmu M) showed almost no change in the presence of other amino acids (Hcy, GSH, leucine, arginine, valine, methionine, threonine, isoleucine, and lysine, each 1 mM) (Figure 3a). Hcy and GSH also increased the PL intensity at 540 nm. However, unlike Cys, Hcy and

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**Figure 1.** Molecular structure of 1 showing displacement ellipsoids at 50%.
GSH did not show ratiometric PL changes within 1 h (Figure 3a). These results supported the fact that sensor 1 was highly selective toward Cys (Figure 3b). In the presence of other amino acids, additional treatment of sensor 1 with 1 mM Cys shifted the maximum wavelength to 516 nm, indicating that other amino acids did not interfere with the reaction between sensor 1 and Cys (Figure 3c).

**ECL Properties.** Figure 4a shows the ECL response of sensor 1 in CH$_3$CN/H$_2$O (1:1, v/v, 10 mM HEPES, pH 7.4, 100 mM TPrA, and 0.1 M TBAP as the supporting electrolyte) upon the addition of Cys. Unlike the PL results, the ECL intensity of sensor 1 drastically decreased until the addition of 100 equiv of Cys. Sensor 1 showed almost no change in the presence of other amino acids (Figure 5a).

However, the ECL intensity of sensor 1 dramatically decreased when adding 1 mM Cys to the solution of sensor 1 and other amino acids.

The addition of Hcy (1 mM) to sensor 1 (10 μM) resulted in a ∼30% decrease in the ECL intensity compared to sensor 1 because the reaction rate was lower than that with Cys. Interestingly, the addition of 100 equiv of GSH caused a 2-fold increase in the ECL intensity compared to sensor 1. This phenomenon resulted from PET inhibition through the reduction of acrylate carbon–carbon double bonds, caused by the Michael addition of GSH thiol to acrylate. This hypothesis was confirmed by 7 having an acetate moiety instead of an acrylate moiety (Scheme 2), which showed a 17-fold increase in the ECL intensity compared to sensor 1 (Figure 5b). The addition of 100 equiv of GSH resulted in only a 2-fold increase. This was because only a small amount of bulky GSH participated in the Michael addition reaction. This was further confirmed by competition experiments in which the ECL intensity decreased upon addition of Cys to a mixture of sensor 1 and Hcy or GSH (Figure 5a). This was also supported by MALDI-TOF MS analysis (Figures S4–S7). Therefore, sensor 1 detected Cys selectively and also enabled discrimination between Cys and GSH using ECL analysis.
In order to demonstrate the potential clinical application, ECL experiments were carried out in diluted human serum (10% in 10 mM HEPES buffer solution, deproteinized) and CH₃CN (1:1, v/v, 100 mM TPrA, and 0.1 M TBAP as the supporting electrolyte). Figure 6a shows a similar tendency to those in a CH₃CN-buffered system (see Figure 4). The ECL intensity of 1 gradually decreased with addition of Cys as expected. To demonstrate the stability of 1 in human serum, chronoamperometry (CA) coupled ECL experiments were conducted by a 40 cycle continuous scan, and the initial ECL intensity remained almost unchanged (Figure 6b), indicating that sensor 1 was stable. These results suggest that sensor 1 could potentially be applied to ECL clinical diagnosis.

