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Electrogenerated Chemiluminescent Chemodosimeter Based on a Cyclometalated Iridium(III) Complex for Sensitive Detection of Thiophenol

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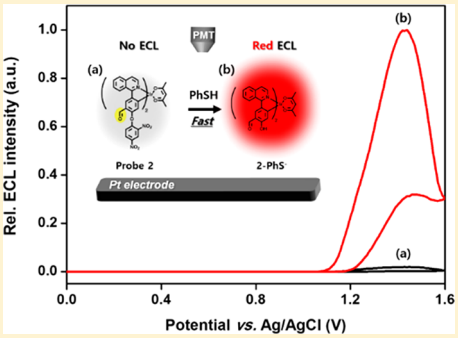
Supporting Information

ABSTRACT: Thiophenol is the simplest aromatic thiol that is utilized for various applications in industry and agriculture. However, it should be used with care because thiophenol is readily absorbed into the human body by inhalation and ingestion, which leads to serious internal injuries. Thus, there is an urgent need for real-time and accurate monitoring of thiophenol. Despite remarkable advantages of electrogenerated chemiluminescence (ECL) analysis, ECL thiophenol probes have never been reported. Herein, a new strategy for the rapid detection of thiophenol by use of an ECL turn-on chemodosimeter based on a cyclometalated Ir(III) complex is described. This analytical system showed superior sensitivity [limit of detection (LOD) value, 3.8 nM] in comparison to the conventional fluorescence method. In addition, our system exhibited remarkable selectivity and reaction rate toward thiophenol over other analytes. Moreover, it was successfully applied to quantify thiophenol in real water samples, providing a new proof-of-concept for field monitoring based on ECL.



Electrogenerated chemiluminescence (ECL) is a process in which sequential electron-transfer reactions on the surface of the electrode induce luminescence.[1] It is superior in comparison to conventional fluorescence assays because of its high sensitivity and low background signal. Additionally, ECL provides the possibility of potential point-of-care-testing (POCT) and field monitoring with the simplicity of the equipment and method.[1−4] For these reasons, ECL has emerged as one of the most powerful techniques for the detection of various analytes.[5−14] Therefore, it is possible to develop efficient environmental pollutant sensors for on-site real sample monitoring by introducing the intrinsic features of ECL to a single-molecule receptor. In this regard, our research group has developed single-molecule ECL sensors for various targets.[15−18] Until now, most ECL-based sensors were developed by using tris(2,2′-bipyridine)ruthenium(II) complexes ($[Ru(bpy)_3^{2+}]$) and ruthenium(II) polyimine complexes with characteristic orange/red emissions due to the limited ligand-field splitting energy of the ruthenium metal center.[19] To overcome this limitation, several groups introduced new ECL luminophores with high quantum efficiencies and various emission wavelengths.[20−26] Among them, cyclometalated iridium(III) complexes have been most successfully employed as ECL luminophores because Ir(III) complexes allow tuning of the emission wavelength through ligand modification, along with remarkable ECL quantum yields.[16,27−33]

Thiophenol (PhSH) is an important chemical reagent that is utilized as a raw material and intermediate to produce pharmaceuticals, agrochemicals, polymers, etc.[34−36] Although thiophenol is important for various applications, it should be used with care because of its high toxicity. Thiophenol has a median lethal concentration (LC_{50}) of 0.01−0.4 mM in fish and a median lethal dose (LD_{50}) of 46.2 mg/kg in mice.[37,38] In addition, thiophenol is readily assimilated into the human body by inhalation and ingestion, which leads to serious systemic injury, central nervous system damage, muscular weakness, coma, and even death.[39,40] Therefore, there is a compelling need to develop a real-time and accurate detection method for thiophenol. To date, various methods have been developed for the detection of thiophenol, including surface-enhanced Raman scattering (SERS) spectroscopy,[41] chromatography-coupled spectrometry,[42] and colorimetric[43] and fluorescence assays.[44−48] Among them, the fluorescence assay has been widely used over the past few decades because it is a nondestructive analytical process and can be applied for real-time monitoring.[49−55] Most of the previously reported fluorescence assays could selectively detect thiophenol (pK_a 6.5) over aliphatic thiols ($pK_a \approx 8.5$) under physiological conditions because of the different degree of deprotonation. However, this method requires not only heavy equipment but also the expertise to conduct a complicated analytical procedure, which restricts rapid on-site detection.

