

New 7-Hydroxycoumarin-Based Fluorescent Chemosensors for Zn(II) and Cd(II)

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Five new 4- or 8-substituted-7-hydroxycoumarin derivatives (**1-5**) were synthesized as fluorescent sensors for metal ions. Fluorescent changes and selectivity for metal ions were compared based on the introduction of different ligands and/or testing with different substitution positions of 7-hydroxycoumarin in aqueous solution. Especially, probes **2**, **3** and **5** displayed large fluorescence enhancements with Zn²⁺ and Cd²⁺. Probes **2** and **3** showed moderate selectivity for Zn²⁺ over Cd²⁺. On the other hand, probe **4** showed large fluorescence quenching effects upon the addition of Ag⁺ and Hg²⁺.

Key Words: Fluorescent chemosensors, Coumarin, Zn²⁺ sensor, Cd²⁺ sensor, Fluorescence

Introduction

Fluorescent sensors are powerful tools to monitor ions because of their simplicity and high sensitivity of fluorescence.¹ The development of fluorescent sensors for transition metal ions has received considerable attention, because these metal ions are essential trace elements in biological systems and also significant environmental pollutants.² Among these metal ions, Zn²⁺ is involved in a variety of physiological and pathological processes, such as Alzheimer's disease, epilepsy, ischemic stroke and infantile diarrhea.³ It is also reported that zinc ion is a potent killer of neurons via oxidative stress.⁴ Accordingly, the development of a fluorescent probe for zinc ion in the presence of a variety of other metal ions has received great attention.^{5,6}

Coumarin derivatives have been actively utilized as fluorescent chemosensors for various analytes, including Zn²⁺.^{7,8} For example, Kikuchi *et al.* recently introduced di-(2-picolyl)amine (DPA) and *N,N*-dipicolylethylenediamine at the 8-position of 7-hydroxycoumarins and reported them as Zn²⁺ selective probes.^{8a} Brückner *et al.* reported 7-hydroxycoumarin derivatives bearing DPA ligand at the 3-position and also utilized it as a Zn²⁺ probe.^{8b} A coumarin-based fluorogenic probe bearing the 2-picolyl unit was recently reported as a Cu²⁺ selective fluorescent probe by Kim *et al.*^{8k}

In a continuation of our work on fluorescent chemosensors,⁹ we have synthesized five new 7-hydroxycoumarin derivatives (Figure 1). For **1** and **2**, an iminodiacetic acid diethyl ester group was introduced at the 4- or 8-positions for the comparison of their selectivity and fluorescent changes towards metal ions. Iminodiacetic acid was introduced at the 4-position of 7-hydroxycoumarin in the case of **3**. On the other hand, sulfur containing ligand was employed on the 8-position for **4**, which indeed showed different selectivity for metal ions. Coumarin derivative **5** bears DPA units, respectively. Among the metal ions examined, derivatives **2**, **3** and **5** displayed large fluorescence enhancements with Zn²⁺. To different extents, Cd²⁺ competed with Zn²⁺, however, the cellular concentration of the toxic Cd²⁺ ion is ex-

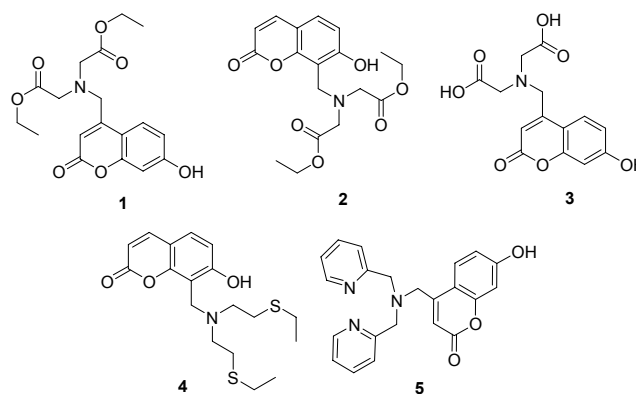


Figure 1. Structures of compound 1-5.

pected to be low. Even though there have been intensive studies on coumarins, we successfully demonstrated that ligand variation, along with alteration of position for the substitution on 7-hydroxycoumarin, can provide different selectivities and sensitivities for metal ions at pH 7.4.

