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Journal Name

ARTICLE

Received 00th January 20xx, Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/



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Mercury, one of the most prevalent toxic metal elements, poses severe risks to human health and environment. Several molecular probes have been developed for colorimetric and fluorometric detection of the Hg(II) ion. Nevertheless, development of a rapid, selective, and sensitive probe for Hg(II) ion remains important. Herein, we report three Ir(III) complex–based phosphorescence and electrochemiluminescence chemodosimeters for the selective detection of Hg(II) ion. The acetylacetonate ancillary ligand of probe **1** reacts selectively with Hg(II) ion, inducing phosphorescence enhancement with concomitant blue–shift. Meanwhile, Hg(II) ion selectively quenches the phosphorescence of probe **2**. Probes **1** and **2** showed low detection limits (LOD) of 73 and 160 nM, respectively. In addition, the Hg(II) ion level was successfully monitored by the electrochemiluminescence decrement of probe **9** with a good linear correlation between 0 and 40 μ M, with a detection limit of 170 pM.

Introduction

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Mercury, one of the most prevalent toxic metal elements, poses severe risks to human health and environment. It easily passes through biological membranes like skin, respiratory tissues, and gastrointestinal tissues, causing brain, kidneys, and lung damage. The gradual accumulation of mercury in the human body also induces Hunter–Russell syndrome, Alzheimer's disease, and Minamata disease.¹

Concerns over Hg(II) ion toxicity have stimulated the development of efficient methods for the detection of Hg(II) ion. Various methods have been utilized to monitor Hg(II) ion levels, including ion sensing, mass spectrometry, and surface plasmon resonance.² However, most methods require expensive equipment, sophisticated and professional instrumentation, and complicated sample preparation procedures. Thus, colorimetric and fluorometric chemodosimeters and chemosensors have attracted much interest due to their advantages like rapid response, simple manipulation, and high sensitivity and selectivity. Conventional chemodosimeters possessing ethynyl or vinyl ether group have been extensively used for the selective detection of Hg(II) ion via oxymercuration reactions.³ Chemosensors based on azathia crown ether or their acyclic analogues are also well known for the detection of Hg(II) ion.⁴ Nevertheless, most of these systems have limitations like delayed response, low selectivity and sensitivity, and fluorescence quenching upon the addition of Hg(II) ion.

Therefore, construction of a selective probe with fast response to quantify Hg(II) ion in environmental samples is still highly desirable.

In this regard, a chemodosimetric approach coupled with phosphorescence and electrochemiluminescence (ECL) can provide the advantages listed above. ECL involves the generation of radical species at the electrode surface, which undergo complicated electron transfer reactions to form excited states that generate light. Hence, light emission can only be obtained with appropriate energy level differences, resulting in extremely high sensitivity due to the absence of background signal.^{5, 6}

Some reports in the 1970s showed that Hg(II) ion could form covalent bonds with the central α -carbon of acetylacetone.⁷ Therefore, we propose that acetylacetone could be developed as a recognition unit to distinguish Hg(II) ion from other metal ions. Meanwhile, Ir(III) complexes are useful phosphorescent dyes and possess several advantages, including relatively long emissive lifetime, high photoluminescence efficiency and quantum yields, flexibility in colour tuning and thermal stability.⁸ For this reason, a lot of Ir(III) complex-based sensors have been published in literatures.⁹ Moreover, Ir(III) complexes have been used as ECL dyes due to their good radical stability.¹⁰ Thus, we report acetylacetonato Ir(III) complexes for the selective detection of Hg(II) ion via phosphorescence and electrochemiluminescence dual techniques and the mechanism for its Hg(II) ion sensing via ¹H NMR and mass spectra.