In this study, a turn-on ECL chemodosimeter (probe **2**) based on a cyclometalated Ir(III) complex was developed for rapid and quantitative detection of thiophenol. Probe **2** selectively reacted with two molecules of thiophenol to

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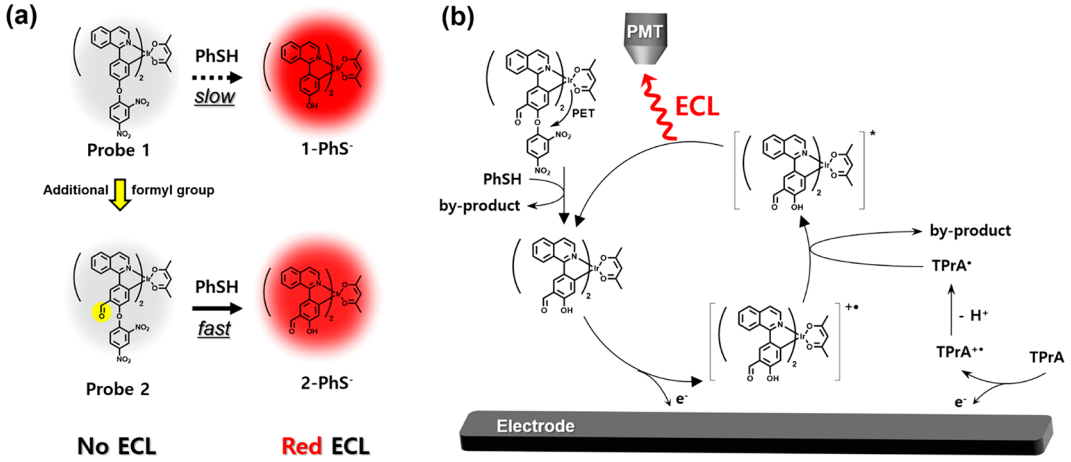
Scheme 1. (a) Design of Probe 2 for Rapid Detection of Thiophenol and (b) Proposed Sensing Mechanism of Probe 2 for Detection of Thiophenol through Electrogenerated Chemiluminescence Analysis



produce **2**-PhS$^-$ with ECL turn-on. The design concept of probe **2** toward thiophenol was based on the following (Scheme 1a): (i) $(piq)_2Ir(acac)$ (where piq = 1-phenylisoquinoline and acac = acetylacetone) was selected as a luminophore, as it is known to exhibit strong ECL emission;[27] (ii) 2,4-dinitrophenyl (DNP) group, a well-known photoinduced electron transfer (PET) quencher, was introduced as a reaction site for thiophenol;[56] and (iii) an electron-withdrawing formyl group was introduced to accelerate the reaction rate.[57−59] On the basis of this rationale, it was expected that probe **2** would rapidly react with the thiophenolate moiety via nucleophilic aromatic substitution (S_NAr) to generate **2**-PhS$^-$. Next, **2**-PhS$^-$ was directly oxidized on the Pt electrode to generate (**2**-PhS$^-$)$^{+\bullet}$, which was converted to the singlet excited state, (**2**-PhS$^-$)*, by one electron transfer from the tripropylamine radical (TPrA$^\bullet$), which eventually emitted ECL, as shown in Scheme 1b.[1,2] The present ECL analysis system for detection of thiophenol showed superior sensitivity and a lower limit of detection (LOD) value (3.8 nM) than the conventional photoluminescence method. It also showed remarkable selectivity and reaction rate toward thiophenol over other analytes, which is highly desirable for on-site detection. Moreover, probe **2** was successfully utilized to quantify thiophenol in real water samples, suggesting a new proof-of-concept for ECL-based field monitoring.

EXPERIMENTAL SECTION

Materials and Instruments. All reagents were purchased from either TCI (Tokyo Chemical Industry, Tokyo, Japan), Alfa Aesar (Tewksbury, MA), or Sigma−Aldrich (St. Loius., MO). All reagents were used without further purification. Deuterated solvents for NMR spectra were acquired from Cambridge Isotopic Laboratories (Cambridge, MA). Analytical thin-layer chromatography was carried out with Kiesegel 60F-254 plates from Merck. Column chromatography was performed on silica gel 60 (230−400 mesh) from Silicycle. High-resolution mass spectrometric (HRMS) data (JEOL, JMS-700) with fast atom bombardment (FAB) positive mode were received directly from the National Center for Inter-University Research Facilities (NCIRF). Mass spectra were recorded on a matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) Microflex instrument from Bruker. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or deuterated dimethyl sulfoxide (DMSO-d_6) on a Bruker Avance DPX-300 or Varian/Oxford As-500 instrument. All chemical shifts were reported in parts per million (ppm), with the residual proton signals of deuterated solvents as an internal reference. All absorption spectra were obtained on a Jasco V-730 spectrometer, and fluorescence spectra were obtained on a Jasco FP-6500 spectrometer or a SpectraMax M2 spectrophotometer. The bandwidth of all spectrometers for excitation and emission was fixed to 5 nm except for the SpectraMax M2 spectrophotometer (sensitivity = high). For photophysical experiments, 2 mM stock solutions of probes **1** and **2** were prepared in DMSO and diluted with acetonitrile (CH_3CN). Various analyte stock solutions were prepared in deionized water and diluted to 10 mM concentration, while thiophenol was dissolved and diluted with acetonitrile.