**Sensing Mechanism.** Density functional theory (DFT) calculations revealed that the HOMO of sensor 1 was mainly distributed on the phenyl ring and Ir(III) center and the LUMO was distributed on the acrylate moiety, unlike the LUMO that is generally distributed on the pyridine ring of the main ligand in Ir(ppy)_2acac (Figure 7b). DFT calculations supported the observation that the fluorescence of sensor 1 was almost quenched by PET. It also suggested that sensor 1 was able to show a turn-on signal in response to Cys by blocking PET. We also evaluated HOMO/LUMO energy levels using cyclic voltammetry (CV) measurements (Figure 7a, Table S2). Both the HOMO and LUMO levels of sensor 1 were elevated upon the reaction with Cys. In coreactant ECL, the LUMO energy level of sensor 1 after the reaction with Cys rose above the energy level of the TPrA radical, which prevented electron transfer from the HOMO of the TPrA radical to the LUMO of the 1-Cys adduct, resulting in quenching of the ECL intensity. We also conducted MALDI-TOF MS analysis to confirm the products formed upon addition of biothiols (Figures S4−S7), which suggested that Cys underwent conjugate addition to the acrylate moiety of sensor 1 followed by cyclization to provide compound 2. In the case of 1 + Cys (100 equiv), the reaction rate was so fast that it was not possible to identify the reaction intermediate of 1 + Cys (Figure S4). However, the reaction with relatively small amounts of Cys (0.3 and 30 equiv) revealed that Cys reacted sequentially with two acrylate groups of sensor 1 (Figure S5). However, the reaction of sensor 1 with Hcy was slower than that with Cys, resulting in the formation of compound 2 and other intermediates (Figure S6). Interestingly, GSH was added to only one of the two acrylate groups of sensor 1 (Figure S7). This suggested that PET via electron transfer to acrylate carbon−carbon double bonds was partially blocked by the 1-GSH adduct. As a result, the addition of sensor 1 to GSH resulted in only a 2-fold increase in the ECL intensity. This result showed the possibility of detecting GSH by an acrylate-containing sensor using the ECL method.

**CONCLUSIONS**

We developed cyclometalated iridium(III)-complex-based chemodosimetric sensors for the selective detection of Cys.
Electron-withdrawing acrylate was introduced to the pyridyl group (sensor 1) of the main ligand to control the HOMO and LUMO energy levels of iridium(III) complexes. Sensor 1 reacted with cysteine selectively by intramolecular conjugate-addition/cyclization, resulting in phosphorescence enhancement and ECL quenching in the blue-shifted region. Particularly, the reaction of sensor 1 with cysteine drastically increased the LUMO energy level of the reaction product (1-Cys adduct), leading to ratiometric emission changes from yellow to green. Additionally, the coreactant ECL system prevented electron transfer from the HOMO of the TPrA radical to the LUMO of 1-Cys adduct, thus showing a high turn-off ratio of the ECL intensity. The addition of GSH led to a 2-fold increase in the ECL intensity because PET was partially blocked. Sensor 1 was successfully applied to detect Cys in human serum by the ECL method. To the best of our knowledge, this is the first case of a cyclometalated iridium(III)-complex-based cysteine-selective PL and ECL dual-channel sensor. We expect that the rational design of efficient PL and ECL dual-channel sensors will be useful for diagnostic technology.

**EXPERIMENTAL SECTION**

**Materials and Instruments.** All reagents were obtained from Acros Organics (USA), Alfa Aesar (USA), Sigma-Aldrich (MO, USA), and TCI (Tokyo, Japan). Unless otherwise noted, all commercial reagents were used without purification. 1H and 13C NMR spectra were recorded on a Bruker Avance DPX-300 or Agilent 400-MR DD2 Magnetic Resonance System. Absorption spectra were measured on a Beckman Coulter DU 800 Series spectrophotometer. Fluorescence emission spectra were measured on a JASCO FP-6500 spectrometer, with a bandwidth of 5 nm for excitation and emission. High-resolution mass spectrometry (HRMS) data were received from the National Center for Inter-University Research Facilities (NCIRF). Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass measurements were performed using a Microflex (Bruker Daltonics). The sensor solutions (1, 12) for all photophysical experiments were prepared from 2 mM stock solution in dimethyl sulfoxide (DMSO), diluted with acetonitrile (CH3CN), and stored in a refrigerator for use. Amino acids were dissolved in 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4).