Experimental

Synthetic procedures

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

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The probes **1**, **2** and **9** were synthesized as shown in Scheme 1. First, the main ligands 2–phenylpyridine, 2–(4– acetylphenyl)pyridine) and 1–phenylisoquinoline were either purchased or synthesized by conventional Suzuki cross– coupling reaction. The corresponding Ir(III) complex dimers were synthesized in refluxing 2–ethoxyethanol. Then ancillary ligands were attached in the presence of a suitable base and the final products were purified by silica gel column chromatography. All the synthesized compounds were fully characterized by ¹H NMR and ¹³C NMR (ESI).

Materials and methods

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All the reagents were purchased from Sigma–Aldrich Corp., Tokyo Chemical Industry, and Acros Organics and were used without further purification. All metal ion sources were in nitrate forms with the highest purity available. Analytical thin layer chromatography was performed using Merck silica gel 60 F254 on aluminium foil. SiliaFlash[®] P60 (230–400 mesh) from SILICYCLE was used as the stationary phase in chromatographic separation. All the ¹H and ¹³C NMR spectra were obtained using Bruker Avance DPX–300 or Agilent 400–MR DD2 Magnetic Resonance System. Chemical shifts (δ) were reported in ppm (chloroform = CDCl₃, acetonitrile = CD₃CN or dimethyl sulfoxide = DMSO–d₆).

Absorption spectra were recorded on a Beckman DU 800 Series spectrometer. Fluorescence emission spectra were recorded on a JASCO FP–6500 spectrometer. The probes **1**, **2** and **9** solutions for all the photophysical experiments were prepared from 2 mM stock solution in DMSO and stored in a refrigerator.

Electrochemical and electrochemiluminescence studies

The electrochemical study was performed with a CH Instruments 650B Electrochemical Analyzer (CH Instruments, Inc., TX, USA). ECL spectra were gathered using a chargecoupled device (CCD) camera (LN/CCD 1752-PB/VSAR, Princeton Instruments, NJ, USA) with temperature maintained below -120 °C using liquid N2. The ECL intensity profile was obtained using a low-voltage photomultiplier tube (PMT) module (H-6780, Hamamatsu photonics K. K., Tokyo, Japan) operated at 1.4 V. A 250 μ L–sized ECL cell was directly mounted on the CCD or PMT module with home-made support mounts during the experiments. All the ECL data were collected via simultaneous cyclic voltammetry (CV). The ECL solutions commonly contained tri(*n*-propyl)amine (TPA, Sigma-Aldrich, MO, USA) as a coreactant and 0.1 M tetrabutylammonium perchlorate (TBAP, TCI) as a supporting electrolyte in acetonitrile (CH₃CN, spectroscopy grade, ACROS). TPA was selected as it has been widely studied and is known for its useful electrochemical properties. The ECL measurements were carried out under ambient conditions. The electrochemical measurements were referenced with respect to a Ag/Ag⁺ reference electrode in organic solvents, or to a Ag/AgCl reference electrode in aqueous media. Especially, the potential values measured under organic conditions were calibrated against the saturated calomel electrode (SCE) using ferrocene

as an internal reference (E°(F_c⁺/F_c) = 0.424 V v_{Se}SCE)_e An At working electrode was polished with 0.05 9M aluminas (Buenler, IL, USA) on a felt pad followed by sonication in 1:1 mixed solution of deionized water and absolute ethanol for 5 min. The working electrode was dried by ultra-pure N₂ gas for 1 min. None of the solutions were reused. The reported ECL values were obtained by averaging the values of at least three repeated experiments. Electrochemical oxidative and reductive behaviours were investigated by applying cyclic voltammetry to individual solutions.

Procedures for synthesis of the Ir(III) complexes

Synthesis of 6. 4-Acetylphenyl boronic acid (892 mg, 5.44 mmol), 2-bromopyridine (660 mg, 4.18 mmol), tetrakis(triphenyl phosphine)palladium (144 mg, 0.125 mmol) and K₂CO₃ (1733 mg, 12.5 mmol) were dissolved in THF (15 mL) and H₂O (15 mL). The mixture was refluxed at 80 °C for 5 h and was allowed to cool to room temperature. The residue was extracted with CH₂Cl₂ and the organic layer was dried over anhydrous Na₂SO₄, filtered, and evaporated under reduced pressure to obtain the crude product. It was then purified by silica gel column chromatography with hexane and ethyl acetate. The product was obtained as a white solid with an isolated yield of 72% (580 mg).