Experimental Details

Materials and methods. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Flash chromatography was carried out on silica gel 60 (230 - 400 mesh ASTM; Merck). ¹H NMR and ¹³C NMR spectra were recorded using Bruker 250 MHz or Varian 500 MHz. Chemical shifts were given in ppm and coupling constants (*J*) in Hz. Fluorescence emission spectra were obtained using RF-5301/PC Spectrofluorophotometer (Shimadzu).

Preparation of fluorometric metal ion titration solutions. Stock solutions (0.01 M) of the perchlorate salts of Ag⁺, Ca²⁺, Cd²⁺, Co²⁺, Cu²⁺, Hg²⁺, K⁺, Li⁺, Mg²⁺, Mn²⁺, Na⁺, Ni²⁺, Pb²⁺, Zn²⁺ ions in distilled water were prepared. Stock solutions of host (0.6 mM) in DMSO were also prepared. Test solutions were prepared by placing 30 L of the probe stock solution into a test tube,

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adding an appropriate aliquot of each metal stock, and diluting the solution to 3 mL with 0.02 M HEPES (pH 7.4).

Synthesis of compound 1. 4-Chloromethyl-7-hydroxycoumarin **6** (1 g, 4.8 mmol), iminodiacetic acid diethyl ester (1.08 g, 5.7 mmol), triethylamine (0.72 g, 0.99 mL, 7.1 mmol) and the catalytic amount of KI were refluxed in CH₂Cl₂ (50 mL) for 14 h. After cooling to ambient temperature, the reaction mixture was washed with water (60 mL) and dried over Na₂SO₄. Then solvent was evaporated under vacuum. Crude product was purified on silica column using hexane-ethyl acetate (6:4) mixture as eluent. The second fraction of the column came out as a right product (0.5 g, 29%). mp 141 °C; ¹H NMR (CDCl₃, 250 MHz) δ 7.95 (d, 1H, *J* = 8.7 Hz), 6.92 (d, 1H, *J* = 2.4 Hz), 6.89 (dd, 1H, *J* = 8.7 Hz, *J* = 2.4 Hz), 6.40 (s, 1H), 4.22 (q, 4H, *J* = 7.1 Hz), 4.10 (s, 2H), 3.59 (s, 4H), 1.30 (t, 6H, *J* = 7.1 Hz); ¹³C NMR (CDCl₃, 62.5 MHz) δ 171.0, 162.3, 160.9, 155.4, 153.0, 126.6, 113.3, 111.8, 111.5, 103.1, 61.0, 54.8, 50.0, 14.2; HRMS(FAB) *m/z* 363.1315 (M+H)⁺, calc. for C₁₈H₂₁NO₇ = 363.1318.

Synthesis of compound 2. To a solution of the iminodiacetic acid diethyl ester (0.58 g, 3.1 mmol) in 30 mL of acetonitrile was added aqueous formaldehyde (37%) (0.27 mL, 3.1 mmol). After 0.5 h of gentle refluxing at 60 °C, the 7-hydroxycoumarin **7** (0.5 g, 3.1 mmol) in 30 mL of acetonitrile was added. The reaction was refluxed for 12 hr and solvents were removed under reduced pressure. The residue was purified by silica column using methylene chloride as eluent. The first fraction of the column came out as a white solid product (0.2 g, 18%). mp 120 °C; ¹H NMR (CDCl₃, 250 MHz) δ 10.7 (hydroxyl group, 1H), 7.56 (d, 1H, *J* = 9.5 Hz), 7.21 (d, 1H, *J* = 4.3 Hz), 6.78 (d, 1H, *J* = 8.5 Hz), 6.13 (d, 1H, *J* = 9.4 Hz), 4.20 ~ 4.12 (m, 6H), 3.47 (s, 4H), 1.26-1.18 (m, 10H); ¹³C NMR (CDCl₃, 62.5 Hz) δ 170.6, 161.8, 160.9, 153.6, 144.1, 128.5, 114.2, 111.8, 111.5, 108.4, 61.4, 54.5, 47.9, 14.1; HRMS(FAB) *m/z* 363.1320 (M+H)⁺, calc. for C₁₈H₂₁NO₇ = 363.1318.

