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PAPER

Tetrahydroindazolone substituted 2-aminobenzamides as fluorescent probes: switching metal ion selectivity from zinc to cadmium by interchanging the amino and carbamoyl groups on the fluorophore†

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Three fluorescent probes CdABA′, CdABA and ZnABA′, which are structural isomers of ZnABA, have been designed with *N*,*N*-bis(2-pyridylmethyl) ethylenediamine (BPEA) as chelator and 2-aminobenzamide as fluorophore. These probes can be divided into two groups: CdABA, CdABA′ for Cd^{2+} and ZnABA, ZnABA′ for Zn^{2+}. Although there is little difference in their chemical structures, the two groups of probes exhibit totally different fluorescence properties for preference of Zn^{2+} or Cd^{2+}. In the group of Zn^{2+} probes, ZnABA/ZnABA′ distinguish Zn^{2+} from Cd^{2+} with $F_{Zn}{}^{2+}$–$F_{Cd}{}^{2+}$ = 1.87–2.00. Upon interchanging the BPEA and carbamoyl groups on the aromatic ring of the fluorophore, the structures of ZnABA/ZnABA′ are converted into CdABA/CdABA′. Interestingly, the metal ions selectivity of CdABA/CdABA′ was switched to discriminate Cd^{2+} from Zn^{2+} with $F_{Cd}{}^{2+}$–$F_{Zn}{}^{2+}$ = 2.27–2.36, indicating that a small structural modification could lead to a remarkable change of the metal ion selectivity. ^{1}H NMR titration and ESI mass experiments demonstrated that these fluorescent probers exhibited different coordination modes for Zn^{2+} and Cd^{2+}. With CdABA′ as an example, generally, upon addition of Cd^{2+}, the fluorescence response possesses PET pathway to display no obvious shift of maximum λ_{em} in the absence or presence of Cd^{2+}. However, an ICT pathway could be employed after adding Zn^{2+} into the CdABA′ solution, resulting in a distinct red-shift of maximal λ_{em}.

Introduction

Cadmium is considered as a toxic and inessential element for life, and generally exists in the form of compounds with low quality in nature. However, Cd can be accumulated in organisms and get into the human body by the food chain, causing severe lesions and cancers in the brain, bone, kidney, liver and gastrointestinal tract *etc*.[1] Therefore, considerable efforts have been expended on developing highly sensitive and selective detecting methods for Cd^{2+}, among which fluorescent probes have attracted great attention due to their simplicity, high degree of specificity and low detection limit.[2]

In the past decade, many fluorescent sensor molecules have been reported to measure Cd^{2+} *in vivo* or *in vitro*,[3] and all of them employed traditional dyes as fluorophores, including fluorescein,[3q] rhodamine,[3k] cyanine,[3b] anthracene/phenanthrene,[3l,s,t] quinoline/naphthyridine,[3a,e–h,j,m,r,u] coumarin,[3n] naphthalimide,[3d,p] BODIPY[3i,o] *etc*. More importantly, there is always interference between Cd^{2+} and Zn^{2+} during the fluorometric detection, because Cd and Zn are both group IIb elements, which process very similar coordinating functions to the fluorescent sensors. For instance, pyridyl-containing ligands BPA (*N*,*N*-bis(2-pyridylmethyl)diamine)[3c,d,o,p,4] and BPEA (*N*,*N*-bis(2-pyridylmethyl) ethylenediamine)[3g,h,n,5] are widely used as chelators for both Zn^{2+} and Cd^{2+} fluorescent sensors; however, it is difficult to predict in which situation BPA/BPEA has more preference of Zn^{2+} to Cd^{2+}, and *vice versa*. Therefore, to date, it still a challenge to develop Cd^{2+}-specific fluorescent probes, especially with new fluorophores, that can distinguish Cd^{2+} from Zn^{2+} in biological and environmental systems.

In the previous study, ZnABA[6] was found to be a Zn^{2+}-specific fluorescent probe (Fig. 1) with 2-aminobenzamide as fluorophore and BPEA as chelator. As a part of our efforts to further synthesize various structural isomers of ZnABA and investigate their fluorescent properties, Herein, we reported the new discovery of fluorescent probes for Cd^{2+} based on 2-aminobenzamide.

Results and discussion

There are three functional groups on the benzene ring of ZnABA: carbamoyl, BPEA and tetrahydroindazolone. To further

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†Electronic supplementary information (ESI) available: Fig. S1–S6 and copies of NMR spectra for all the new compounds. CCDC 875185 for **CdABA′** and 875205 for **8b**. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob25852h

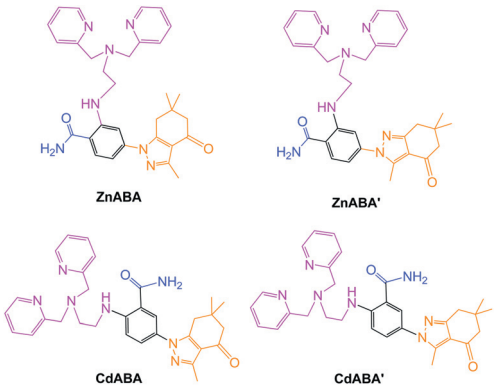
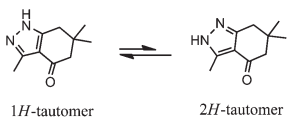


Fig. 1 Structures of ZnABA and its structural isomers. ZnABA has been previously reported.[6] The others are newly designed.