**Electrochemical Analysis and Electrochemiluminescence (ECL) Measurements.** Electrochemical studies were carried out using a CH Instruments 650B electrochemical potentiostat. Cyclic voltammetry (CV) and chronoamperometry (CA) were performed on individual solutions to investigate electrochemical oxidative and reductive behaviors. ECL intensity profiles were acquired using a low-voltage photomultiplier tube module (H-6780 series, Hamamatsu Photonics K. K., Tokyo, Japan) operated at 1.0 V. A 25 μL ECL cell was directly mounted on the PMT module with a custom-made mounting support during experiments. All electrochemical and ECL data were referenced to a Ag/Ag+ reference electrode. All potential values were calibrated against the saturated calomel electrode (SCE) by measuring the oxidation potential of 1 mM ferrocene (vs Ag/Ag+) as the standard ($E_{\text{ox}}(\text{Fc}^+/\text{Fc}) = 0.424$ V vs SCE). CV and CA for ECL experiments were performed using a Pt disk electrode (2 mm diameter) between 0 and 2.0 V at a scan rate of 0.1 V/s.

**Figure 3.** (a) Phosphorescence spectra of 1 (10 μM) at 1 h after addition of 100 equiv of various amino acids (Cys, Hcy, GSH, leucine, arginine, valine, methionine, threonine, isoleucine, lysine). (b) Corresponding PL intensity changes of 1 for various amino acids. (c) PL competition assays carried out by addition of 1 mM Cys to 10 μM 1 in the presence of 1 mM various amino acids. Conditions: CH3CN/ H2O (1:1, v/v, pH 7.4, 10 mM HEPES, 25 °C) ($\lambda_{ex} = 400$ nm, bandwidth: 5/5 nm).
Solutions used for ECL experiments contained a 10 μM sensor, 0.1 M tetrabutylammonium perchlorate (TBAP, TCI) supporting electrolyte in CH₃CN (spectroscopy grade, Acros), and 100 mM tripropylamine (TPrA, Sigma-Aldrich, MO, USA) coreactant. The electrochemical and ECL solutions were freshly prepared for each experiment, and the Pt working electrode was polished with alumina (Buehler, IL, USA) on a felt pad and sonicated in a 1:1 mixture of absolute ethanol and deionized water for 10 min. Then the electrode was blown with high-purity N₂ gas for 1 min. A single solution was used for each experiment and discarded after data collection. ECL values were obtained by averaging the values from at least three data sets with good reliability in CH₃CN/H₂O solution (1:1, v/v, 10 mM HEPES, pH 7.4).

**Sample Pretreatment.** Human serum (from human male AB plasma, USA origin, sterile-filtered, Aldrich) samples were centrifuged at 12000 rpm for 15 min to get a supernatant and then diluted 10-fold with HEPES buffer solution (10 mM, pH 7.4) before analysis.

**Synthesis of the Ir(III) Complexes (Scheme 2).**

**Synthesis of 3.** A mixture of 2-bromo-5-hydroxypyridine (1.74 g, 10.02 mmol) and K₂CO₃ (2.71 g, 20.04 mmol) in dimethylformamide (DMF, 100 mL) was stirred at room temperature for 30 min, and iodomethane (0.936 mL, 15.03 mmol) was added slowly. The reaction mixture was stirred at room temperature for 6 h, following which the solvent was removed under reduced pressure and diluted with dichloromethane (CH₂Cl₂, DCM). The organic layer was washed with brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel chromatography using DCM/CH₃OH (100:1, v/v) as an eluent to yield a colorless pale-yellow liquid (1.42 g, 75% yield).

**Synthesis of 4.** A mixture of 3 (1.40 g, 7.44 mmol), phenylboronic acid (1.00 g, 8.20 mmol), K₂CO₃ (3.40 g, 24.6 mmol), and Pd(PPh₃)₄ (258 mg, 0.22 mmol) in tetrahydrofuran (THF, 40 mL) and H₂O (40 mL) was refluxed for 12 h. After cooling to room temperature, the reaction mixture was diluted with DCM, and the organic layer was washed with brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel chromatography using DCM/CH₃OH (50:1, v/v) as the eluent to give a white powder (1120 mg, 81% yield).