Synthesis of compound 3. 4-Chloromethyl-7-hydroxycoumarin **6** (0.5 g, 2.37 mmol) in 20 mL of acetonitrile was added to the mixture of iminodiacetic acid (0.315 g, 2.37 mmol), sodium bicarbonate (0.6 g, 7.12 mmol in 10 mL of water and the catalytic amount of KI. The reaction mixture was stirred at room temperature overnight. Another 30 mL of water was added and the resulting mixture was washed with ethyl acetate (3 × 50 mL) to remove the unreacted starting material. The aqueous residue was evaporated to dryness under vacuum. The light yellow crude product was purified on silica column using ethyl acetate-methanol (7:3) as eluent. Evaporation of the solvents gave 0.2 g (28%) of cream colored solid. mp (decomposition) > 116 °C; ¹H NMR (CD₃OD, 250 MHz) δ 7.80 (d, 1H, *J* = 8.8 Hz), 7.00 (dd, 1H, *J* = 8.8 Hz & *J* = 2.4 Hz), 6.58 (d, 1H, *J* = 2.4 Hz), 6.26 (s, 1H), 4.05 (s, 2H), 3.46 (s, 4H); ¹³C NMR (CD₃OD, 62.5 MHz) δ 175.6, 163.7, 163.0, 156.9, 154.4, 127.7, 114.3, 112.4, 111.8, 103.5, 57.4, 55.6; HRMS(FAB) *m/z* 308.0774 (M+H)⁺, calc. for C₁₄H₁₄NO₇ = 308.0770.

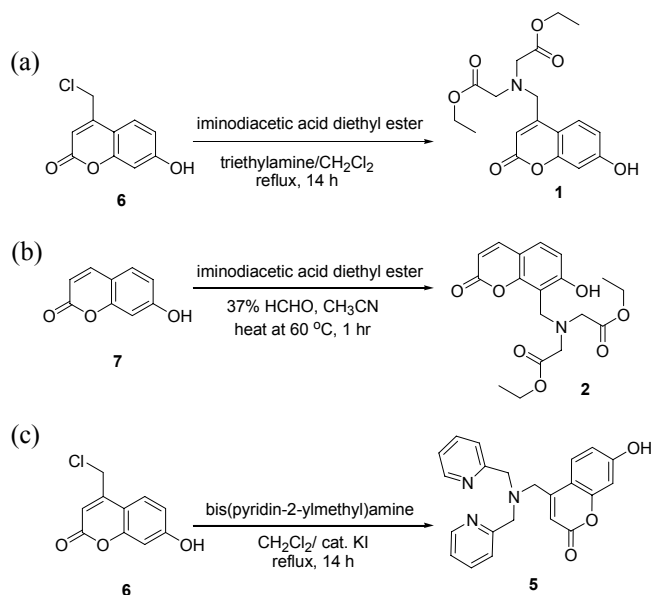
Synthesis of compound 4. To a solution of the bis(2-(ethylthio)ethyl)amine (0.6 g, 3.1 mmol) in 30 mL of acetonitrile was added aqueous formaldehyde (37%) (0.27 mL, 3.1 mmol). After 0.5 h of gentle refluxing at 60 °C, the 7-hydroxycoumarin **7** (0.5 g, 3.1 mmol) in 30 mL of acetonitrile was added followed by triethylamine (1 eq.) and 5 mL of water. The reaction was refluxed

for 12 hr and solvent was removed under reduced pressure. The residue was purified by silica column using hexane-ethyl acetate (7:3) as eluent to give **4** a transparent viscous liquid in a yield of 20%; ¹H NMR (CDCl₃, 250 MHz) δ 7.57 (d, 1H, *J* = 9.5 Hz), 7.23 (d, 1H, *J* = 8.5 Hz), 6.72 (d, 1H, *J* = 8.5 Hz), 6.14 (d, 1H, *J* = 9.5 Hz), 2.80 ~ 2.73 (m, 4H), 2.68 ~ 2.62 (m, 4H), 2.48 (q, 4H, *J* = 7.4 Hz); ¹³C NMR (CDCl₃, 62.5 MHz) δ 162.4, 161.2, 153.0, 144.3, 126.6, 113.9, 111.5, 108.2, 53.1, 49.8, 28.2, 26.2, 14.8; HRMS(FAB) *m/z* 368.1356 (M+H)⁺, calc. for C₁₈H₂₆NO₃S₂ = 368.1354.