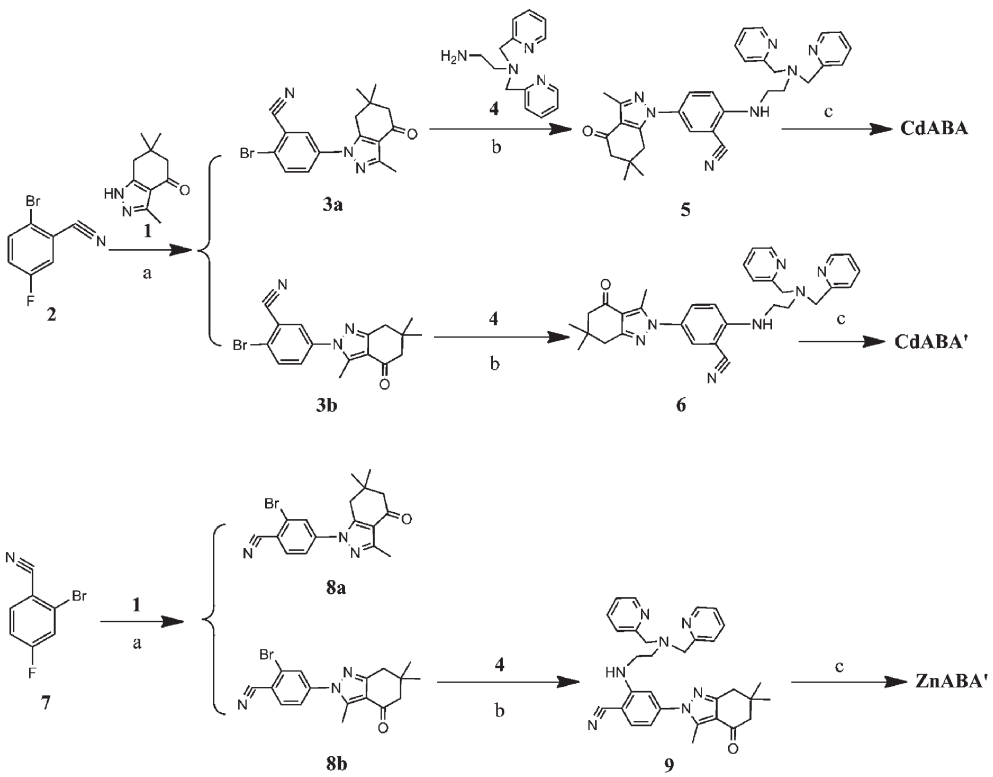
explore the possibilities of developing new fluorescent probes, we interchanged the BPEA and carbamoyl groups on the fluorophore of ZnABA to produce a new compound CdABA, whose chemical structure is shown in Fig. 1.

3,6,6-Trimethyl-1(2),5,6,7-tetrahydro-4*H*-indazol-4-one (**1**) has a equilibrium of two tautomers (Scheme 1).[7] Previously, the 1*H*-tautomer substituted derivative ZnABA has been synthesized.[6] To deeply evaluate the influence of different tautomers of **1** on the fluorescent probes, the 2*H*-tautomer substituted derivatives ZnABA′ and CdABA′ (Fig. 1) were then designed and synthesized in this study.

The synthetic procedures of CdABA, CdABA′ and ZnABA′ are depicted in Scheme 2. For the synthesis of CdABA/CdABA′, firstly, 2-bromo-5-fluorobenzonitrile (**2**) was coupled with tetrahydro-4*H*-indazol-4-one **1** in the presence of K_2CO_3, to afford the desired tetrahydroindazolone 1*H*-tautomer substituted benzonitrile **3a** as the major product (50% yield, Table 1, entry 1), and 2*H*-tautomer compound **3b** as the minor product (<5% yield). To increase the product yield of isomer **3b**, Cs_2CO_3 instead of K_2CO_3 was selected for the reaction, and the yield of 1*H*-tautomer compound **3a** declined to 38% with increasing the yield of 2*H*-tautomer compound **3b** to 19% (Table 1, entry 2), indicating that the stronger base Cs_2CO_3 with greater solubility leads to a much higher ratio of **3b** to **3a** in the aromatic nucleophilic substitution. Next, the Buchwald–Hartwig coupling[8] was carried out under the conditions of catalytic $PdCl_2$, 1,1′-bis(diphenylphosphanyl)ferrocene (DPPF), and NaO*t*Bu in toluene at 100 °C, and then BPEA moiety was incorporated into the aromatic ring of **3a/3b** to give compound **5/6** in 54–66% yield. Finally, hydration[9] of the yielding benzonitrile **5/6** with KOH catalyzed by H_2O_2 in a mixture of EtOH/DMSO afforded the new probe CdABA/CdABA′ in high yield of 82–90%. Additionally, the crystal structure of CdABA′ was obtained by single-crystal X-ray diffraction, which is demonstrated in Fig. 2.[10]



Scheme 1 The equilibrium of two tautomers of tetrahydro-4*H*-indazol-4-one **1**.



Scheme 2 Synthesis of tetrahydroindazolone substituted 2-aminobenzamides. *Reagents and conditions*: (a) K_2CO_3, or Cs_2CO_3, DMF; (b) $PdCl_2$, DPPF, NaO*t*Bu, toluene, 100 °C; (c) KOH, H_2O_2, EtOH/DMSO.

Table 1 Effect of base on the coupling of **1** with bromobenzonitrile

Entry	Substrate	Base	Products (yield)
1	**2**	K_2CO_3	**3a** (50%)/**3b** (<5%)
2	**2**	Cs_2CO_3	**3a** (38%)/**3b** (19%)
3	**7**	K_2CO_3	**8a** (82%)/**8b** (<5%)
4	**7**	Cs_2CO_3	**8a** (57%)/**8b** (28%)

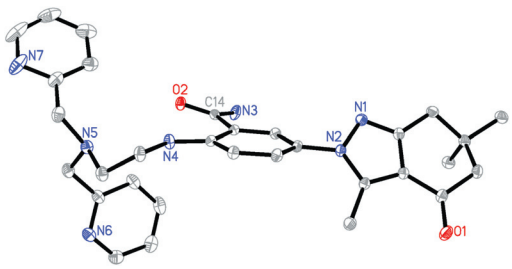


Fig. 2 X-ray crystallographic structure of CdABA′.

Similarly, ZnABA′ was also obtained through above synthetic strategy. **8b** was prepared by the substitution of 2-bromo-4-fluorobenzonitrile (**7**) with indazolone **1**. Notably, Cs_2CO_3 was still a better base for the reaction, since the expected 2*H*-tautomer compound **8b** was obtained in higher yield (28%, Table 1, entry 4). In contrast, the yield of **8b** was lower than 5% with K_2CO_3 as base (Table 1, entry 3), and the 1*H*-tautomer compound **8a** was almost the major product. The crystal structure of **8b** was also confirmed by X-ray diffraction (Fig. S1†).[10]

With all of the newly designed probes CdABA, CdABA′ and ZnABA′ in hand, we screened their fluorescence response to various metal ions. Interestingly, CdABA/CdABA′ was demonstrated to be a Cd^{2+}-specific fluorescent probe, while ZnABA′ was a Zn^{2+} fluorescent probe.