**Synthesis of 5.** A mixture of 4 (1018 mg, 5.50 mmol) and iridium trichloride hydrate (656 mg, 2.20 mmol) in 2-ethoxyethanol (90 mL) and H₂O (30 mL) was refluxed for 24 h. After cooling to room temperature, water (200 mL) was poured into the reaction mixture and stirred at room temperature for 30 min. The resulting precipitate was filtered, washed several times with water, and dried under an IR lamp for 12 h to give a yellow powder (1000 mg, 76% yield).

**Figure 4.** (a) ECL intensity of 10 μM 1 upon addition of Cys (1 mM) as the potential is swept at a working electrode (Pt disk, diameter: 2 mm) over the range of 0–2.0 V vs Ag/Ag⁺ (scan rate: 0.1 V/s). (b) ECL titration curve of 1 (10 μM) upon addition of Cys. LOD = 0.23 μM. Conditions: CH₃CN/H₂O (1:1, v/v, pH 7.4, 10 mM HEPES, 100 mM TPrA, and 0.1 M TBAP as the supporting electrolyte).

**Figure 5.** (a) Competitive ECL binding assays carried out with addition of 1 mM Cys to 10 μM 1 in the presence of 1 mM amino acid. (b) Comparison of ECL intensities of 1 and 7. All the ECL measurements were performed after reaction for 1 h. Conditions: CH₃CN/H₂O (1:1, v/v, pH 7.4, 10 mM HEPES, 100 mM TPrA, and 0.1 M TBAP as the supporting electrolyte).
(d, \( J = 2.7 \) Hz, 2H), 8.20 (d, \( J = 9.1 \) Hz, 2H), 8.09 (d, \( J = 9.1 \) Hz, 2H), 7.86−7.51 (m, 8H), 6.95−6.51 (m, 8H), 6.24 (d, \( J = 7.4 \) Hz, 2H), 5.64 (d, \( J = 7.4 \) Hz, 2H), 3.95 (d, \( J = 8.4 \) Hz, 12H).

**Synthesis of 6.** To a stirred solution of 5 (500 mg, 0.42 mmol) in dry DCM (25 mL) at 0 °C, BBr3 (1 M in DCM, 2.50 mL, 2.50 mmol) was added slowly under a N₂ atmosphere. The reaction mixture was stirred at room temperature for 12 h. Water was poured into the reaction mixture and stirred at room temperature for 30 min. The resulting precipitate was filtered, washed several times with water, and dried under an IR lamp for 12 h to give a dark green powder (320 mg, 67% yield). ¹H NMR (300 MHz, DMSO-d₆) δ 10.80 (s, 2H), 10.53 (s, 2H), 9.48 (s, 2H), 9.37 (s, 2H), 8.06 (d, \( J = 8.9 \) Hz, 2H), 7.96 (d, \( J = 8.9 \) Hz, 2H), 7.50 (ddd, \( J = 12.1, 10.6, 4.8 \) Hz, 8H), 6.92−6.58 (m, 8H), 6.16 (d, \( J = 7.5 \) Hz, 2H), 5.71 (d, \( J = 7.5 \) Hz, 2H).

**Synthesis of 2.** A mixture of 6 (100 mg, 0.16 mmol), acetylacetone (0.161 mL, 1.58 mmol), and Na₂CO₃ (167 mg, 1.58 mmol) in 2-ethoxyethanol (10 mL) was refluxed for 1 h. The reaction mixture was cooled to room temperature, filtered with Celite, and washed with ethyl acetate (EA). Following solvent evaporation, the residue was triturated with acetone and hexane. The resulting precipitate was filtered, washed several times with hexane, and dried under an IR lamp for 6 h to give a pale yellow powder (35 mg, 63% yield). ¹H NMR (300 MHz, acetone-d₆) δ 9.25 (s, 2H), 8.26 (d, \( J = 2.5 \) Hz, 2H), 7.92 (d, \( J = 8.8 \) Hz, 2H), 7.47 (ddd, \( J = 11.7, 5.3 \) Hz, 4H), 6.71 (t, \( J = 7.0 \) Hz, 2H), 6.57 (d, \( J = 10.6 \), 4.1 Hz, 2H), 6.22 (d, \( J = 7.4 \) Hz, 2H), 5.30 (s, 1H), 1.72 (s, 6H). ¹³C NMR (100 MHz, DMSO-d₆) δ 184.11, 158.60, 154.13, 146.26, 145.00, 136.11, 132.78, 127.05, 124.93, 122.42, 120.51, 119.94, 100.74, 28.79. HRMS (FAB+, m-NBA): m/z observed 632.1290 (calculated for C₂₇H₂₃IrN₂O₄ [M]+ 632.1288).