Electrochemical and Electrogenerated Chemiluminescence Measurements. Electrochemical studies were performed with a CH Instruments 660 electrochemical analyzer (CH Instruments, Inc., Bee Cave, TX). Cyclic voltammetry (CV) was applied to individual solutions in order to investigate electrochemical properties with a CH Instruments 650B Electrochemical Analyzer (CH Instruments, Inc., Bee Cave, TX). Electrochemical properties were examined by CV with a scan rate of 0.1 V/s and by differential pulse voltammetry (DPV) under the following conditions: sample width, 17 ms; pulse amplitude, 50 mV; pulse width, 50 mV; pulse period, 200 ms; and quiet time, 2 s. All potential values were adjusted relative to the ferrocene/ferrocenium (Fc/Fc^+) redox couple by measuring the oxidation potential of 1 mM ferrocene (vs Ag/Ag^+) as a standard.

The ECL intensity profile was obtained by use of a low-voltage photomultiplier tube (PMT) module (H-6780, Hamamatsu Photonics K.K., Tokyo, Japan) during the CV process in the range 0−1.6 V vs Ag/AgCl (scan rate 0.1 V/s). A 25 μL volume ECL cell was directly mounted on the PMT module with a homemade mounting support during the experiments. The ECL spectrum was obtained by measuring ECL intensities with a series of optical filters (550, 580, 600, 620, 650, 670, and 690 nm).[60,61] All ECL data were collected by simultaneous CV in solution. ECL solutions commonly contained 100 mM TPrA (tripropylamine, Sigma−Aldrich, St. Louis, MO) and 0.1 M tetrabutylammonium perchlorate (TBAP, TCI) as the supporting electrolyte in acetonitrile (CH_3CN, spectroscopy grade, Acros). Especially, TPrA was

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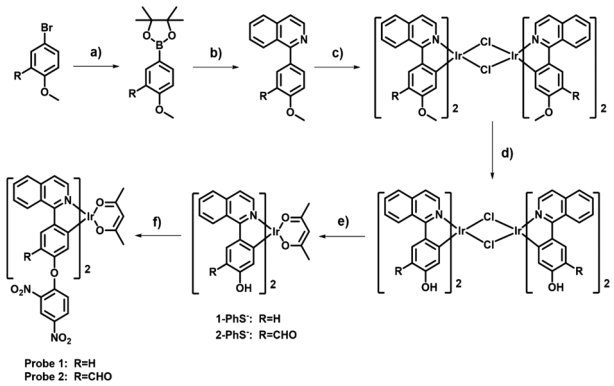
selected and used as an ECL coreactant. All the electrochemical and ECL experiments were referenced with respect to an Ag/Ag^+ reference electrode in organic solvent or to an Ag/AgCl reference electrode in aqueous solution. CV for ECL experiments was applied to the solutions at a scan rate of 0.1 V/s. The electrochemical and ECL solutions were freshly prepared in each experiment, and the Pt working electrode was polished with 0.05 μm alumina (Buehler, Lake Bluff, IL) on a felt pad. Then the electrode was blown with ultrapure N_2 gas for 1 min. A single solution was only used for one experiment and discarded after collection of data. The reported ECL values were obtained by averaging the values from at least three experiments with good reliability.

Synthesis of Probes 1 and 2. Probes **1** and **2** were synthesized in six steps as shown in Scheme 2. Synthesis began with commercially available 4-bromoanisole and 5-bromo-*o*-anisaldehyde to afford boron compounds for the next steps. After the main ligands with methyl ether protecting group were obtained thorough conventional borylation/Suzuki cross-coupling reactions, the corresponding Ir(III) complex dimers were synthesized in refluxing 2-ethoxyethanol. Then demethylation of aryl methyl ethers was conducted by boron tribromide (BBr_3), followed by chelation of acetylacetone (acac) as an ancillary ligand under basic conditions to furnish **1**-PhS^- and **2**-PhS^-. Finally, probes **1** and **2** were synthesized by introduction of the dinitrophenyl (DNP) group via S_NAr. Details of synthesis as well as spectral data (^1H NMR, ^{13}C NMR, and HRMS) of new compounds are described in Supporting Information.