Synthesis of compound 5. 4-Chloromethyl-7-methoxycoumarin **6** (1 g, 4.75 mmol) and dipicolylamine (1.89 g, 9.49 mmol) were refluxed in methylene chloride (60 mL) for 14 h in the presence of a catalytic amount of KI. After cooling to ambient temperature, the solvent was evaporated and added dichloromethane (60 mL). The crude product was filtered and dried in vacuum. The crude product (1.4 g, 79%) was crystallized in aqueous ethanol to give **5** in 28 % yield (500 mg). mp 242.3 °C; ¹H NMR (DMSO-*d*₆) δ 10.6 (hydroxyl group, 1H), 8.50 (m, 2H), 7.77 (td, 2H, *J* = 7.6 Hz & 1.6 Hz), 7.64 (d, 1H, *J* = 8.7 Hz), 7.47 (d, 2H, *J* = 7.8 Hz), 7.26 (dd, 2H, *J* = 7.3 Hz & 4.9 Hz), 6.74 (d, 1H, *J* = 8.7 Hz & 2.3 Hz), 6.67 (d, 1H, *J* = 2.2 Hz), 6.45 (s, 1H), 3.86 (s, 2H), 3.82 (s, 4H); ¹³C NMR (DMSO-*d*₆) δ 160.9, 160.4, 158.4, 154.9, 153.5, 148.9, 136.6, 126.3, 122.8, 122.3, 112.6, 110.6, 109.8, 102.1, 59.7, 54.1; HRMS(FAB) *m/z* 374.1503 (M+H)⁺, calc. for C₂₂H₂₀N₃O₃ = 374.1505.

Results and Discussion

Synthesis. The derivatives **1-5** were synthesized by three representative methods as shown in Scheme 1. Coumarin derivatives **1**, **3** and **5**^{8j} were synthesized using 4-chloromethyl-7-hydroxycoumarin (**6**). For example, **6**, iminodiacetic acid diethyl ester, triethylamine and the catalytic amount of KI were refluxed in CH₂Cl₂ for 14 h. After the solvent was evaporated under vacuum, crude product was purified on a silica column



Scheme 1. Representative syntheses of compounds **1**, **2** and **5**.

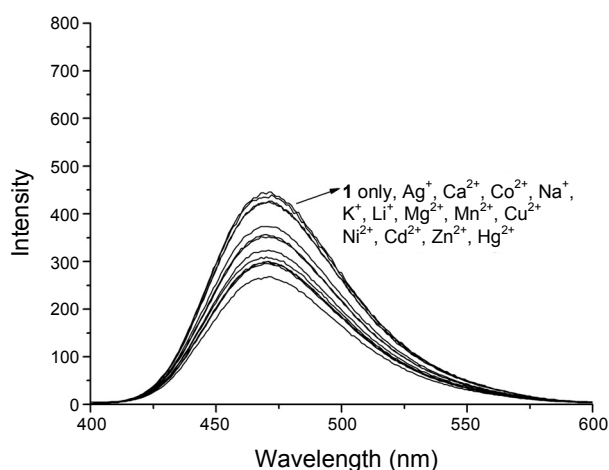


Figure 2. Fluorescence spectra of **1** (6 μM) with various metal ions (60 μM) in DMSO-HEPES buffer (0.02 M, pH 7.4) (1:99, v/v) (λ_{ex} = 380 nm, slit: 3 nm/5 nm).

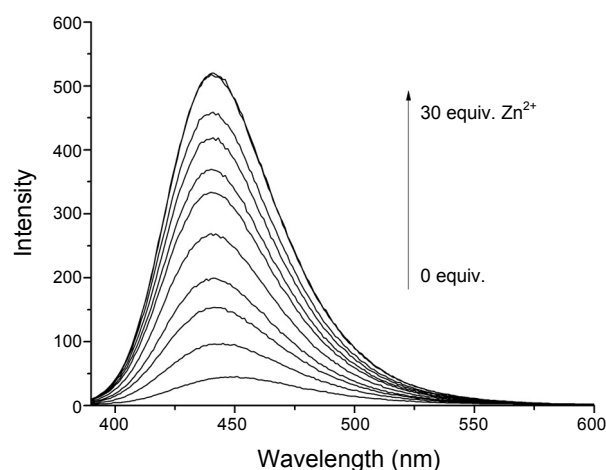


Figure 4. Fluorescence titrations of **2** (6 μM) with Zn^{2+} in DMSO-HEPES buffer (0.02 M, pH 7.4) (1:99, v/v) (λ_{ex} = 380 nm, Slit: 1.5 nm/3 nm).

