

Synthesis and fluorescence characteristics of selective ratiometric fluorescent sensors for silver ions based on 3-indolyl-4-indazolyl maleimide derivatives

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Abstract Selective ratiometric fluorescent sensor **b** for detection of silver ions and its counterparts (sensor **a and c**) were efficiently synthesized based on 3-indolyl-4-indazolyl maleimide, and their photophysical properties and activities towards metal ions were examined. The experimental results were verified by density functional theory (DFT) and time-dependent DFT (TDDFT) calculations. The results demonstrated that sensor **b** could selectively and sensitively recognize Ag^+ compared with other competing metal ions in tetrahydrofuran (THF). It probably forms a 1:1 complex with Ag^+ , displaying a 52-nm bathochromic shift of the fluorescence emission spectrum. Moreover, the binding mechanism was investigated using binding stoichiometry titration and ¹H, ¹³C nuclear magnetic resonance (NMR).

Keywords Ratiometric fluorescent sensor · Bathochromic shift · Maleimide derivatives · DFT calculations

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Introduction

Methods for monitoring heavy transition metals (HTMs) have been developed continuously at an unabated pace for use in various chemical [1], biological [2], environmental [3, 4], and physiological [5] application fields. Traditional techniques for HTM detection include atomic absorption spectroscopy (AAS) [6], flame atomic absorption spectrometry (FAAS) [7], capillary electrophoresis (CE) [8], inductively coupled plasma–atomic emission spectroscopy (ICP-AES) [9], inductively coupled plasma–atomic emission spectroscopy (ICP-AES) [9], inductively coupled plasma–atomic (ICP-MS) [10], inductively coupled plasma–optical emission spectrometry (ICP-OES) [11], and flow injection analysis (FIA) [12]. Compared with such conventional instrumental techniques, which are relatively expensive and time-consuming, fluorescent chemosensors, which can be used for selective detection of HTM, have attracted unprecedented attention. This can be attributed to their high sensitivity and selectivity, versatility, rapid response, low cost, simple operation, and low detection limit, and the widespread availability of equipment for such analysis [13–17].

Silver, one of the most common HTMs, has been extensively utilized together with its compounds in chemistry, pharmacy, electrical industry, as well as photographic and imaging industry [18]. Silver ions exert strong inhibitory and bactericidal effects; moreover, they exhibit broad-spectrum antimicrobial activity even at low concentration [19]. However, large doses of silver ions will inevitably lead to some negative effects, such as argyria, characterized by grayish-blue pigmentation of the skin and eye [20–22]. Silver ions can bind with sulfhydryl terminals in food via soft–soft acid–base interaction. Ratiometric [23–29], "turn-on" [30–34], and "turn-off" chemosensors [35–37] have been recognized as promising methods for detection of silver ions. Therefore, fluorescent chemosensors with high sensitivity and selectivity for detection of silver ions have become popular in the field of academic research.

In this work, novel selective ratiometric fluorescent **sensor b** for detection of silver ions and its counterparts (**sensor a and c**) were synthesized based on 3-indolyl-4-indazolyl maleimide, and their structure was characterized. The results show that the fluorescent maleimide derivatives with analogous structure exhibited large Stokes shift [38] and aggregation-induced enhanced emission [39]. Moreover, N and O atoms in maleimide might be potential binding sites for HTMs, encouraging us to explore the feasibility of their use for detection of HTMs. Furthermore, **sensor b** formed a 1:1 complex with Ag⁺ and displayed a bathochromic shift in the fluorescence emission spectrum. The binding mechanism was verified by comparing the different responses of **sensors a–c** to Ag⁺ using binding stoichiometry titration and ¹H, ¹³C NMR.

Experimental

General methods

Materials and equipments

All chemicals used in the current study were purchased from commercial vendors and used as received without further purification, unless otherwise noted. All solvents were purified and dried according to standard methods prior to use. Nuclear magnetic resonance (¹H and ¹³C) spectra were recorded using a Bruker AM 500 spectrometer (Bruker), with chemical shifts reported as ppm at 500 and 125 MHz, respectively, with CDCl₃ as internal standard. Melting points (m.p.) were recorded by an Electrothermal X-4 digital melting point apparatus.

Absorbance, emission, and quantum yield

Ultraviolet–visible (UV–Vis) absorption spectra were measured using a UV-2550. Fluorescence spectra and solid emission spectra were determined using a F-7000 fluorescence spectrophotometer. The $\Phi_{\rm f}$ value in solution was determined in accordance with the general method using quinine sulfate ($\Phi = 0.55$ in 50 mM H₂SO₄ solution) as standard. Moreover, the compounds were diluted in organic solvent prior to use at final concentration of 5×10^{-5} mol L⁻¹. All solvents used in photochemical measurements were spectroscopically pure. All experiments were repeated to confirm the reproducibility of the results.