Scheme 2. Synthetic Route for Probes 1 and 2[a]



[a]Reagents and conditions: (a) bis(pinacolato)diboron, KOAc, $Pd(dppf)Cl_2$, 1,4-dioxane, 90 °C; (b) 1-chloroisoquinoline, $Pd(PPh_3)_4$, $NaHCO_3$, THF, H_2O, 80 °C; (c) $IrCl_3 \cdot xH_2O$, 2-ethoxyethanol, H_2O, 100 °C; (d) BBr_3, CH_2Cl_2, 0 °C → rt; (e) acetylacetone, Na_2CO_3, 2-ethoxyethanol, 80 °C; (f) 1-chloro-2,4-dinitrobenzene, K_2CO_3, DMF, 100 °C. $Pd(dppf)Cl_2$ = [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), KOAc = potassium acetate, THF = tetrahydrofuran, DMF = *N*,*N*-dimethylformamide.

RESULTS AND DISCUSSION

Time-Dependent Photoluminescence Response and Sensing Mechanism. To prove the rate-accelerating effect of the formyl group, control probe **1** was synthesized. Probes **1** and **2** showed significant turn-on photoluminescence (PL) response upon reaction with thiophenol (Figure 1a,b). Reaction rates of probes **1** and **2** were compared by measuring

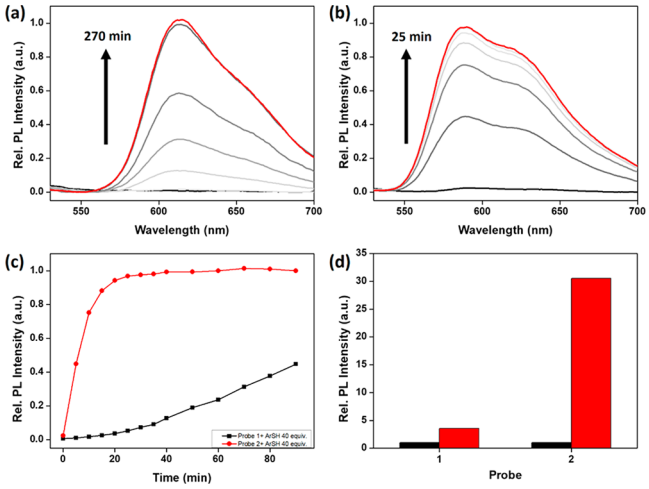


Figure 1. (a, b) Time-dependent measurements of PL response (λ_{ex} = 450 nm) of (a) probe **1** (10 μM) and (b) probe **2** (10 μM) in the presence of thiophenol (400 μM) in H_2O/CH_3CN (1:1 v/v, pH 7.4, 10 mM HEPES). (c) Relative PL intensity changes at 613 nm for probe **1** (black line) and at 589 nm for probe **2** (red line) in the presence of thiophenol. (d) Relative PL intensity of probes **1** and **2** before (black bar) and after (red bar) reaction with 400 μM thiophenol for 25 min in CH_3CN/H_2O (1:1 v/v, pH 7.4, 10 mM HEPES).

the time-dependent changes of PL in the presence of 40 equiv of thiophenol in aqueous medium [pH 7.4, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer/CH_3CN = 1:1 v/v]. As shown in Figure 1c, probe **2** reached saturation within 25 min, whereas probe **1** took 270 min to reach a plateau (Figure S1). In addition, the PL intensity of probe **2** at ~25 min after addition of thiophenol showed a 31-fold increase at 589 nm, while probe **1** showed only a 4-fold increase in the PL intensity at 613 nm (Figure 1d). This indicated that the formyl group adjacent to DNP played a significant role in improving the reactivity of the probe. The strong electron-withdrawing formyl group assists in efficient cleavage of the DNP group from the Meisenheimer-type intermediate.[57−59] It can also stabilize both the intermediate and the reaction product (**2**-PhS^-) by a resonance-assisted hydrogen bond.[62] Moreover, probe **2** exhibited stable PL emission over the pH range 5−11 (Figure S2). These results indicated that our design concept for probe **2** was effective for rapid detection of thiophenol.