All the characterization data are identical to previous reports.

Synthesis of 7. Compound 6 (310.4 mg, 1.58 mmol) and Ir(III) chloride hydrate (189 mg, 0.63 mmol) were dissolved in 2–ethoxyethanol (9 mL) and H₂O (3 mL). The mixture was refluxed for 24 h and cooled to room temperature. Then water (50 mL) was added and the resulting reddish orange precipitate was filtered to give a crude cyclometalated Ir(III) chlorobridged dimer with an isolated yield of 49% (152.5 mg).

Synthesis of 1.11 Compound 7 (96.5 mg, 0.08 mmol), acetylacetone (39.4 mg, 0.39 mmol) and Na₂CO₃ (41.3 mg, 0.39 mmol) were dissolved in 2-ethoxyethanol (1 mL) in a roundbottom flask. The mixture was heated and stirred at 50 °C for 1 h. The reaction mixture was then cooled down to room temperature and extracted twice with CH₂Cl₂. The combined organic layer was dried over anhydrous Na₂SO₄ and filtered. Volatiles were removed under reduced pressure. The residue was purified by silica gel column chromatography with dichloromethane and methanol. The product was obtained following the addition of ether and the red orange solid was filtered with an isolated yield of 80% (87.5 mg). ¹H NMR (300 MHz, DMSO- d_6 , δ): 8.50 (d, J = 5.5 Hz, 2H), 8.31 (d, J = 8.0 Hz, 2H), 8.06 (t, J = 7.7 Hz, 2H), 7.85 (d, J = 8.1 Hz, 2H), 7.53 (t, J = 6.5 Hz, 2H), 7.38 (d, J = 8.0 Hz, 2H), 6.61 (s, 2H), 5.29 (s, 1H), 2.26 (s, 6H), 1.74 (s, 6H); ¹³C NMR (75 MHz, DMSO-d₆, δ): 198.37, 184.77, 166.53, 150.64, 148.64, 147.13, 138.91, 136.18, 131.57, 124.37, 124.28, 121.98, 120.83, 100.91, 28.65, 26.86; HRMS (FAB) m/z: [M]⁺ calc. for C₃₁H₂₇IrN₂O₄ 684.1600, found 684.1602.

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Scheme 1. a) Pd(PPh₃)₄, K₂CO₃, THF, H₂O, reflux; b) IrCl₃·xH₂O, 2–ethoxyethanol, H₂O, reflux; c) acetylacetone, Na₂CO₃, 2–ethoxyethanol, 50 °C; d) picolinic acid, Na₂CO₃, 2–ethoxyethanol, 50 °C; e) AgBF₄, acetonitrile, reflux. (THF = tetrahydrofuran)

Synthesis of 3.¹² Compound 7 (264.1 mg, 0.16 mmol), picolinic acid (60 mg, 0.49 mmol) and Na₂CO₃ (52 mg, 0.49 mmol) were dissolved in 2–ethoxyethanol (3 mL) in a round–bottom flask. The mixture was heated and stirred at reflux for 24 h. The reaction mixture was then cooled down to room temperature, and extracted with CH₂Cl₂. The organic layer was dried over anhydrous Na₂SO₄ and filtered. The residue was purified by silica gel column chromatography with dichloromethane and methanol. The product was obtained after ether was added and the orange solid was filtered with an isolated yield of 83% (188.5 mg). ¹H NMR (300 MHz, DMSO–*d*₆, δ): 8.60 (d, J = 5.5 Hz, 1H), 8.39 (t, J = 7.0 Hz, 2H), 8.16–8.10 (m, 2H), 8.05 (d, J = 8.4 Hz, 2H),