Theoretical calculations

All structures were optimized based on density functional theory (DFT) using the B3LYP functional and 6-311G basis sets. The fluorescence emission and excitation wavelengths of the compounds were calculated in accordance with time-dependent density functional theory (TDDFT). All calculations were performed using Gaussian 09W [40, 41].

Synthesis

Synthesis of 3-chloro-4-(1H-indol-3-yl)-1-phenyl-1H-pyrrole-2,5-dione (1)

To a stirred mixture of 1.8 g (75 mmol) Mg and 0.1 g (catalytic amount) iodine in degassed anhydrous ether (15 mL), 9.8 g (90 mmol) bromoethane in anhydrous ether (20 mL) was added dropwise under nitrogen atmosphere. After addition, the mixture was stirred under reflux for 30 min. Then, 9.1 g (78 mmol) indole in anhydrous benzene (10 mL) was added dropwise to the mixture. After stirring for 30 min at reflux temperature, indole Grignard reagent was obtained. To a stirred solution of 12.2 g (50 mmol) 3,4-dichloro-1-phenyl-1*H*-pyrrole-2,5-dione and anhydrous THF (20 mL), indole Grignard reagent prepared above was added

dropwise at room temperature. After stirring at room temperature for 4 h, the reaction mixture was poured into solution of HCl in water (1 M, 100 mL), extracted using ethyl acetate (3 × 100 mL), washed with saturated NaCl solution (3 × 300 mL), concentrated to give crude product under reduced pressure, and recrystallized from ethyl acetate to yield 11.8 g (73%) compound 1 as red solid. M.p. 203–204 °C. ¹H NMR (500 MHz, DMSO-d₆) δ (ppm): 7.19–7.26 (2H, m), 7.45–7.54 (6H, m), 7.97 (d, J = 8.0 Hz, 1H), 8.13 (1H, s), 12.18 (1H, s).

Synthesis of 3-(1H-indol-3-yl)-4-(1-methyl-1H-indazol-3-yl)-1-phenyl-1H-pyrrole-2,5-dione (2)

A mixture of 1.27 g (3.5 mmol) 1, 1.47 g (3.5 mmol) 1-methyl-3-(tributylstannyl)-1H-indazole, 0.1 g (catalytic amount) anhydrous LiCl, and 0.1 g (catalytic amount) Pd(Ph₃)₂Cl₂ in anhydrous toluene (100 mL) was stirred for 1.5 h at reflux temperature under nitrogen atmosphere. After cooling to room temperature, the reaction mixture was poured into water (100 mL) and extracted using ethyl acetate (3×100 mL). The combined organic phase was washed with saturated NaCl solution $(3 \times 300 \text{ mL})$ and concentrated to give crude product under reduced pressure, which was purified by silica gel chromatography elution (silica gel, ethyl acetate:petroleum ether = 1:4, v/v) to afford 1.28 g (77%) **2** as red solid. M.p. > 250 °C. ¹H NMR (500 MHz, CDCl₃) δ (ppm): 9.12 (s, 1H), 8.06 (d, J = 2.9 Hz, 1H), 7.76 (d, J = 8.3 Hz, 1H), 7.60–7.50 (m, 4H), 7.47-7.37 (m, 3H), 7.35-7.25 (m, 1H), 7.14-7.08 (m, 2H), 6.74 (t, J = 7.5 Hz, 1H), 6.37 (d, J = 8.1 Hz, 1H), 4.02 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 169.1 (1C), 168.7 (1C), 139.4 (1C), 135.6 (1C), 133.5 (1C), 133.2 (1C), 131.2 (1C), 131.0 (1C), 127.7 (2C), 126.4 (1C), 125.4 (2C), 125.3 (1C), 123.8 (1C), 122.3 (1C), 121.1 (1C), 120.8 (1C), 120.4 (1C), 120.2 (1C), 119.9 (1C), 118.9 (1C), 110.9 (1C), 108.2 (1C), 104.5 (1C), 34.8 (1C). HRMS (ESI) m/z calcd. for $C_{26}H_{19}N_4O_2^+$ (M + H)⁺ 419.15025, found 419.15057.