The photophysical properties of all the 2-aminobenzamides used in this study, including previously reported Zn^{2+} probe ZnABA, are summarized in Table 2. Among these probes, CdABA′ and ZnABA have greater ε values (2.68–2.88 × 10^4 M^{-1} cm^{-1}), whereas CdABA and ZnABA′ show much smaller ε values (1.17–1.26 × 10^4 M^{-1} cm^{-1}). CdABA′ has a maximal absorption peak at 260 nm with a long tail to 400 nm before titration (Fig. 3a). Upon addition of Cd^{2+} (0–2.0 equiv.), the absorbance at 260 nm decreased and one isosbestic point at 283 nm appeared, suggesting that the CdABA′–Cd^{2+} complex could be formed. Meanwhile, the maximum fluorescence emission of CdABA′ was at 454 nm with a very low quantum yield of 0.06 in the absence of Cd^{2+} (Table 2). After Cd^{2+} (0–2.0 equiv.) was added into the probe solution, the maximum fluorescence emission peak didn't change, and the fluorescence intensity showed a 5.2-fold enhancement with remarkably improved quantum yield of 0.23 (Fig. 4a). Considering above results on UV and fluorescent studies, it is suggested that the observed fluorescence enhancement of CdABA′ should be attributed to the fact that the lone pair of the tertiary nitrogen of BPEA chelates to Cd^{2+}, and then the photo-induced electron transfer (PET) process is prevented to bring out an efficient off-on fluorescence response.

Table 2 Absorption and fluorescence properties of all probes used in this study

Compound	ε^a (M^{-1} cm^{-1})	$\lambda_{em}{}^b$ (nm)	Φ^c
CdABA[d]	11 700	456	0.20
CdABA + Cd^{2+} [e]	12 600	456	0.53
CdABA′[d]	26 820	454	0.06
CdABA′ + Cd^{2+} [e]	25 880	454	0.23
ZnABA [d,f]	28 835	434	0.0096
ZnABA + Zn^{2+} [e,f]	21 900	446	0.077
ZnABA′ [d]	14 700	436	0.015
ZnABA′ + Zn^{2+} [e]	14 700	448	0.059

[a] Data were evaluated at the maximum λ_{abs} of 260 nm. [b] The excitation wavelength was 260 nm. [c] The fluorescence quantum yields were obtained by using quinine sulfate (in 0.05 M H_2SO_4, Φ = 0.55) as the standard. [d] Data were determined in the absence of Zn^{2+} or Cd^{2+}. [e] Data were determined in the presence of 1.0 equiv. Zn^{2+} or Cd^{2+}. [f] See ref. 6.

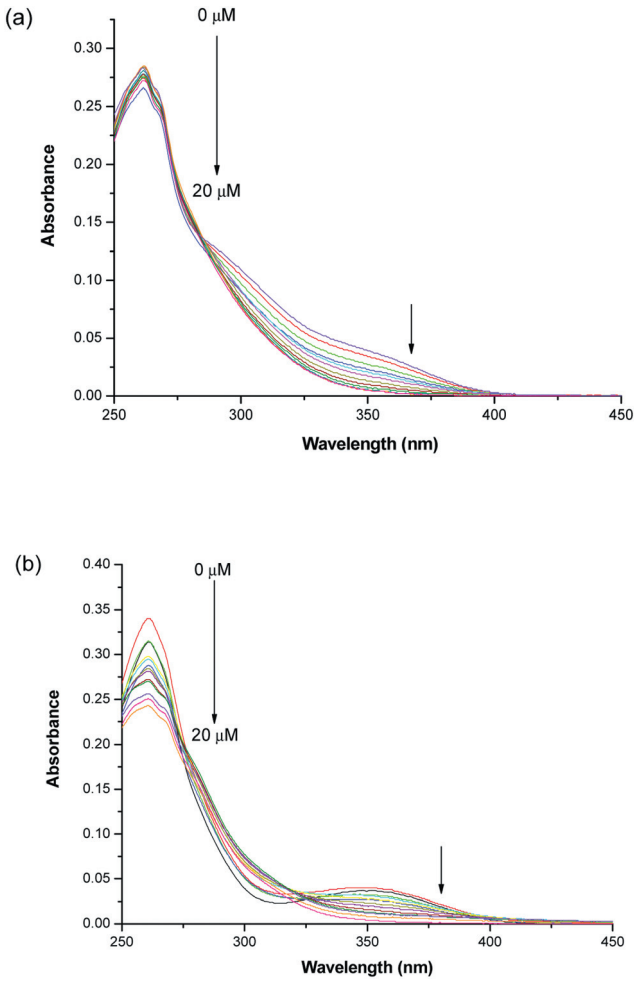


Fig. 3 UV-vis spectra of (a) CdABA′ and (b) ZnABA′ (10 μM; in HEPES buffer, 25 mM, 0.1 M $NaClO_4$, pH = 7.4, I = 0.1) upon addition of Cd^{2+} and Zn^{2+} (0–20 μM).

The only difference in structure between CdABA and CdABA′ is the tetrahydroindazolone moiety with 1*H*-tautomer and 2*H*-tautomer respectively. Upon addition of Cd^{2+} (1.0 equiv.), the fluorescence emission intensity of CdABA was