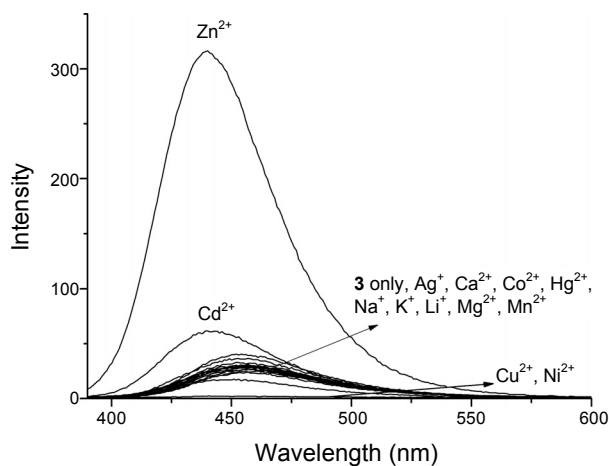


Figure 3. Fluorescence spectra of **2** (6 μM) with various metal ions (60 μM) in DMSO-HEPES buffer (0.02 M, pH 7.4) (1:99, v/v) (λ_{ex} = 380 nm, slit: 3 nm/3 nm).

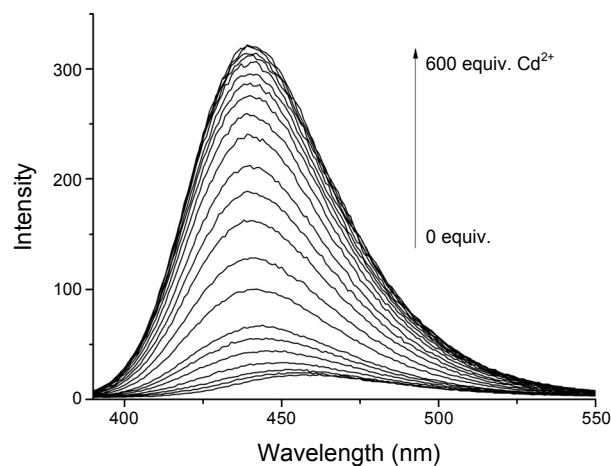


Figure 5. Fluorescence titrations of **2** (6 μM) with Cd^{2+} in DMSO-HEPES buffer (0.02 M, pH 7.4) (1:99, v/v) (λ_{ex} = 380 nm, Slit: 1.5 nm/3 nm).

using hexane-ethyl acetate (6:4) mixture as the eluent to give **1** in 29% yield. On the other hand, 8-substituted derivatives **2** and **4** were synthesized in a single step by the Mannich-type reaction using aqueous formaldehyde. The detailed synthetic procedures are explained in the experimental section of this paper and NMR spectra are reported in the supporting information.

Fluorescent changes of 1-5 with metal ions. The emission spectra of **1-5**, and their fluorescence titrations, were recorded in HEPES buffer solutions (20 mM, pH 7.4) containing 1% DMSO. The solutions of Ag^+ , Ca^{2+} , Cd^{2+} , Co^{2+} , Cu^{2+} , Hg^{2+} , K^+ , Li^+ , Mg^{2+} , Mn^{2+} , Na^+ , Ni^{2+} , Pb^{2+} and Zn^{2+} ions (10 equiv.) were then used to evaluate the metal ion selectivities of **1-5**. From the job-plot analysis, probes **1-5** displayed 1:1 stoichiometry with metal ions.