Synthesis of 3-(1-methyl-1H-indol-3-yl)-4-(1-methyl-1H-indazol-3-yl)-1-phenyl-1H-pyrrole-2,5-dione (sensor a)

To solution of 105 mg (0.25 mmol) **2** in anhydrous DMF (20 mL), 10.5 mg (60%, 0.26 mmol) NaH was added at 0 °C. After addition, the mixture was stirred at room temperature for 1 h. Then, 71 mg (0.5 mmol) CH₃I in anhydrous DMF (5 mL) was added dropwise at 0 °C; after stirring at room temperature for 4 h, the reaction mixture was poured into water (100 mL) and extracted using ethyl acetate (3 × 100 mL). The combined organic phase was washed with saturated NaCl solution (3 × 100 mL) and concentrated to give crude product under reduced pressure. The crude product was purified by silica gel chromatography elution (silica gel, ethyl acetate:petroleum ether = 1:5, v/v) to afford 79.6 mg (74%) **3** as red solid. M.p. > 250 °C. ¹H NMR (500 MHz, CDCl₃) δ (ppm): 8.17 (s, 1H), 7.79 (d, *J* = 8.3 Hz, 1H), 7.58–7.50 (m, 4H), 7.47–7.37 (m, 3H), 7.29 (d, *J* = 7.4 Hz, 1H), 7.18–7.10 (m, 2H), 6.77–6.71 (m, 1H), 6.31 (d, *J* = 8.1 Hz, 1H), 4.00 (s, 3H), 3.90 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 169.2 (1C), 168.0 (1C), 139.3 (1C), 135.8 (1C), 133.7 (1C), 133.4 (1C), 132.0 (1C), 131.9 (1C), 127.8 (2C), 126.4 (1C), 125.5 (2C), 125.2 (1C), 123.6 (1C), 122.4

(1C), 121.1 (1C), 120.6 (1C), 120.4 (1C), 120.3 (1C), 119.8 (1C), 118.1 (1C), 110.7 (1C), 108.4 (1C), 104.6 (1C), 39.6 (1C), 34.6 (1C). HRMS (ESI) *m*/*z* calcd. for $C_{27}H_{21}N_4O_2^+$ (M + H)⁺ 433.16590, found 433.16577.

Synthesis of 3-(1-methyl-1H-indazol-3-yl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole- 2,5-dione (sensor b)

A mixture of 43 mg (0.1 mmol) **sensor a** and 2 g (overdose) NH₄OAc was heated for 6 h at 140 °C under nitrogen environment. After cooling, the mixture was poured into water (100 mL), adjusted to weak alkalinity with Na₂CO₃, and extracted using ethyl acetate (3 × 100 mL). The organic layer was washed with saturated NaCl solution (3 × 300 mL), dried over Na₂SO₄, and concentrated in vacuum. Silica gel chromatography elution was used to wash the crude product [silica gel, dichloromethane (DCM):methanol = 200:1, v/v] to afford 23.7 mg **sensor b** (yield, 67%) as red solid. M.p. 202–203 °C. ¹H NMR (500 MHz, CDCl₃) δ (ppm): 8.10 (s, 1H), 7.70–7.53 (m, 1H), 7.60 (s, 1H), 7.48–7.36 (m, 2H), 7.31–7.23 (m, 1H), 7.11 (d, *J* = 8.2 Hz, 2H), 6.71 (t, *J* = 7.6 Hz, 1H), 6.24 (d, J = 8.1 Hz, 1H), 4.01 (s, 3H), 3.89 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 172.1 (1C), 171.9 (1C), 140.1 (1C), 136.7 (1C), 134.7 (1C), 134.5 (1C), 134.0 (1C), 126.0 (1C), 125.1 (1C), 123.0 (1C), 122.0 (1C), 121.9 (1C), 121.6 (1C), 121.4 (1C), 120.6 (1C), 119.7 (1C), 109.5 (1C), 108.9 (1C), 104.4 (1C), 35.5 (1C), 33.0 (1C). HRMS (ESI) *m/z* calcd. for C₂₁H₁₇N₄O₂⁺ (M + H)⁺ 357.13460, found 357.13455.