8.01–7.93 (m, 2H), 7.71–7.60(m, 3H), 7.53 (t, J = 6.1 Hz, 2H), 7.46 (dd, J = 8.1, 1.4 Hz, 1H), 7.37 (t, J = 6.5 Hz, 1H), 6.78 (s, 1H), 6.59 (d, J = 1.3 Hz, 1H), 2.32 (s, 3H), 2.26 (s, 3H); ¹³C NMR (75 MHz, DMSO– d_6 , δ): 198.35, 198.32, 172.17, 166.76, 165.98, 151.32, 149.98, 149.60, 149.54, 149.33, 148.77, 148.47, 146.99, 139.44, 139.10, 139.10, 137.14, 136.67, 131.16, 130.70, 129.69, 128.17, 125.17, 124.85, 124.82, 124.54, 122.59, 122.56, 121.27, 121.23, 26.96, 26.88; HRMS (FAB) m/z: [M]⁺ calc. for C₃₂H₂₄IrN₃O₄ 707.1396, found 707.1398.

Synthesis of 5. A solution of dichlorobridged Ir dimer **7** (65 mg, 0.04 mmol) and silver tetrafluoroborate (19 mg, 0.1 mmol) in

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acetonitrile was refluxed for 12 h under nitrogen atmosphere. The AgCl formed as a by–product was removed through Celite[®] filtration. The resulting solution was evaporated then chromatographed over silica gel with acetonitrile as an eluent to yield **5**. Yellow orange: Yield = 80% (42 mg) ¹H NMR (400 MHz, CD₃CN, δ): 9.18 (m, 1H), 8.16 (m, 2H), 7.76 (d, J = 8.1 Hz, 1H), 7.60 (m, 1H), 7.47 (dd, J = 8.1, 1.7 Hz, 1H), 6.57 (d, J = 1.5 Hz, 1H), 2.25 (d, J = 3.4 Hz, 6H); ¹³C NMR (75 MHz, CD₃CN, δ): 197.86, 165.57, 151.46, 149.25, 142.95, 139.43, 136.99, 129.81, 124.85, 124.16, 123.39, 121.02, 119.87, 25.79, 0.77; HRMS (FAB) m/z: [M – BF₄⁻ – 2CH₃CN]⁺ calc. for C₂₆H₂₀IrN₂O₂ 585.1154, found 585.1155.

Synthesis of 8. Compound **8** was synthesized analogously to **7** using phenylpyridine (271.6 mg, 1.75 mmol) instead of **6** and Ir(III) chloride hydrate (200 mg, 0.7 mmol). Yield = 67% (250 mg)

Synthesis of 2.¹³ Compound **2** was synthesized analogously to **1** using compound **8** (150 mg, 0.14 mmol) instead of **7** and acetylacetone (42 mg, 0.4 mmol). Yellow solid: Yield = 82% (138 mg) ¹H NMR (300 MHz, DMSO– d_6 , δ): 8.48 (d, J = 5.2 Hz, 2H), 8.13 (d, J = 8.0 Hz, 2H), 7.93 (dd, J = 11.1, 4.3 Hz, 2H), 7.68 (d, J = 7.5 Hz, 2H), 7.39 (t, J = 6.5 Hz, 2H), 6.77 (t, J = 7.1 Hz, 2H), 6.60 (t, J = 7.3 Hz, 2H), 6.07 (d, J = 7.4 Hz, 2H), 5.26 (s, 1H), 1.72 (s, 6H); ¹³C NMR (75 MHz, DMSO– d_6 , δ): 184.43, 168.02, 148.23, 147.86, 145.54, 138.36, 133.07, 128.80, 124.43, 122.92, 120.78, 119.33, 100.78, 28.70; HRMS (FAB) m/z: [M]⁺ calc. for C₂₇H₂₃IrN₂O₂ 600.1389, found 600.1390.