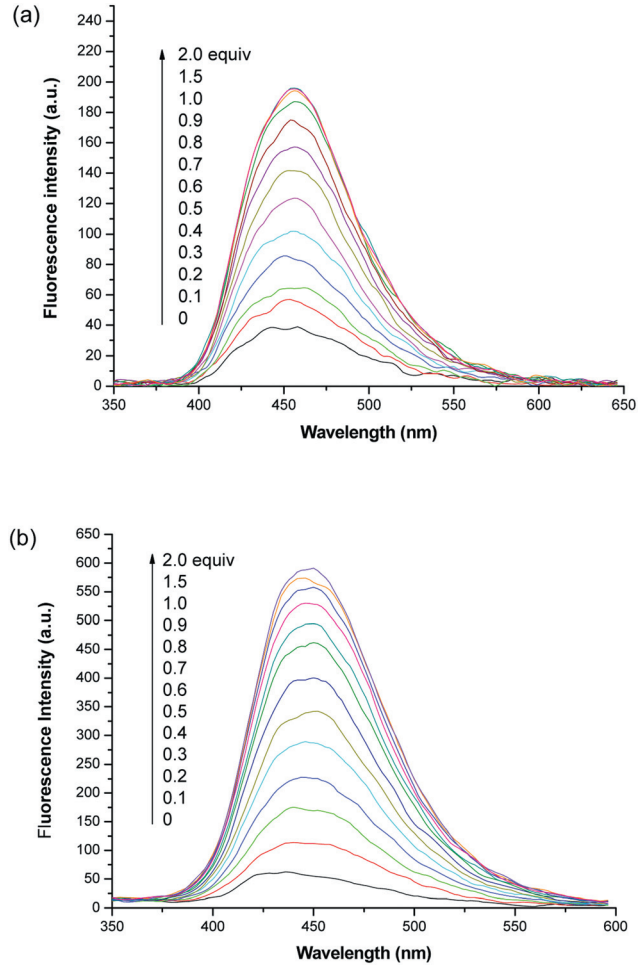


Fig. 4 Fluorescence emission spectra (λ_{ex} = 260 nm) of (a) CdABA′ and (b) ZnABA′ (10 μM, 25 mM HEPES buffer, 0.1 M $NaClO_4$, pH = 7.4, I = 0.1) upon addition of Cd^{2+} or Zn^{2+} [added as $Cd(ClO_4)_2$ or $Zn(ClO_4)_2$, 0–20 μM].

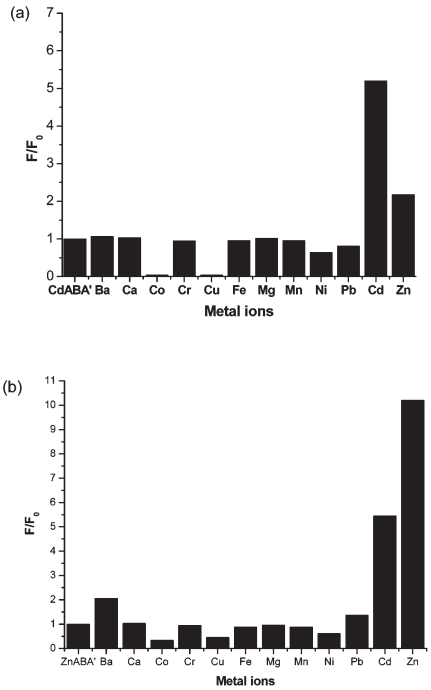


Fig. 5 Selectivity of (a) CdABA′ and (b) ZnABA′ towards various metal ions. *Experimental conditions*: CdABA′/ZnABA′ (10 μM, 25 mM HEPES buffer, 0.1 M $NaClO_4$, pH = 7.4, I = 0.1), 10 μM Ba^{2+}, Ca^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+}, λ_{ex} = 260 nm, λ_{em} = 454 nm for CdABA′ and λ_{em} = 448 nm for ZnABA′.

enhanced 3.4-fold (Fig. S2†), which is slightly lower than that of CdABA′. On the whole, CdABA has very similar photophysical properties to CdABA′, indicating that 1*H*-tautomer and 2*H*-tautomer of tetrahydroindazolone have no distinguishable influence on the probe properties.

For ZnABA′, its UV titration spectra show two absorption bands centered at 260 and 354 nm. On the addition of 0–2.0 equiv. Zn^{2+}, the intensities of both bands decreased (Fig. 3b). Two isosbestic points at 272 and 321 nm were observed due to the formation of the ZnABA′-Zn^{2+} complex. The quantum yields of ZnABA′ in the absence and presence of 1.0 equiv. Zn^{2+} were 0.015 and 0.059 respectively, and its fluorescence intensity was enhanced by 10.2-fold (Fig. 4b). Moreover, the maximal emission peak of ZnABA′ showed a 12 nm red-shift, which is totally different from the case of CdABA′ with no change of the emission peak. The results can be explained by the intermolecular charge transfer (ICT) effect of the aromatic plane with BPEA as the conjugated electron donor and tetrahydroindazolone as the receptor. Upon Zn^{2+} binding to ZnABA′, the electron-donating ability of the 2-amino group in the BPEA moiety drops down, and the electron-withdrawing ability of the 1-carbamoyl group goes up, which leads to the two obvious isosbestic points in its UV spectra and the red-shift of its emission peak. In general, ZnABA′ displayed similar fluorescence properties to ZnABA, which has been reported before.[6]

The selectivity of CdABA′ towards various common metal ions was examined and the results are shown in Fig. 5a. No change could be observed upon addition of the metal cations such as Ba^{2+}, Ca^{2+}, Cr^{3+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, Ni^{2+} and Pb^{2+}, whereas the transition-metal ions Co^{2+} and Cu^{2+} quenched the fluorescence to some extent. Besides, both Zn^{2+} and Cd^{2+} induced the fluorescence enhancement. The fluorescence enhancement (5.2-fold) of CdABA′ towards Cd^{2+} is higher than that towards Zn^{2+} (2.2-fold), suggesting that CdABA′ has a selectivity to prefer Cd^{2+} to Zn^{2+} ($F_{Cd^{2+}}$–$F_{Zn^{2+}}$ = 2.36). CdABA also exhibited the preference for Cd^{2+} compared to Zn^{2+}. Upon addition of Cd^{2+} and Zn^{2+} (1.0 equiv.), the fluorescence intensity of CdABA increased 3.4-fold and 1.5-fold, respectively (Fig. S3†), and the value of $F_{Cd^{2+}}$–$F_{Zn^{2+}}$ is 2.27. Based on the results, CdABA has a comparable selectivity to CdABA′ to discriminate Cd^{2+} from Zn^{2+} in aqueous solutions.

As for the Zn^{2+} probes, the value of $F_{Zn^{2+}}$–$F_{Cd^{2+}}$ is 1.87 for ZnABA′ (Fig. 5b), and 2.00 for ZnABA,[6] which provides a approximate selectivity to distinguish Zn^{2+} from Cd^{2+}.