To elucidate the sensing mechanism of probe **2** for thiophenol, ^1H NMR and MALDI-TOF MS analyses of probe **2** were performed in the presence of 40 equiv of thiophenol. As depicted in Figure S3, the ^1H NMR spectrum of the reaction mixture was identical and could be superimposed with those of **2**-PhS^-, 2,4-dinitrophenyl phenyl sulfane (DN), and thiophenol. Next, the *m*/*z* signal of probe **2** was compared in the absence and presence of thiophenol by MALDI-TOF MS analysis. This comparison showed that the *m*/*z* signal (1121.533) of probe **2** disappeared and that (789.118) of **2**-PhS^- appeared upon the addition of thiophenol (Figure S4). These observations indicated that the DNP group of probe **2** was cleaved through the S_NAr reaction with thiophenol, forming **2**-PhS^- and DN.

Photophysical Properties of Probe 2. The UV−visible absorption spectra of **1**-PhS^-, **2**-PhS^-, probes **1** and **2**, and DN in CH_3CN/H_2O (1:1 v/v, pH 7.4, 10 mM HEPES) are shown

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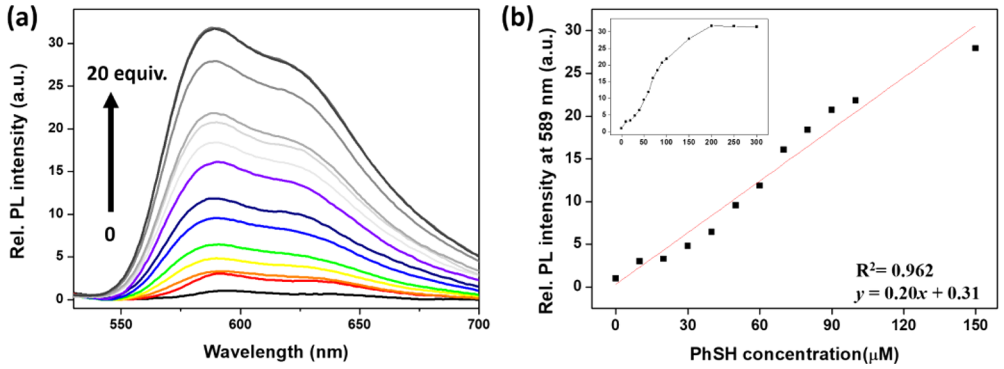

Figure 2. PL emission spectra (λ_{ex} = 450 nm) of probe **2** (10 μM) in the presence of increasing amounts of thiophenol. (CH_3CN/H_2O = 1:1 v/v, pH 7.4, 10 mM HEPES). (b) Linear plot of PL intensity at 589 nm upon addition of varying concentrations of thiophenol.

in Figure S5a. All the iridium complexes displayed intense absorption bands below 375 nm, which correspond to spin-allowed ligand-centered (^{1}LC) transitions. In addition, the weak and broad absorption bands in the range 380−550 nm were assigned to metal-to-ligand charge transfer (MLCT) transitions.[63] In the case of DN, the intense absorption band appeared around 350 nm. However, the absorption spectra of probes in the presence of 40 equiv of thiophenol did not show any noticeable changes (Figure S5b,c).

The sensitivity of probe **2** was investigated in aqueous medium (pH 7.4, 10 mM HEPES buffer/CH_3CN = 1:1 v/v) by measuring the PL intensity at 589 nm with the concentration of thiophenol in the range 0−300 μM. As shown in Figure 2a, the PL intensity of probe **2** increased significantly upon gradual addition of thiophenol until saturation was reached after addition of more than 20 equiv of thiophenol. The PL intensity showed an almost linear correlation (R^2 = 0.962) from 0 to 15 equiv of thiophenol, as depicted in Figure 2b, and thus the LOD was calculated as 39 nM [signal-to-noise (S/N) ratio = 3]. We then conducted a competitive PL analysis of probe **2** for thiophenol by adding various anions (counterion, Na^+) and biothiols. As shown in Figure 3, the addition of a large excess (80 equiv) of other analytes to probe **2** did not induce significant changes in the PL intensity. However, upon further addition of 40 equiv of thiophenol to the mixtures, intense PL signals were observed.

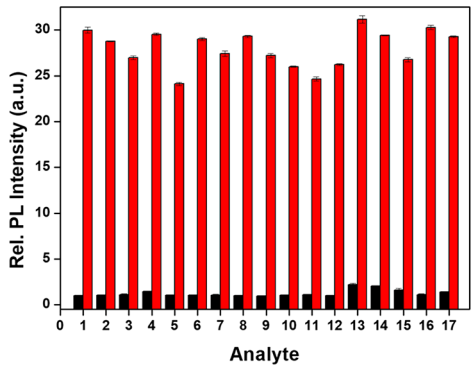

Figure 3. PL emission intensites at 589 nm (λ_{ex} = 450 nm) of probe **2** (10 μM) upon addition of each analyte (800 μM) in the absence (black bar) and presence of 400 μM thiophenol (red bar) in CH_3CN/H_2O (1:1 v/v, pH 7.4, 10 mM HEPES): (1) none, (2) CO_3^{2-}, (3) $C_2O_4^{2-}$, (4) CN^-, (5) HCO_3^-, (6) N_3^-, (7) NO_3^-, (8) OAc^-, (9) F^-, (10) Cl^-, (11) Br^-, (12) I^-, (13) SO_4^{2-}, (14) HS^-, (15) cysteine (Cys), (16) glutathione (GSH), and (17) homocysteine (Hcy).