Synthesis of 4.¹⁴ Compound **4** was synthesized analogously to **3** using compound **8** (30 mg, 0.03 mmol) instead of **7** and picolinic acid (10 mg, 0.08 mmol). Yellow solid: Yield = 80% (29 mg) ¹H NMR (300 MHz, DMSO– d_6 , δ): 8.53 (d, J = 5.4 Hz, 1H), 8.20 (t, J = 8.0 Hz, 2H), 8.15–8.04 (m, 2H), 7.96–7.86 (m, 2H), 7.81 (t, J = 8.2 Hz, 2H), 7.66 (d, J = 4.8 Hz, 1H), 7.58 (dd, J = 13.4, 5.8 Hz, 2H), 7.37 (t, J = 6.3 Hz, 1H), 7.21 (t, J = 6.3 Hz, 1H), 6.94–6.82 (m, 2H), 6.78 (t, J = 7.4 Hz, 1H), 6.71 (t, J = 7.3 Hz, 1H), 6.26 (d, J = 7.4 Hz, 1H), 6.07 (d, J = 7.3 Hz, H); ¹³C NMR (75 MHz, DMSO– d_6 , δ): 172.17, 168.27, 167.36, 151.55, 150.40, 148.75, 148.52, 148.10, 147.78, 145.06, 144.53, 139.15, 138.58, 138.58, 132.45, 132.25, 130.12, 129.44, 129.44, 128.03, 125.29, 124.69, 123.76, 123.43, 121.63, 121.38, 119.82, 119.74; HRMS (FAB) m/z: [M]⁺ calc. for C₂₈H₂₀IrN₃O₂ 623.1185, found 623.1186.

Synthesis of 10. Compound **10** was synthesized analogously to **6** using 1–chloroisoquinoline (1000 mg, 6.1 mmol) and boronic acid (964 mg, 7.9 mmol). White solid: Yield = 93% (1170 mg)

Synthesis of 11. Compound **11** was synthesized analogously to **7** using phenylisoquinoline (1170 mg, 5.7 mmol) instead of **6** and Ir(III) chloride hydrate (682 mg, 2.28 mmol). Yield = 70 % (1035 mg)

Synthesis of 9.¹⁵ Compound 9 was synthesized analogously to 1 using compound 11 (500 mg, 0.38 mmol) instead of 7 and acetylacetone (115 mg, 1.15 mmol). Red solid: Yield = 78% (440 mg) ¹H NMR (400 MHz, DMSO– d_6 , δ): 8.95 (d, J = 8.0 Hz, 2H),

8.34 (d, J = 6.4 Hz, 2H), 8.19 (d, J = 8.0 Hz, 2H), 8,14 $_{A}$ (Q) (m, 2H), 7.82 (m, 6H), 6.84 (t, J = 7.1 Hz, 2H), 6.56 (t, 9.39/9.20H2), 2H), 6.12 (d, J = 7.1 Hz, 2H), 5.25 (s, 1H), 1.67 (s, 6H); ¹³C NMR could not be obtained because of poor solubility; HRMS (FAB) m/z: [M]⁺ calc. for C₃₅H₂₇IrN₂O₂ 700.1702, found 700.1704.

Results and discussion

Spectroscopic property

The photophysical properties of the probes were investigated and using UV–Vis absorption photoluminescence spectroscopies, including titration studies with Hg(II) ion (Fig. S1 and Fig. 1). As shown in Fig. 1, the phosphorescence intensity of the probe **1** (10 μ M in acetonitrile/water = 9/1, λ_{ex} = 400) increased gradually with concomitant blue shift from 600 nm to 521/550 nm. The enhancement reached a plateau after the addition of 3 equivalents of Hg(II) ion. It is worth noting that the spectroscopic change occurred almost instantaneously. We also found that the phosphorescence changed in a stepwise manner. The ratio of the intensity at 521 nm to that at 600 nm (I_{521}/I_{600}) showed only a small change when $3-9 \mu M$ of the Hg(II) ion was added, followed by strong enhancement in phosphorescence upon varying Hg(II) concentration between 9 and 30 µM. This



Figure 1. (a) Titration curves of **1** (10 μ M) in the presence of various amounts of Hg²⁺ ion (0–30 μ M, instant) in CH₃CN/water (9/1). (b) Phosphorescence intensity changes of **1** (10 μ M) upon the addition of Hg²⁺ ion (0–100 μ M). LOD = 73 nM. Inset: Plot of I₅₂₁/I₆₀₀ vs [Hg²⁺] (9–30 μ M) showing the linear relationship.