All the fluorescent probes used in this work have a 1 : 1 binding ratio with Zn^{2+} or Cd^{2+}, which is determined by the Job plot (Fig. S4†). The dissociation constants of CdABA′/CdABA for Cd^{2+} and ZnABA′/ZnABA for Zn^{2+} were determined by fluorescence spectroscopy in metal–ligand-buffered solutions with different Cd^{2+}/Zn^{2+} concentrations. CdABA′ and CdABA responded to the picomolar concentration of free Cd^{2+} (K_d =

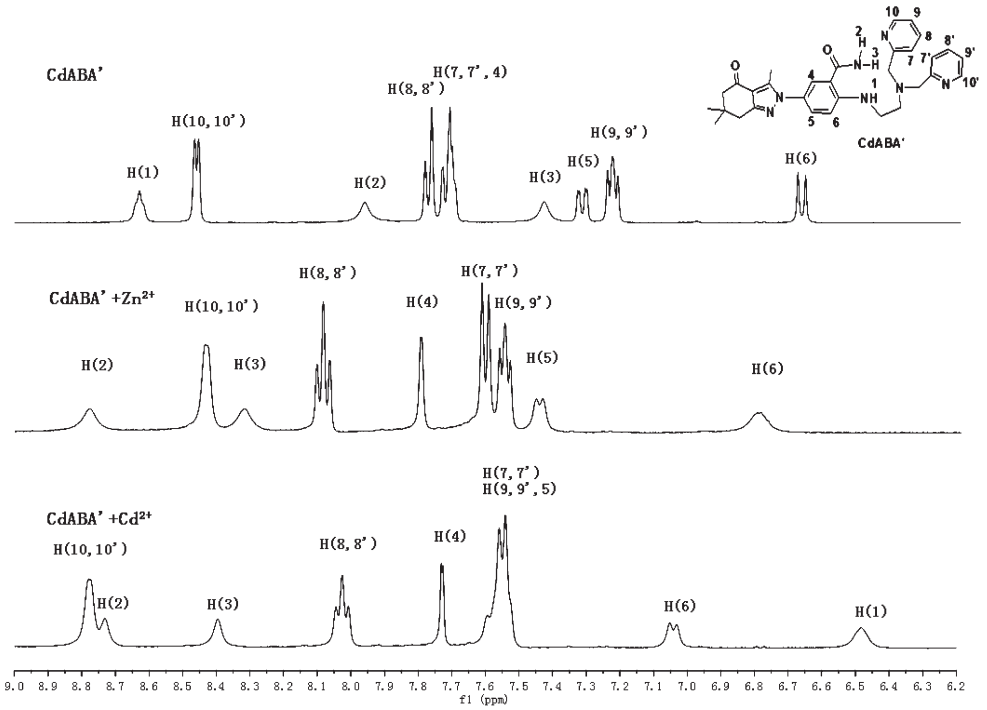


Fig. 6 ^{1}H NMR spectra of CdABA′, CdABA′–Cd^{2+} complex and CdABA′–Zn^{2+} complex in DMSO-d_6.

1.66–1.86 pM), indicating the high binding affinity of CdABA′/CdABA for Cd^{2+}. In contrast, the competitive binding experiments afforded the estimated K_d of 1.06–2.06 nM for Zn^{2+}-ZnABA′ or Zn^{2+}-ZnABA complex. Therefore, CdABA′/CdABA has more sensitivity to detect Cd^{2+} than ZnABA′/ZnABA to detect Zn^{2+}.

Notably, above results and Fig. S5† show the maximum λ_{em} of the probes used in this work always exhibits a red-shift after the addition of Zn^{2+}, while there is no obvious changes of λ_{em} upon addition of Cd^{2+}, which indicates that there could be different mechanisms of coordination for Zn^{2+} and Cd^{2+}. To further understand the mechanisms, the ^{1}H NMR titration experiments of CdABA′ with Zn^{2+} and Cd^{2+} in DMSO-d_6 have been taken to provide further evidence. As shown in Fig. 6, addition of 1.0 equiv. of Zn^{2+} or Cd^{2+} promoted downfield shifts of the H(5), H(6), H(8,8′) and H(9,9′) protons and upfield shifts of the H(7,7′) protons, indicating that the chemical circumstances of these protons have been changed through the interaction between Zn^{2+}/Cd^{2+} and CdABA′. Moreover, the H(2) and H(3) protons of amide experienced approximate 0.8–1.0 ppm downfield shifts with Zn^{2+}/Cd^{2+} adding into the CdABA′ solution, which demonstrates the amide group directly participated the metal ion coordination. In addition, a huge upfield shift of the H(1) proton from 8.63 to 6.48 was observed as a result of the aniline nitrogen atom coordinating with Cd^{2+} to form $[CdABA' + Cd]^{2+}$. However, the disappearance of H(1) signal in CdABA′ − Zn^{2+} proton NMR spectrum implied that the $[CdABA' + Zn - H]^+$ could be formed in the solution, which is consistent to the reports that zinc ion usually cause deprotonation during the coordination with probes.[3h,3p] The ESI mass spectra also provided the supplemental evidence for the existence of the singly charged $[CdABA' + Zn - H]^+$ complex (Fig. S6†).

Conclusion

Three novel fluorescent probes, CdABA′, CdABA and ZnABA′ have been designed with BPEA as chelator and the 2-aminobenzamide as fluorophore. All the probes used in this study are the structural isomers of ZnABA, which has been previously reported as Zn^{2+} probe. For the preparation of the probes, three reactions including the aromatic nucleophilic substitution, the Buchwald–Hartwig coupling and the subsequent hydration were employed to successfully afford the desired fluorescent molecules. It should be noted that the utilization of Cs_2CO_3 as base is crucial to the synthesis of 2*H* indazolone tautomer substituted benzonitriles.

These probes can be divided into two groups, CdABA, CdABA′ for Cd^{2+} and ZnABA, ZnABA′ for Zn^{2+}. ZnABA and CdABA, ZnABA′ and CdABA′ are positional isomers on the benzene ring; while the couples of ZnABA and ZnABA′, CdABA and CdABA′ are 1*H* or 2*H* indazolone tautomer substituted functional isomers. Although there is little difference in the chemical structures of these 2-aminobenzamide probes, these two groups of probes exhibit totally different fluorescence properties for the preference of Zn^{2+} or Cd^{2+}. In the group of probes for Zn^{2+}, ZnABA/ZnABA′ prefers Zn^{2+} to Cd^{2+} with F_{Zn}^{2+}–F_{Cd}^{2+} = 1.87–2.00. It is noted that the structure of ZnABA/ZnABA′ is converted into CdABA/CdABA′ by interchanging the BPEA and carbamoyl groups on the 2-aminobenzamide fluorophore. Interestingly, the metal ions selectivity of CdABA/CdABA′ was switched to prefer Cd^{2+} to Zn^{2+} with F_{Cd}^{2+}–F_{Zn}^{2+} = 2.27–2.36, indicating that a small structural modification could lead to a remarkable change in the metal ion selectivity.