These results indicate that probe **2** is highly selective toward thiophenol without interference from other analytes.

Electrogenerated Chemiluminescence Properties of Probe 2. ECL intensity of probe **2** was measured in aqueous medium [pH 7.4, HEPES buffer/CH_3CN = 1:1 v/v, with 0.1 M TPrA, 0.1 M HEPES, and 0.1 M tetrabutylammonium perchlorate (TBAP) as the supporting electrolyte]. During CV in the range 0−1.6 V, no change occurred in ECL intensity for probe **2**. However, there was a concentration-dependent increase in ECL intensity (up to 52-fold) until more than 20 equiv of thiophenol had been added (Figure 4a). In particular, ECL intensity at 1.4 V had an almost linear relationship (R^2 = 0.995) with the concentration of thiophenol in the range 0−200 μM (Figure 4b). Furthermore, ECL of probe **2** showed sufficiently stable maximum intensities at 1.4 V under various concentrations of thiophenol (Figure S6). The LOD was

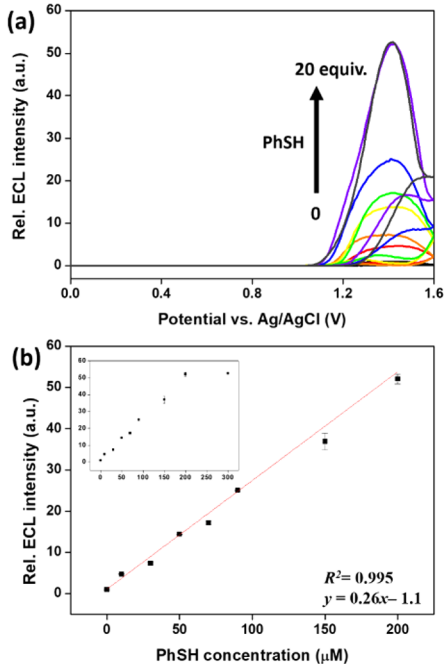

Figure 4. (a) ECL intensities of probe **2** (10 μM) upon addition of thiophenol in CH_3CN/H_2O (1:1 v/v, pH 7.4, 100 mM TPrA, 100 mM HEPES, and 0.1 M TBAP as the supporting electrolyte) while the potential is swept at a Pt disk electrode (diameter 2 mm) in the range 0−1.6 V (scan rate 0.1 V/s). (b) Linear plot of ECL intensity at 1.4 V upon addition of varying concentrations of thiophenol.

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estimated to be 3.8 nM (S/N ratio = 3), which is superior to that (39 nM) determined from the PL assay. To assess the detection sensitivity of probe **2**, we examined the changes in ECL intensity of probe **2** in the presence of low concentrations of thiophenol. As shown in Figure S7, probe **2** showed a linear increase in ECL intensity upon addition of thiophenol in the range 0−100 nM. This is the first ECL method capable of detecting thiophenol at nanomolar concentrations.

A competitive ECL analysis was carried out to evaluate the selectivity of probe **2** for thiophenol by adding various anions (counterion, Na^+) and biothiols. As depicted in Figure 5a, the addition of a large excess (80 equiv) of other analytes to probe **2** did not induce any significant changes in ECL intensity. However, upon the addition of 40 equiv of thiophenol to the mixtures, remarkable changes in ECL intensities were observed, except in the case of iodide, which is an oxidation-sensitive anion (Figure 5b).[64,65] Because competitive PL analysis revealed that there was no interference from anions, including iodide, in the detection of thiophenol, this result confirmed that the iodide anion genuinely had no effect on the PL selectivity of probe **2** but did affect the electrochemical process.