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Figure 2. (a) Titration curves of **2** (10 μ M) in the presence of various amounts of Hg²⁺ ion (0–40 μ M, instant) in CH₃CN/water (9/1). (b) Phosphorescence intensity changes of **2** (10 μ M) upon the addition of Hg²⁺ ion (0–40 μ M). LOD = 160 nM. Inset: Plot of I₅₂₅ vs [Hg²⁺] (12–40 μ M) showing the linear relationship.

phenomenon might indicate a 2:1 stoichiometry of the reaction between probe **1** and Hg(II) ion (*vide infra*). A good linear relationship between the phosphorescence intensity ratio and the Hg(II) ion concentration was observed in the stiff–enhancing region (9–30 μ M), which had a higher coefficient of determination (R²) value than that of the probe **2**. The estimated limit of detection (LOD) was 73 nM.

Meanwhile, the phosphorescence intensity of **2** (10 μ M in acetonitrile/water = 9/1, λ_{ex} = 400) decreased at 525 nm until 4 equivalents of Hg(II) ion were added (Fig. 2). **2** also showed relatively small changes in the phosphorescence intensity when 3–9 μ M of Hg(II) ion was added, and the phosphorescence decreased gradually after the addition of 12–40 μ M of Hg(II) ion (Fig. 2). A 35–fold decrement in the phosphorescence intensity of **2** indicates a LOD of 160 nM. Again, the spectral changes were found to be instantaneous after the addition of Hg(II) ion.

The binding ratio between the probe and Hg(II) ion was confirmed by the Job's plot (Fig. S2), which exhibited maximum phosphorescence intensity near a molar fraction of 0.33. This implies 2:1 binding stoichiometry of the probe to Hg(II) ion, suggesting that a Hg(II) ion bridges two ancillary acetylacetonate ligands of **2** (*vide infra*).

To further evaluate the interference of other metale ions, competition assay was performed (FigD3).19 Here ions, competing ions (50 μ M) such as Ag⁺, Al³⁺, Ca²⁺, Cd²⁺, Cd²⁺, Cu²⁺, Fe²⁺, K⁺, Mg²⁺, Mn²⁺, Na⁺, Ni²⁺, Pb²⁺, and Zn²⁺ resulted in small changes in the phosphorescence of 1 and 2. This indicates that these chemodosimeters display high selectivity toward Hg(II) ion. In the presence of competing ions, additional treatment of 1 and 2 with 50 μ M of Hg(II) ion led to an increase and decrease in the phosphorescence intensity, respectively, implying that other cations cannot interfere with the reaction between 1 (or 2) and Hg(II) ion.

Mass analysis

We then carried out ESI–MS and MALDI–TOF MS analysis to confirm the product formed following the addition of Hg(II) ion (Fig. S3–S6). Before adding Hg(II) ion, molecular ion peaks of **1** and **2** appeared at 684.218 and 600.116 (m/z), respectively. After the addition of a small amount of Hg(II) ion (0.5 equivalent) the molecular ion peak completely disappeared. In the case of **2**, the major peak appeared at 501.093 (m/z) which corresponds to (ppy)₂Ir⁺. In addition, the minor peaks were shown at 542.120 (m/z), representing (ppy)₂Ir⁺(CH₃CN). Mass spectral data of **1** was similar to that of **2**. The major peak appeared at 585.114 (m/z) which corresponds to (Acppy)₂Ir⁺ (Ac



Selectivity



Figure 4. Phosphorescence intensity changes of (a) 3 (10 μM) and (b) 4 (10 μM) upon the addition of Hg^{2+} ion (0–100 $\mu M).$

= acetyl) and the additional minor peak at 626.141 (m/z), which represents (Acppy)₂Ir⁺(CH₃CN).