As shown in Figure 2, probe **1**, bearing an iminodiacetic acid diethyl ester ligand at the 8-position, did not display significant changes in its fluorescence emissions upon addition of various metal ions, even though small fluorescence quenching effects

were observed with several metal ions, including Cu^{2+} , Hg^{2+} and Ni^{2+} . On the other hand, probe **2**, which has an iminodiacetic acid diethyl ester ligand at the 8-position, displayed a selective and large fluorescence enhancement with Zn^{2+} even though there was a small fluorescence enhancement with Cd^{2+} and fluorescence quenching effects with Cu^{2+} and Ni^{2+} (Figure 3). Fluorescence titrations of probe **2** with Zn^{2+} are shown in Figure 4, and those for Cd^{2+} are illustrated in the Figure 5. The association constants were calculated as $1.7 \times 10^4 \text{ M}^{-1}$ and $1.4 \times 10^3 \text{ M}^{-1}$, respectively (error <10%).¹⁰

Generally, fluorescence enhancement of probe **2** with metal ions were larger than those of probe **1**, which means that the photo-induced electron transfer (PET) process of benzylic amine is greater for the 8-substituted derivative. The quantum yields of **1** and **2** were calculated as 0.19 and 0.03, respectively, compared to 4-methylumbelliferone (7-hydroxy-4-methyl coumarin) as a standard.¹¹ In addition, ~10 nm blue shifts were observed upon addition of Zn^{2+} and Cd^{2+} , which can be att-

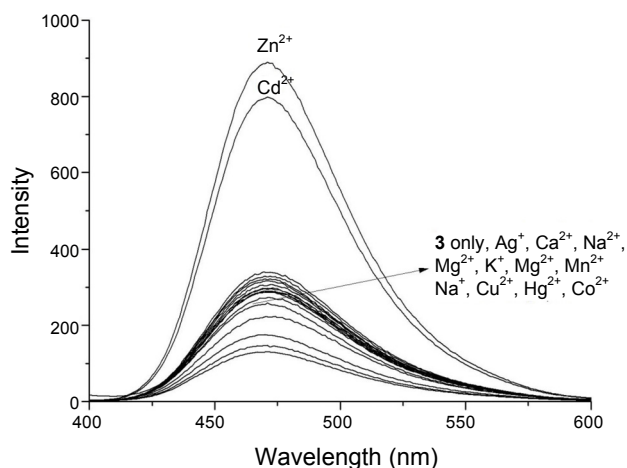


Figure 6. Fluorescence spectra of **3** (6 μM) with metal ions (60 μM) in DMSO-HEPES buffer (0.02 M, pH 7.4) (1:99, v/v) ($\lambda_{\text{ex}} = 380$ nm, slit: 3 nm/5 nm).

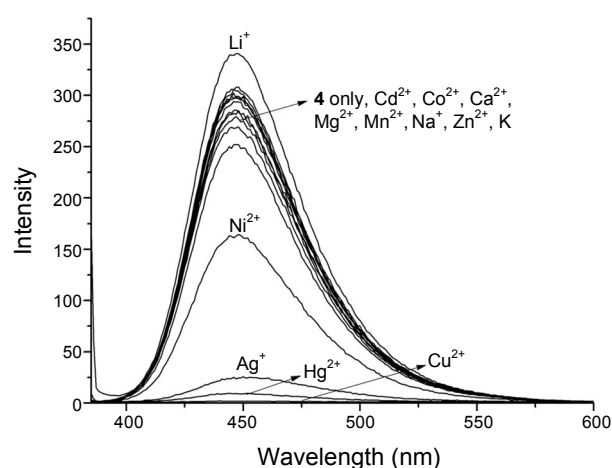


Figure 8. Fluorescence spectra of **4** (6 μM) with metal ions (60 μM) in DMSO-HEPES buffer (0.02 M, pH 7.4) (1:99, v/v) ($\lambda_{\text{ex}} = 380$ nm, slit: 1.5 nm/3 nm).