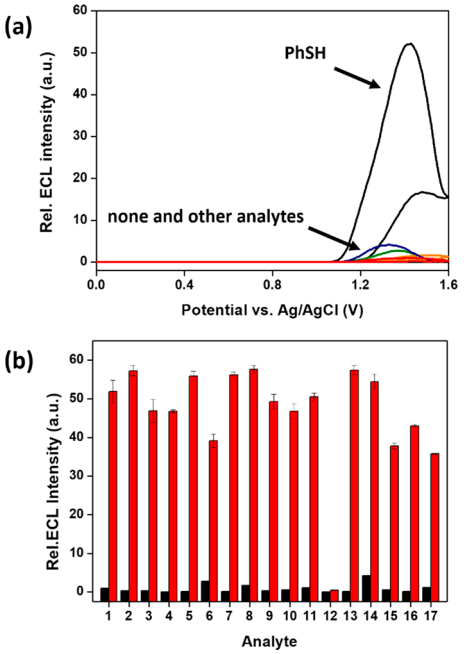

Figure 5. (a) ECL intensities of probe **2** (10 μM) in the presence of various analytes (800 μM each; thiophenol 400 μM) in CH_3CN/H_2O (1:1 v/v, pH 7.4, 0.1 M TPrA, 0.1 M HEPES, and 0.1 M TBAP as the supporting electrolyte) while the potential is swept at a Pt disk electrode (diameter 2 mm) in the range 0−1.6 V (scan rate 0.1 V/s). (b) ECL intensities at 1.4 V of probe **2** (10 μM) upon addition of 800 μM various analytes in the absence (black bar) and presence of 400 μM thiophenol (red bar): (1) none, (2) CO_3^{2-}, (3) $C_2O_4^{2-}$, (4) CN^-, (5) HCO_3^-, (6) N_3^-, (7) NO_3^-, (8) OAc^-, (9) F^-, (10) Cl^-, (11) Br^-, (12) I^-, (13) SO_4^{2-}, (14) HS^-, (15) Cys, (16) GSH, and (17) Hcy.

Electrogenerated Chemiluminescence Mechanism Studies. Density functional theory (DFT) calculations were applied to probes **1** and **2** and their reaction products (**1**-PhS^- and **2**-PhS^-) to support the photoinduced electron transfer (PET) sensing mechanism (Figure S8). The highest occupied molecular orbitals (HOMO) of probes **1** and **2** were mainly delocalized over the iridium center and phenyl group, and their lowest occupied molecular orbitals (LUMO) were localized on the DNP group, while the LUMOs of **1**-PhS^- and **2**-PhS^- were localized primarily on the isoquinoline group. This strongly suggested that the turn-on response of probes **1** and **2** relied on the PET mechanism. In addition, **2**-PhS^- had a larger HOMO−LUMO gap than **1**-PhS^- because of the strongly electron-withdrawing formyl groups on the phenyl rings of the piq ligands. These groups strongly stabilized the HOMO/LUMO levels (0.66 and 0.51 eV, respectively), increasing the HOMO−LUMO energy gap. This result also explains the blue-shifted emission maximum of **2**-PhS^- (589 nm) as compared to that of **1**-PhS^- (613 nm).

To confirm the theoretical predictions and ECL mechanism, CV and DPV were conducted to estimate the experimental HOMO/LUMO levels of **1**-PhS^- and **2**-PhS^- (Table S2). It was found that the LUMO of DNP (−4.36 eV)[17] lay between the HOMO and LUMO levels of **1**-PhS^- and **2**-PhS^-, which implied that their PL would be quenched by DNP via the PET process (Figure S9a). The HOMO/LUMO energy levels of **1**-PhS^- and **2**-PhS^- were compared with the singly occupied molecular orbital (SOMO) energy level of the TPrA radical ($TPrA^\bullet$) and the HOMO energy level of TPrA to clarify the ECL mechanism.[27] As indicated in Figure S9b, the LUMO levels of **1**-PhS^- (−3.28 eV) and **2**-PhS^- (−3.31 eV) were sufficiently lower than the SOMO level of $TPrA^\bullet$ that an electron can be smoothly transferred from $TPrA^\bullet$ to the LUMO of **1**-PhS^- and **2**-PhS^-, which produced singlet exited states in both **1**-PhS^- and **2**-PhS^-. Additionally, it was investigated whether **1**-PhS^- and **2**-PhS^- were capable of oxidizing TPrA through the catalytic pathway, which is critical for high efficiency of ECL. The HOMO level of **1**-PhS^- (−5.39 eV) was similar to that of TPrA (−5.38 eV), while the HOMO level of **2**-PhS^- (−5.88 eV) lay at a lower level than that of TPrA (Figure 6). Therefore, it was expected that **2**-PhS^- would produce $TPrA^\bullet$ more efficiently than **1**-PhS^- via a smooth catalytic process. Ir(III) complexes with a formyl group on the main ligand exhibited weaker PL intensities compared to those without a formyl group.[16,66−68] As expected, **2**-PhS^- exhibited much weaker PL intensity than **1**-PhS^-. However, the ECL intensity of **2**-PhS^- was comparable to that of **1**-PhS^-, which could be explained by