Control experiments

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Figure 5. Normalized intensity of 1 upon the addition of 50 μ M of Hg²⁺ ion and 5.

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even after the addition of 10 equivalents of Hg(II) ion (Fig. 4). These results suggested that the acetylacetohate3 Mgand Would be associated with the reaction with Hg(II) ion.

Furthermore, we synthesized $(Acppy)_2 Ir(CH_3 CN)_2$ (5) from the dimer form of Acppy (vide supra). As shown in Fig. 5, the phosphorescence spectrum was almost identical to that of 1 treated with Hg(II) ion. This indicates that the reaction of 1 with Hg(II) ion results in the decomposition to (Acppy)₂Ir(CH₃CN)₂, and this decomposition is a key process of the sensing mechanism. As well, the phosphorescence of $2 + 50 \mu M Hg(II)$ ions in CH₃CN is the same as that of (ppy)₂Ir(CH₃CN)₂ (Fig. S7), which indicates that 2 reacts with Hg(II) ions in CH₃CN to generate (ppy)₂Ir(CH₃CN)₂ (see also Scheme 2).

Crystal Structure

No crystal structure determination of Ir(III) complex reacted with Hg(II) ion has been published to date. As shown in Fig. 6, the structure of (ppy)₂ Ir (CH₃CN)₂⁺ complex was unambiguously



Figure 7. Comparison of NMR spectra of 1, 1 + 5 equivalents of Hg²⁺ ion and 5. For detailed integration values of 1 and 5, see Fig. S14 and Fig. S22. O: acetonitrile (solvent), \square : water, \triangle : H_a, \diamond : H_b, \Leftrightarrow : acetyl–H, x: CH₃CN (Ir coordinated)



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Scheme 2. The expected sensing mechanism of 1 for Hg²⁺ ion.



Figure 9. ECL responses of 9 (10 $\mu M)$ in the presence of 50 μM of Hg^{2+} ion and various metal ions in CH_3CN/water (9/1).

revealed on the basis of a single–crystal X–ray diffraction analysis. It is definite that acetylacetonate of the Ir(III) complex was detached immediately following the addition of Hg(II) ion, resulting in the formation of acetylacetone–Hg(II) complex.¹⁶



Figure 8. ECL intensity of 10 μ M of **9** upon the addition of Hg²⁺ ion (0–100 μ M) in CH₃CN/water (9/1) (30 mM TPA, and 0.1 M TBAP as a supporting electrolyte). The potential was swept at a Pt disk electrode (diameter: 2 mm) over the range 0–1.4 V vs Ag/AgCl (scan rate: 0.1 V/s). Inset: Plot of ECL intensity vs [Hg²⁺] (0–40 μ M) showing the linear relationship.



Figure 6. Single–crystal X–ray structure of **2** after the addition of 0.5 equivalent of Hg^{2+} ion (thermal ellipsoids are drawn at 50% probability level). Colour code: C, black; N, blue; H, white; Ir, dark blue.

NMR study

In order to gain deeper understanding about the sensing mechanism, NMR experiments in CD₃CN were performed. As shown in Fig. 7, H_a proton of **1** at 5.3 ppm disappeared, whereas H_b protons at 1.76 ppm shifted downfield. In addition, the resonance peaks of **1** following the addition of Hg(II) ions were very similar to those of probe **5**, which was also shown in the control experiments (Fig. 5). The NMR spectra of **2** showed a similar pattern change after the addition of Hg(II) ions (Fig. S8). In this case, the shifted H_b protons (δ = 2.3) appeared to be similar to those of the mixture of acetylacetone and Hg(II) ion (Fig. S9).