**Synthesis of 1.** A mixture of 2 (30 mg, 0.05 mmol) and triethylamine (26 μL, 0.19 mmol) in dry DCM (5 mL) was stirred at 0 °C for 30 min, and then acryloyl chloride (9.6 μL, 0.12 mmol) was added slowly. The reaction mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure and diluted with DCM. The organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography using EA/hexane (1:5, v/v) as the eluent to give an orange-yellow powder (25 mg, 71% yield). ¹H NMR (300 MHz, CDCl₃) δ 8.44 (d, \( J = 2.3 \) Hz, 2H), 7.88 (d, \( J = 8.9 \) Hz, 2H), 7.68 (dd, \( J = 11.7, 5.3 \) Hz, 4H), 7.53 (d, \( J = 7.6 \) Hz, 2H), 6.84 (t, \( J = 7.0 \) Hz, 2H), 6.76−6.58 (m, 4H), 6.44−6.26 (m, 4H), 6.12 (d, \( J = 10.4 \) Hz, 2H), 5.28 (s, 1H), 1.83 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 185.05, 166.32, 163.76, 146.65, 145.23, 143.91, 141.19, 133.90, 132.97, 120.59, 119.94, 100.74, 28.79. HRMS (FAB+, m-NBA): m/z observed 740.1497 (calculated for C₃₃H₂₇IrN₂O₆ [M]+ 740.1500).

**Figure 6.** (a) ECL titration curve of 1 (10 μM) upon addition of Cys in human serum. (b) Continuous cyclic ECL scans of 1 (10 μM) in human serum at 0−1.5 V. Conditions: diluted serum (1:10, v/v, 10 mM HEPES, deproteinized) and CH₃CN (1:1, v/v, 100 mM TPrA, and 0.1 M TBAP as the supporting electrolyte). CA parameters were selected as follows: initial E: 0 V, high E: 1.5 V, number of steps: 80 (40 cycles), pulse width: 0.5 s, sample interval: 1 ms.

**Figure 7.** (a) Cyclic voltammograms of 1 and 1-Cys adduct. (b) HOMO/LUMO distributions obtained by DFT calculations and energy levels calculated from CV analysis of 1 and 1-Cys adduct.
Synthesis of 7. A mixture of 2 (34 mg, 0.05 mmol) and triethylamine (30 μL, 0.21 mmol) in dry DCM (10 mL) was stirred for 30 min at room temperature, and then acetic anhydride (18 μL, 0.16 mmol) was added slowly. The reaction mixture was refluxed for 12 h. The solvent was removed under reduced pressure and diluted with DCM. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel chromatography using EA/hexane (1:5, v/v) as the eluent to give a yellow powder (32 mg, 83% yield). ¹H NMR (300 MHz, CDCl₃) δ 8.39 (d, J = 2.3 Hz, 2H), 7.86 (d, J = 8.9 Hz, 2H), 7.63 (dd, J = 8.9, 2.5 Hz, 2H), 7.53 (t, J = 9.6 Hz, 2H), 6.83 (t, J = 6.9 Hz, 2H), 6.73 (dd, J = 10.5, 4.2 Hz, 2H), 6.29 (d, J = 7.4 Hz, 2H), 5.27 (s, 6H), 3.39 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 185.06, 168.69, 166.23, 145.51, 145.28, 143.92, 141.21, 132.98, 130.57, 123.90, 120.97, 118.32, 100.75, 98.67, 85.06, 84.93, 83.70, 78.70. HRMS (FAB+, m-NBA): m/z observed 716.1505 (calculated for C₃₁H₂₇IrN₂O₆ [M⁺]: 716.1500).