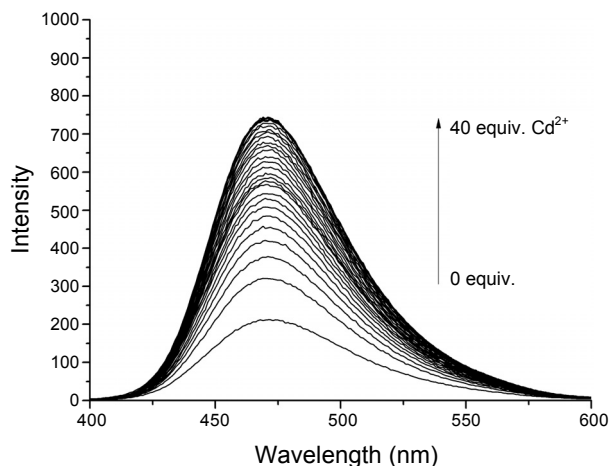
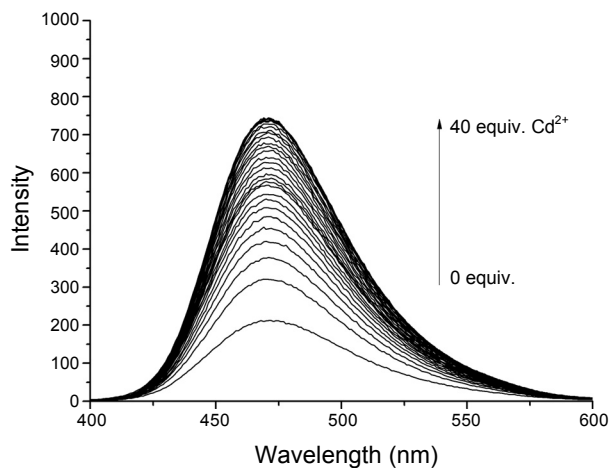


Figure 7. Fluorescence titrations of **3** (6 μM) with Zn^{2+} (upper) and Cd^{2+} (bottom) in DMSO-HEPES buffer (0.02 M, pH 7.4) (1:99, v/v) ($\lambda_{\text{ex}} = 380$ nm, slit: 3 nm/5 nm).

ributed to the decreased electron donating ability of $-\text{O}^- - \text{Zn}^{2+}$ compared to the $-\text{O}^-$ of phenolate.^{8a}

Among the metal ions examined, probe **3**, which has an imi-

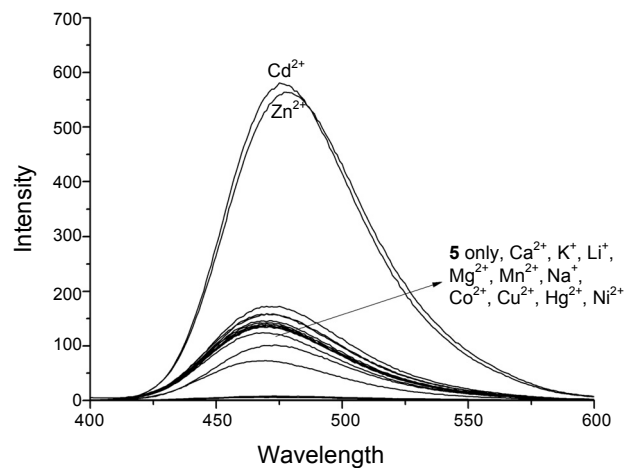


Figure 9. Fluorescence spectra of **5** (6 μM) with metal ions (60 μM) in DMSO-HEPES buffer (0.02 M, pH 7.4) (1:99, v/v) ($\lambda_{\text{ex}} = 380$ nm, slit: 3 nm/3 nm).

nodiacetic acid group at the 4-position, showed large fluorescence enhancements only with Zn^{2+} and Cd^{2+} (Figure 6). As compared to probe **2**, probe **3** displayed less selectivity for Zn^{2+} over Cd^{2+} , however, the fluorescent changes for other metal ions were relatively reduced. For example, the fluorescence quenching effect with Cu^{2+} was significantly reduced. From the fluorescence titrations (Figure 7), the association constants were calculated as $4.2 \times 10^5 \text{ M}^{-1}$ for Zn^{2+} , and $1.3 \times 10^4 \text{ M}^{-1}$ for Cd^{2+} , respectively (error < 10%).¹⁰ Two carboxylate oxygens vs phenolate could be the reason for the larger association constants of probe **3** compared to those of probe **2**. In addition, iminodiacetic acid ligand provides better binding affinities towards Zn^{2+} and Cd^{2+} than the iminodiacetic acid diethyl ester ligand in probe **1**.