^{1}H NMR titration and ESI mass experiments showed that these fluorescent probes exhibited different coordination modes

for Zn^{2+} and Cd^{2+}. With CdABA′ as an example, generally, upon addition of Cd^{2+}, the fluorescence response possesses PET pathway to display no obvious shift of maximum λ_{em} in the absence or presence of Cd^{2+}. However, an ICT pathway could be employed when adding Zn^{2+} into the CdABA′ solution, as a consequence a distinct red-shift of maximal λ_{em} is observed.

The current study should be helpful for the design and synthesis of other metal fluorescent probes with 2-aminobenzamide as the fluorophore. Also, the deep investigations of these probes concerning biological activities and applications are under way and will be reported in due course.

Experimental

General

All chemicals were purchased as reagent grade and used without further purification. Buchwald–Hartwig cross-coupling reactions were performed in flame-dried glassware under argon. Toluene was distilled from calcium hydride. The reactions were monitored by analytical thin-layer chromatography (TLC) on silica gel F_{254} glass plates and visualized under UV light (254 and 365 nm) and/or by staining with ninhydrin. Flash column chromatography was performed on silica gel (200–300 mesh). ^{1}H NMR spectra were recorded with a Bruker Avance III 400 MHz NMR spectrometer at 20 °C. Chemical shifts (in ppm) were determined relative to tetramethylsilane (δ = 0 ppm) in deuteriated solvents. Coupling constants in Hz were measured from the one-dimensional spectra. ^{13}C NMR or ^{13}C attached-proton-test (^{13}C-Apt) spectra were recorded with the 400 MHz NMR spectrometer (100 MHz) and calibrated with $CDCl_3$ (δ = 77.23 ppm). High-resolution mass spectra were recorded with a Waters LCT Premier XE mass spectrometer. UV absorption and emission spectra were recorded with a GBC Cintra 10e UV/Vis spectrophotometer and a Varian Cary Eclipse spectro-fluorimeter, respectively, in a quartz cell with a 1 cm path length.

2-Bromo-5-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)-benzonitrile (3a). Indazolone **1**[7] (250 mg, 1.47 mmol, 1.47 equiv.) was dissolved in DMF, and 2-bromo-5-fluorobenzonitrile (**2**) (200 mg, 1.00 mmol, 1.00 equiv.) and K_2CO_3 (350 mg, 2.54 mmol, 2.54 equiv.) were added. The reaction mixture was stirred at room temperature for 30 min and then heated in an 80 °C oil bath until the starting material had been completely consumed as detected by TLC. The solution was then allowed to cool to room temperature, and the DMF was evaporated under vacuum to leave a yellowish oil. The crude oil was then diluted with DCM (150 mL), washed with saturated NaCl solution (3 × 30 mL), and dried with $MgSO_4$. After removal of the solvent, the mixture was purified by column chromatography (hexanes–EtOAc, 5 : 1) and then recrystallized (CH_2Cl_2–hexanes) to give **3a** (175 mg, 0.50 mmol, 50%) as a colorless solid. Mp. 152–153 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 6 H), 2.42 (s, 2 H), 2.53 (s, 3 H), 2.81 (s, 2 H), 7.65 (dd, J = 4.39, 1.28 Hz, 1 H), 7.80–7.83 (m, 2 H). ^{13}C-Apt (100 MHz, $CDCl_3$): δ 13.28, 28.40 (2C), 35.94, 37.45, 52.19, 116.16, 116.95, 117.88, 123.85, 128.11, 128.14, 134.25, 138.35, 148.92, 150.99, 193.00. HRMS (ESI) calcd for $C_{17}H_{17}N_3OBr$: 358.0555 $[M + H]^+$; Found 358.0563.

2-({2-[Bis(pyridin-2-ylmethyl)amino]ethyl}amino)-5-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)benzonitrile (5). A Schlenk flask was charged with compound **3a** (96.3 mg, 0.275 mmol, 1.00 equiv.), BPEA[11] (66.4 mg, 0.274 mmol, 1.00 equiv.), sodium *tert*-butoxide (96 mg, 1.00 mmol, 2.60 equiv.), palladium chloride (10 mg, 0.06 mmol, 0.20 equiv.), 1,1′-bis-(diphenylphosphanyl)ferrocene (DPPF; 48 mg, 0.086 mmol, 0.31 equiv.), and toluene (8 mL) under argon. The flask was immersed in an oil bath at 100 °C with stirring until the starting material had completely disappeared as judged by TLC analysis. The solution was then allowed to cool to room temperature, diluted with DCM (100 mL), filtered through Celite, and concentrated. The crude product was purified by column chromatography (CH_2Cl_2–MeOH, 40 : 1) on silica gel to give **5** (93 mg, 0.18 mmol, 66%) as a dark-red viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (s, 6 H), 2.38 (s, 2 H), 2.52 (s, 3H), 2.67 (s, 2 H), 2.96 (t, J = 5.60 Hz, 2 H), 3.28 (s, 2H), 3.93 (s, 4 H), 6.11 (bs, 1 H), 6.62 (d, J = 9.00 Hz, 1 H), 7.18 (dd, J = 6.70, 5.50 Hz, 1 H), 7.42 (dd, J = 9.00, 2.50 Hz, 1 H), 7.59 (d, J = 7.80 Hz, 2 H), 7.71 (td, J = 7.70, 1.50 Hz, 2 H), 8.58 (d, J = 4.30 Hz, 2 H). ^{13}C-Apt (100 MHz, CDCl3): δ 13.25, 28.38, 28.42, 29.66, 35.75, 36.82, 40.40, 51.58, 52.35, 60.15, 95.55, 111.32, 116.51, 117.02, 122.28, 122.32, 123.27, 123.29, 127.41, 130.15, 130.19, 131.25, 136.86 (2 C), 148.84, 149.10, 149.12, 149.62, 149.92, 158.69, 193.20 ppm. HRMS (ESI) calcd for $C_{31}H_{34}N_7O$: 520.2825 $[M + H]^+$; Found 520.2834.