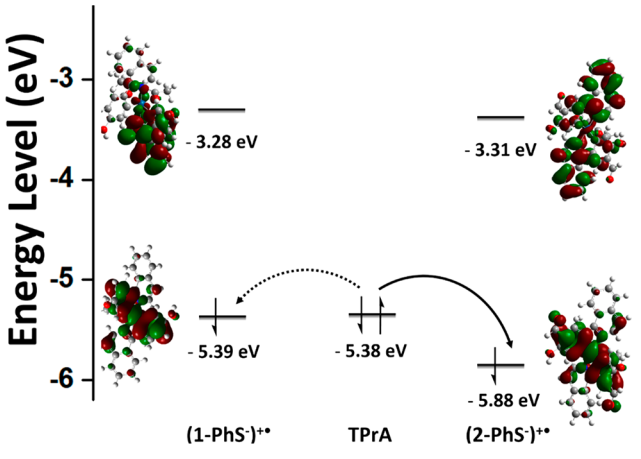

Figure 6. HOMO/LUMO energy levels, calculated from CV and DPV measurements, electronic distributions of **1**-PhS^- and **2**-PhS^-, and generation of $TPA^{+\bullet}$ through the catalytic pathway.

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the smooth catalytic process of **2**-PhS$^-$ (Figure S10). It is noteworthy that the relative maximum ECL intensities of **1**-PhS$^-$ and **2**-PhS$^-$ were about twice that of $Ru(bpy)_3^{2+}$ (where bpy = 2,2′-bipyridyl). Furthermore, ECL efficiencies of **1**-PhS$^-$ and **2**-PhS$^-$ were higher than that of $Ru(bpy)_3^{2+}$. The ECL spectrum of **2**-PhS$^-$ was similar to the PL spectrum of **2**-PhS$^-$, which indicates that the ECL signal genuinely originated from **2**-PhS$^-$ (Figure S11).

Quantification of Thiophenol in Water Samples. The ECL assay coupled with probe **2** was applied to the quantification of thiophenol in real water samples by the standard addition method. Environmental water samples were collected from the Han River in Seoul, and all ECL measurements were performed in the same manner as described. The concentration of the diluted analytical solution was determined by comparing ECL intensity with the titration curve in Figure 2. The actual sample concentrations before dilution were successfully calculated by multiplying with dilution factor 2.5 (Table 1). Probe **2** showed good recovery of thiophenol in the range 96−98%. All these results revealed that the present ECL system has great potential for field monitoring of thiophenol in real samples.

Table 1. Quantification of Thiophenol in Water Samples from the Han River[a,b]

	rel ECL intensity		
[PhSH][c] (μM)	exptl (n = 3)	calcd	recovery (%)
0	0.0031 ± 0.0002		
50	0.1174 ± 0.0021	0.1216	95.94 ± 1.72
100	0.2152 ± 0.0053	0.2218	97.12 ± 2.40
200	0.4151 ± 0.0107	0.4222	98.20 ± 2.53

[a]All ECL intensities of probe **2** (10 μM) were measured during the CV process in the range 0−1.6 V vs Ag/AgCl (scan rate 0.1 V/s). [b]Analytical solutions were prepared by diluting 0.8 mL of Han River samples containing various concentrations of thiophenol with 0.2 mL of 1.0 M HEPES buffer (pH 7.41, 1.0 M TPrA) and 1.0 mL of 0.20 M TBAP in acetonitrile. [c]Concentration of thiophenol in Han River samples.

CONCLUSIONS

In conclusion, a turn-on cyclometalated Ir(III) complex-based ECL chemodosimeter was developed for the detection of thiophenol. To the best of our knowledge, this is the first example of thiophenol detection based on ECL. Probe **2** was rationally designed to improve the reactivity toward thiophenol without loss of selectivity. The present system showed superior sensitivity and a lower LOD value (3.8 nM) compared to the values obtained by conventional PL methods. In addition, probe **2** could be successfully used for quantification of thiophenol in real water samples, which provides a new proof-of-concept for field monitoring based on ECL. It is expected that our strategy will be helpful for developing ECL-based analysis systems for the detection of other biologically and environmentally important small molecules.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b03445.

Additional text and two schemes with detailed procedures for synthesis of probes **1** and **2**; 19 figures showing UV−vis absorption spectra, additional PL spectra, MALDI-TOF mass spectra, additional ECL studies, DFT calculations, electrochemical properties, and NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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