As shown in Figure 8, probe **4**, which bears bis(2-(ethylthio)ethyl)amine, showed quite a different binding selectivity. Hg^{2+} , Cu^{2+} and Ag^{+12} induced large fluorescence quenching effects and a relatively smaller fluorescence quenching effect was ob-

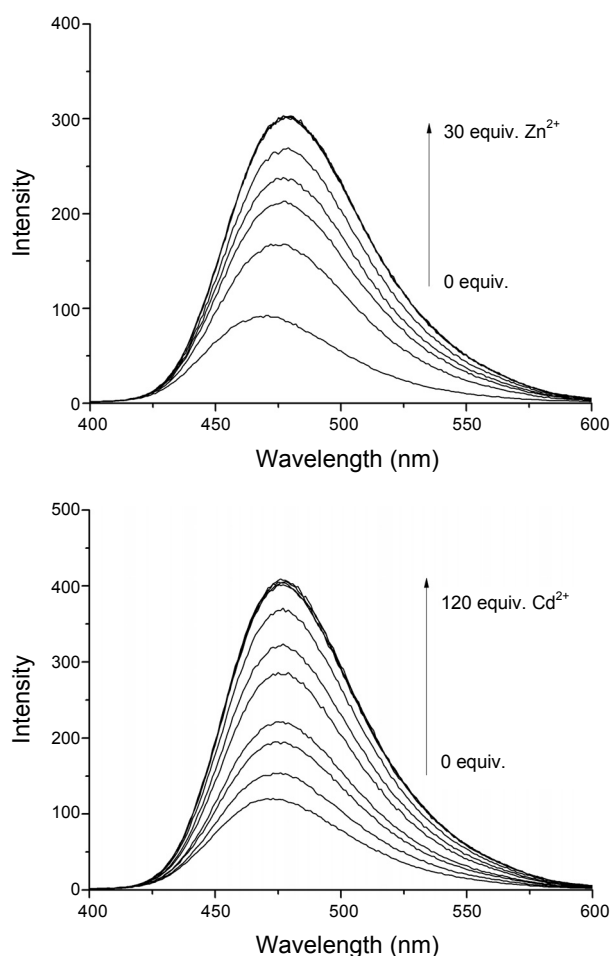


Figure 10. Fluorescence titrations of **5** (6 μM) with Zn^{2+} (upper) and Cd^{2+} (bottom) in DMSO-HEPES buffer (0.02 M, pH 7.4) (1:99, v/v) ($\lambda_{\text{ex}} = 380 \text{ nm}$, slit: 3 nm/3 nm).

served with Ni^{2+} . From the fluorescence titrations, the association constants were calculated as $3.3 \times 10^5 \text{ M}^{-1}$ for Hg^{2+} , $4.6 \times 10^4 \text{ M}^{-1}$ for Cu^{2+} and $1.9 \times 10^6 \text{ M}^{-1}$ for Ag^+ , respectively (error < 10%).¹⁰ Sulfur atoms on the ligand could be the reason for the binding affinity for Ag^+ and Hg^{2+} .

Probe **5**, in which DPA ligand was introduced at the 4-position, showed large fluorescence enhancements with Zn^{2+} and Cd^{2+} and fluorescence quenching effects with Cu^{2+} and Hg^{2+} (Figure 9). From the fluorescence titrations (Figure 10), the association constants were calculated as $1.4 \times 10^5 \text{ M}^{-1}$ for Zn^{2+} , and $1.2 \times 10^4 \text{ M}^{-1}$ for Cd^{2+} , respectively (error < 10%).¹⁰ Addition of Zn^{2+} and Cd^{2+} induced a slight red shift from 468 nm to 478 nm (10 nm) and from 468 nm to 476 nm (7 nm), respectively (Figure 10). Usually, fluorescent probes bearing DPA ligand displayed selective fluorescence enhancement with Zn^{2+} ,^{5,13} however, there are reports which show Cd^{2+} selectivity¹⁴ or Cu^{2+} selectivity.¹⁵

Conclusions

In current study, we synthesized new 4- or 8-substituted-7-hydroxycoumarin derivatives (**1-5**). Introduction of different ligands and/or different substitution positions of 7-hydroxycou-

marin induced different fluorescent changes and selectivities for metal ions. Especially, probes **2**, **3** and **5** displayed large fluorescence enhancements with Zn^{2+} and Cd^{2+} in aqueous solutions. Probes **2** and **3** showed a moderate selectivity for Zn^{2+} over Cd^{2+} . On the other hand, probe **4** showed large fluorescence quenching effects upon the addition of Ag^+ and Hg^{2+} . We successfully demonstrated that ligand variation, along with alteration of position for the substitution on 7-hydroxycoumarin, can provide different selectivities and sensitivities for metal ions at pH 7.4.

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