2-({2-[Bis(pyridin-2-ylmethyl)amino]ethyl}amino)-5-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)benzamide (CdABA). KOH (70 mg, 1.24 mmol, 7.75 equiv.) and compound **5** (84.6 mg, 0.16 mmol, 1.00 equiv.) were added to a solution of EtOH and DMSO (4 : 1, 3.50 mL). The reaction mixture was stirred in a 50 °C oil bath, and H_2O_2 (30%, 0.4 mL) was slowly added dropwise through a syringe over 0.5 h. Then the resulting solution was stirred for another 3.5 h until the disappearance of benzonitrile, as shown by TLC. After removal of the solvent by rotary evaporation, the mixture was diluted with DCM (150 mL), washed with saturated NaCl solution (3 × 30 mL), dried with $MgSO_4$, filtered, and concentrated. The mixture was purified by column chromatography (CH_2Cl_2–MeOH, 20 : 1) to give CdABA (73.4 mg, 0.136 mmol, 82%) as a colorless solid. Mp. 235–236 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08 (s, 6 H), 2.35 (s, 2 H), 2.52 (s, 3H), 2.65 (s, 2 H), 2.95 (t, J = 5.90 Hz, 2 H), 3.36 (t, J = 6.00 Hz, 2H), 3.97 (s, 4 H), 6.65 (d, J = 8.90 Hz, 1 H), 7.13–7.20 (m, 2 H), 7.23 (dd, J = 8.90, 2.10 Hz, 1 H), 7.55 (d, J = 2.10 Hz, 1 H), 7.65–7.80 (m, 4 H), 8.53 (d, J = 4.50 Hz, 2 H). ^{13}C-Apt (100 MHz, $CDCl_3$): δ 13.31, 28.34 (2C), 35.66, 36.68, 40.41, 52.31, 52.55, 60.41 (2C), 111.77, 113.77, 116.15, 122.11 (2C), 123.81 (2C), 124.90, 125.66, 128.71, 136.66 (2C), 148.82 (2C), 149.08, 149.18, 149.46, 159.07 (2C), 171.06, 193.43. HRMS (ESI) calcd for $C_{31}H_{35}N_7O_2Na$: 560.2750 $[M + Na]^+$; Found 560.2740.

2-Bromo-5-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-2-yl)benzonitrile (3b). Compound **1** (170 mg, 1.00 mmol, 1.00 equiv.) was dissolved in DMF, and 2-bromo-5-fluorobenzonitrile (**2**, 200 mg, 1.00 mmol, 1.00 equiv.) and Cs_2CO_3 (648 mg, 2.00 mmol, 2.00 equiv.) were added. The reaction mixture was stirred at room temperature for 30 min and then heated in an

80 °C oil bath until the starting material had been completely consumed as detected by TLC. The solution was then allowed to cool to room temperature, and the DMF was evaporated in vacuum to leave a yellowish oil. The crude oil was then diluted with DCM (150 mL), washed with saturated NaCl solution (3 × 30 mL), and dried with $MgSO_4$. After removal of the solvent, the mixture was purified by column chromatography (hexanes–EtOAc, 6 : 1) and then recrystallized (CH_2Cl_2–hexanes) to give **3b** (66.4 mg, 0.19 mmol, 19%) as a colorless solid. Mp. 152–153 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 6 H), 2.40 (s, 2 H), 2.66 (s, 3 H), 2.73 (s, 2 H), 7.60 (dd, J = 8.70, 2.50 Hz, 1 H), 7.83 (dd, J = 5.60, 3.00, 2 H). ^{13}C-Apt (100 MHz, $CDCl_3$): δ 12.27, 28.44 (2C), 34.99, 36.87, 53.34, 116.04, 116.84, 117.01, 124.70, 129.64, 134.12, 138.26, 141.77, 156.94, 194.81. HRMS (ESI) calcd for $C_{17}H_{17}N_3OBr$: 358.0555 $[M + H]^+$; Found 358.0549.

2-({2-[Bis(pyridin-2-ylmethyl)amino]ethyl}amino)-5-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-2-yl)benzamide (CdABA′). A Schlenk flask was charged with compound **3b** (78.0 mg, 0.22 mmol, 1.00 equiv.), BPEA (63.4 mg, 0.26 mmol, 1.17 equiv.), sodium *tert*-butoxide (96 mg, 1.00 mmol, 4.50 equiv.), palladium chloride (10 mg, 0.06 mmol, 0.27 equiv.), DPPF (48 mg, 0.086 mmol, 0.39 equiv.), and toluene (8 mL) under argon. The flask was immersed in an oil bath at 100 °C with stirring until the starting material had completely disappeared as judged by TLC analysis. The solution was then allowed to cool to room temperature, was diluted with DCM (100 mL), filtered through Celite, and concentrated. The crude product was then purified further by column chromatography (CH_2Cl_2–MeOH, 40 : 1) on silica gel to give **6** (61.6 mg, 0.12 mmol, 54%) as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (s, 6 H), 2.37 (s, 2 H), 2.52 (s, 3H), 2.70 (s, 2 H), 2.96 (t, J = 5.60 Hz, 2 H), 3.28 (s, 2H), 3.94 (s, 4 H), 6.12 (bs, 1 H), 6.61 (d, J = 9.00 Hz, 2 H), 7.18 (dd, J = 6.70, 5.50 Hz, 2 H), 7.37 (dd, J = 9.00, 2.50 Hz, 1 H), 7.46 (d, J = 2.50 Hz, 1 H) 7.60 (d, J = 7.80 Hz, 2 H), 7.71 (td, J = 7.70, 1.60 Hz, 2 H), 8.57 (d, J = 4.70 Hz, 2 H). Then, by using the same procedure as that described for the preparation of CdABA, compound **6** was hydrated with KOH catalyzed by H_2O_2 to give CdABA′ in 90% yield as a colorless solid. Mp. 235–236.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (s, 6 H), 2.37 (s, 2 H), 2.50 (s, 3H), 2.70 (s, 2 H), 2.93 (t, J = 5.70 Hz, 2 H), 3.33 (t, J = 5.80 Hz, 2H), 3.94 (s, 4 H), 5.87 (bs, 2 H), 6.63 (d, J = 8.90 Hz, 1 H), 7.14–7.18 (m, 2 H), 7.24 (dd, J = 8.90, 2.40 Hz, 1 H), 7.48 (d, J = 2.40 Hz, 1 H), 7.68 (t, J = 7.60 Hz, 2 H), 7.76 (d, J = 7.80 Hz, 2 H), 8.52 (d, J = 4.70 Hz, 2 H). ^{13}C-Apt (100 MHz, $CDCl_3$): δ 11.97, 28.49 (2C), 29.67, 35.08, 40.32, 52.51, 53.29, 60.35 (2C), 111.80, 113.52, 115.51, 122.21 (2C), 123.41 (2C), 125.50, 125.83, 130.05, 136.78 (2C), 141.76, 148.78 (2C), 149.70, 155.73, 158.83 (2C), 170.90, 194.90. HRMS (ESI) calcd for $C_{31}H_{36}N_7O_2$: 538.2930 $[M + H]^+$; Found 538.2929.