Synthesis of 8. A mixture of 2-bromopyridine (181 μL, 1.90 mmol), 4-methoxyphenylboronic acid (317 mg, 2.09 mmol), and Pd(PPh₃)₄ (66 mm, 0.06 mmol) in THF (15 mL) and H₂O (15 mL) was refluxed for 12 h. After cooling to room temperature, the reaction mixture was diluted with DCM, and the organic layer was washed with brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel chromatography using EA/hexane (1:5, v/v) as the eluent to give a white powder (251 mg, 71% yield). ¹H NMR (300 MHz, CDCl₃) δ 8.67 (d, J = 4.7 Hz, 1H), 7.97 (d, J = 8.8 Hz, 2H), 7.84–7.63 (m, 2H), 7.20 (dd, J = 8.5, 3.2 Hz, 1H), 7.02 (d, J = 8.8 Hz, 2H), 3.89 (s, 3H).

Synthesis of 9. A mixture of iridium trichloride hydrate (90 mg, 0.08 mmol) and CuI (90 mg, 0.80 mmol) in 2-ethoxyethanol (10 mL) was refluxed for 1 h. The reaction mixture was cooled to room temperature, filtered with Celite, and washed with EA. The resulting precipitate was filtered, washed several times with hexane, and dried under an IR lamp to give a yellow powder (32 mg, 63% yield). ¹H NMR (400 MHz, DMSO-d₆) δ 8.89 (s, 2H), 8.24 (d, J = 5.5 Hz, 2H), 7.87 (d, J = 8.3 Hz, 2H), 7.78 (t, J = 7.2 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 7.18 (t, J = 6.2 Hz, 2H), 6.17 (dd, J = 8.4, 2.3 Hz, 2H), 5.45 (d, J = 2.3 Hz, 2H), 5.18 (s, 1H), 1.66 (s, 6H). ¹³C NMR (100 MHz, DMSO-d₆) δ 184.14, 168.11, 157.88, 150.37, 147.81, 137.82, 136.73, 125.88, 120.92, 119.84, 117.98, 108.45, 100.69, 28.70. HRMS (FAB+, m-NBA): m/z observed 740.1501 (calculated for C₃₃H₂₇IrN₂O₆ [M⁺]: 740.1500).

Synthesis of 11. A mixture of 10 (90 mg, 0.08 mmol), acetylacetone (0.081 mL, 0.80 mmol), and Na₂CO₃ (83.7 mg, 0.80 mmol) in 2-ethoxyethanol (10 mL) was refluxed for 1 h. The reaction mixture was cooled to room temperature, filtered with Celite, and washed with EA. Following solvent evaporation, the residue was triturated with acetone and hexane. The resulting precipitate was filtered, washed several times with hexane, and dried under an IR lamp for 6 h to give a pale yellow powder (32 mg, 63% yield). ¹H NMR (300 MHz, CDCl₃) δ 8.47 (d, J = 5.3 Hz, 2H), 7.87–7.71 (m, 4H), 7.59 (d, J = 8.5 Hz, 2H), 7.17 (t, J = 5.8 Hz, 2H), 6.70 (dd, J = 8.4, 2.3 Hz, 2H), 6.47 (d, J = 17.3, 1.3 Hz, 2H), 6.18 (dd, J = 17.3, 10.4 Hz, 2H), 6.02–5.82 (m, 4H), 5.22 (s, 1H), 1.79 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 184.93, 167.48, 164.65, 150.42, 148.39, 148.07, 147.27, 131.98, 128.13, 124.86, 124.53, 121.67, 118.61, 114.20, 100.75, 28.29. HRMS (FAB+, m-NBA): m/z observed 740.1501 (calculated for C₃₅H₂₉IrN₂O₆ [M⁺]: 740.1500).

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b01501.

Crystal data, additional PL spectra, MALDI-TOF mass spectra, electrochemical properties, and NMR spectra (PDF)
Crystallographic data for 1 (CIF)

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The manuscript was written through contributions of all authors.

Notes
The authors declare no competing financial interest.

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