The Job's plot for **1** and Hg(II) ion (Fig. S2) reveals that a Hg(II) ion tends to coordinate with two acetylacetonate ligands. For example, a single crystal structure of Hg(II) coordinating with two molecules of 2,2,6,6–tetramethyl–3,5–heptanedione has been reported.^{7, 17} Therefore, we propose a two–step sensing mechanism. In the first step, acetylacetonate readily reacts with Hg(II) ion, inevitably forming a tetrahedral carbon centre and a β –diketone structure, which has weaker coordinating ability. Then, the β –diketone rapidly dissociates from the Ir(III) complex to generate a cationic Ir(III) complex as shown in Scheme 2.¹⁸

ECL property

We further designed and synthesized an Ir(III) complex possessing 1–phenylisoquinoline (piq) groups as main ligands (9). This complex is well known for exhibiting better ECL property than Ir(III) complexes possessing 2–phenylpyridine (ppy) groups as main ligands. This phenomenon is due to the difference of LUMO energy levels, which should be lower than the LUMO of TPA radical for efficient electron transfer. Isoquinoline, which is a stronger electron withdrawing group than pyridine would stabilize LUMO level, resulting in better electron transfer.¹⁹ As expected, among three Ir(III) complexes (**1**, **2**, **9**), the probe **9** showed the highest ECL intensity and turn–

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off ratio (Fig. S10). Therefore, further ECL experiments were mainly conducted with **9**.

ECL measurements were performed in an acetonitrile/water solution mixture (9:1 ratio) with 10 μ M **9** and 30 mM TPA with 0.1 M TBAP as a supporting electrolyte. As shown in Fig. 8, the ECL intensity decreased gradually until the concentration of Hg(II) ion reached 40 μ M. A good linear relationship was observed over the range of 0–40 μ M and the estimated limit of detection (LOD) was 170 pM (signal–to–noise (S/N) ratio=3, n=3). **1** and **2** also showed the decrement of ECL intensity until 80 μ M of Hg(II) ion was added (see Fig. S11 and Fig. S12). In this case, twice the amount of Hg(II) ion was needed for saturation compared to **9**. Interestingly, the ECL intensity of **1** decreased exponentially upon the addition of Hg(II) ion. The estimated limit of detection was 1.9 nM and 0.78 nM for **1** and **2**, respectively, which appear to be much smaller than the LOD determined by photoluminescence.

Interestingly, all probes showed ECL quenching in the presence of Hg(II) ion. This phenomenon might be inevitable as the ECL process can occur only when proper oxidation is allowed. However, the results of CV showed that after the addition of Hg(II) ion, no significant oxidation peaks were observed. Therefore, we can conclude that the proper oxidation does not occur following the addition of Hg(II) ion which is manifested as a quenching of the ECL intensity (Fig. S13).

A selective binding assay of **9** was carried out as shown in Fig. 9. The ECL intensity certainly decreased only in the presence of Hg(II) ion. The addition of other metal ions (50 μ M each), such as Ag⁺, Al³⁺, Ca²⁺, Cd²⁺, Co²⁺, Cu²⁺, Fe²⁺, K⁺, Mg²⁺, Mn²⁺, Na⁺, Ni²⁺, Pb²⁺, and Zn²⁺, caused relatively small changes and the ECL intensities remained high.

Conclusions

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We demonstrated the first example, to the best of our knowledge, of a phosphorescence and electrogenerated chemiluminescence dual-mode chemodosimeters for Hg(II) ion detection. In addition, we demonstrated the sensing mechanism based on the selective reaction of Hg(II) ion with the acetylacetonate ancillary ligand of Ir(III) complexes. We assert that this strategy could emerge as a general tool for the selective detection of Hg(II) ion.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the National Research Foundation (Grant No. 2015M3A6A4076701) funded by the MSIP.

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Acetylacetonato Ir(III) complexes showed turn-on, turn-off, or ratiometric responses in both phosphorescence and electrochemiluminescence channels.