2-Bromo-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-2-yl)benzonitrile (8b). Compound **1** (250 mg, 1.47 mmol, 1.00 equiv.) was dissolved in DMF, and 2-bromo-4-fluorobenzonitrile (**7**; 354 mg, 1.77 mmol, 1.20 equiv.) and Cs_2CO_3 (955 mg, 2.94 mmol, 2.00 equiv.) were added. The reaction mixture was stirred at room temperature for 30 min and then heated in a 40 °C oil bath until the starting material had been completely consumed as detected by TLC. The solution was then allowed to cool to room temperature, and the DMF was evaporated under vacuum to leave a yellowish oil. The crude oil was then diluted with DCM (150 mL), washed with saturated NaCl solution (3 × 30 mL), and dried with $MgSO_4$. After removal of the solvent, the mixture was purified by column chromatography (hexanes–EtOAc, 5 : 1) and then recrystallized (CH_2Cl_2–hexanes) to give **8b** (150 mg, 0.42 mmol, 28%) as a colorless solid. Mp. 159–160 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 6 H), 2.40 (s, 2 H), 2.70 (s, 3 H), 2.73 (s, 2 H), 7.57 (dd, J = 8.40, 2.00 Hz, 1 H), 7.78 (d, J = 8.40, 1 H), 7.93 (d, J = 1.90, 1 H). ^{13}C-Apt (100 MHz, $CDCl_3$): δ 12.50, 28.41 (2C), 34.91, 36.68, 53.36, 114.99, 116.40, 117.15, 123.07, 126.06, 128.85, 134.77, 142.07, 142.67, 157.13, 194.73. HRMS (ESI) calcd for $C_{17}H_{17}N_3OBr$: 358.0555 $[M + H]^+$; Found 358.0556.

2-({2-[Bis(pyridin-2-ylmethyl)amino]ethyl}amino)-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-2-yl)benzamide (ZnABA′). A Schlenk flask was charged with compound **8b** (115.5 mg, 0.33 mmol, 1.20 equiv.), BPEA (66.7 mg, 0.275 mmol, 1.00 equiv.), sodium *tert*-butoxide (96 mg, 1.00 mmol, 3.60 equiv.), palladium chloride (10 mg, 0.06 mmol, 0.22 equiv.), DPPF (48 mg, 0.086 mmol, 0.31 equiv.), and toluene (8 mL) under argon. The flask was immersed in an oil bath at 100 °C with stirring until the starting material had completely disappeared as judged by TLC analysis. The solution was then allowed to cool to room temperature, was diluted with DCM (100 mL), filtered through Celite, and concentrated. The crude product was then purified further by column chromatography (CH_2Cl_2–MeOH, 40 : 1) on silica gel to give **9** (73.9 mg, 0.14 mmol, 53%) as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (s, 6 H), 2.38 (s, 2 H), 2.60 (s, 3H), 2.71 (s, 2 H), 2.93 (t, J = 5.60 Hz, 2 H), 3.27 (d, J = 3.70 Hz, 2H), 3.91 (s, 4 H), 6.09 (bs, 1 H), 6.66–6.68 (m, 2 H), 7.16 (dd, J = 6.70, 5.60 Hz, 2 H), 7.49 (d, J = 8.20 Hz, 1 H), 7.58 (d, J = 7.80 Hz, 2 H), 7.69 (td, J = 7.70, 1.5 Hz, 2 H), 8.55 (d, J = 4.80 Hz, 2 H). Then, by using the same procedure as that described for the preparation of CdABA, compound **9** was hydrated with KOH catalyzed by H_2O_2 to give **ZnABA′** in 90% yield as a colorless solid. Mp. 198–199 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 6 H), 2.38 (s, 2 H), 2.57 (s, 3H), 2.72 (s, 2 H), 2.90 (t, J = 6.00 Hz, 2 H), 3.30 (t, J = 6.00 Hz, 2H), 3.90 (s, 4 H), 5.93 (bs, 2 H), 6.60 (d, J = 8.3 Hz, 1 H), 7.08–7.18 (m, 2 H), 7.49 (d, J = 8.30 Hz, 1 H), 7.65 (t, J = 7.00 Hz, 2 H), 7.72 (d, J = 7.70 Hz, 2 H), 8.48 (d, J = 4.50 Hz, 2 H). ^{13}C-Apt (100 MHz, $CDCl_3$): δ 12.32, 28.48 (2C), 35.02, 36.99, 40.45, 52.57, 53.40, 60.37 (2C), 108.04, 110.68, 113.43, 116.19, 122.09 (2C), 123.29 (2C), 129.28, 136.64 (2C), 141.68, 142.61, 148.80 (2C), 150.51, 156.08, 159.04 (2C), 171.04, 194.93. HRMS (ESI) calcd for $C_{31}H_{36}N_7O_2$: 538.2930 $[M + H]^+$; Found 538.2923.

Spectroscopic materials and methods. Stock solutions (0.001 M) of zinc/cadmium perchlorate were prepared in HEPES buffer (25 mM HEPES, 0.1 M $NaClO_4$, pH = 7.4, I = 0.1). Stock solutions (0.001 M) of ZnABA′, CdABA and CdABA′ were prepared in EtOH. All the fluorescence spectra of ZnABA′, CdABA and CdABA′ were also measured in HEPES buffer [25 mM HEPES, 0.1 M $NaClO_4$, pH = 7.4, I = 0.1, 1% (v/v) EtOH] and

the excitation wavelength was 260 nm with excitation and emission slit widths of 5 nm at room temperature. The dissociation constants of the complexes between metal ions and the probes were determined according to the literature procedures.[3c,h,12]

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