Synthesis of 1-methyl-3-(1-methyl-1H-indazol-3-yl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione (sensor c)

A mixture of 43 mg (0.1 mmol) **sensor a** and 6 mL 32% CH₃NH₂ in ethanol solution was stirred for 6 h at room temperature. The solvent was removed in vacuum, and silica gel chromatography elution was used to wash the crude product (silica gel, DCM:methanol = 200:1, v/v) to afford 25.2 mg **sensor c** (yield, 68%) as red solid. M.p. 241–242 °C. ¹H NMR (500 MHz, CDCl₃) δ (ppm): 8.10 (s, 1H), 7.68 (d, J = 8.2 Hz, 1H), 7.45–7.34 (m, 2H), 7.29 (d, J = 8.6 Hz, 1H), 7.18–7.03 (m, 2H), 6.71 (t, J = 7.6 Hz, 1H), 6.26 (d, J = 8.1 Hz, 1H), 4.00 (s, 3H), 3.87 (s, 3H), 3.23 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ (ppm): 171.9 (1C), 171.5 (1C), 140.8 (1C), 137.4 (1C), 135.2 (1C), 135.1 (1C), 133.8 (1C), 126.51 (1C), 122.6 (1C), 122.7 (1C), 122.5 (1C), 122.4 (1C), 35.9 (1C), 33.4 (1C), 24.2 (1C). HRMS (ESI) m/z calcd. for C₂₂H₁₉N₄O₂⁺ (M + H)⁺ 371.15025, found 371.15012.

Results and discussion

Synthesis

As shown in Scheme 1, according to reported literature [39-43], efficient reactions were used to synthesize the target compounds **sensor a-c** based on 3-indolyl-4-



Scheme 1 Synthesis of compounds sensor a-c

indazolyl maleimide. Using 3,4-dichloro-1-phenyl maleimide as raw material, **sensor a** was synthesized by Grignard reaction (73% yield), Stille coupling reaction (77% yield), and *N*-methyl reaction (74% yield). **Sensor b** and **c** were synthesized from **sensor a** via ammonolysis reaction in 67 and 68% yield, respectively. The structures of compounds **sensor a–c** were further adequately characterized by ¹H NMR, ¹³C NMR, and HRMS.

UV-Vis absorption and fluorescence emission spectra

The fluorescence properties of **sensor** \mathbf{a} - \mathbf{c} were examined in different organic solvents. The photophysical properties of **sensor** \mathbf{a} in different solutions are presented in Fig. 1 and Table 1, while those of **sensor** \mathbf{a} - \mathbf{c} in toluene are presented in Fig. 2 and Table 1.

UV-Vis and fluorescence in solution

The final products **sensor a–c** exhibited favorable stability and solubility in solvents including EtOH, DCM, DMF, THF, toluene, and chloroform. The impact of solvents on the absorption and fluorescence properties of **sensor a** was investigated and is presented in Fig. 1 and Table 1. A sharp absorption peak can be observed for **sensor a** at 454 nm. Besides, it was also seen that the polarity of the solvent had the least effect on the maximum absorption wavelength (λ_{max}) of **sensor a**, suggesting that the dipole moment of the molecules was more or less similar in both excited and ground state [44]. **Sensor a** showed weak fluorescence in protonic solvents. The molar absorption coefficient (ε) of **sensor a** depended on the solvent used, with the most impressive values seen in toluene and THF. Additionally, the fluorescence quantum yield (Φ_f) of **sensor a** reached its highest value of 0.38 in toluene.



Fig. 1 Absorption spectra (a) and fluorescence emission spectra (b) of sensor a $(1 \times 10^{-5} \text{ mol } \text{L}^{-1})$ in different solvents (UV-2550, PMT voltage: 700 V)

Compound	Solvent	$\epsilon (M^{-1} cm^{-1})$	λ_{abs} (nm)	$\lambda_{\rm em}$ (nm)	Stokes shift (nm)	$\Phi_{ m f}$
Sensor a	DCM	7400	454	580	127	0.22
	CHCl ₃	6000	462	584	122	0.30
	EA	8700	447	562	116	0.12
	EtOH	5900	459	589	130	0.00
	Et ₂ O	6900	443	552	108	0.28
	THF	6100	456	562	106	0.25
	DMF	4800	450	586	136	0.03
	CH ₃ CN	5900	447	581	133	0.02
	Toluene	6800	459	566	107	0.38
Sensor b	Toluene	4000	448	543	95	0.27
Sensor c	Toluene	3200	450	552	102	0.42

Table 1 Photophysical properties for compounds sensor a-c

Fluorescence quantum yields measured with quinine sulfate ($\Phi_f = 54.6\%$ in 0.05 M H₂SO₄) as reference

Therefore, toluene was conjectured to be the best solvent when mixed with 3-indolyl-4-indazolyl maleimide.

Various substituents were integrated with the N atom in the maleimide ring of **sensor a-c** to study the effect of the substituent group. As seen from Fig. 2 and Table 1, compared with **sensor b** and **c**, **sensor a** displayed a conspicuous bathochromic shift of around 10 nm, seen in both the absorption and fluorescence emission spectra. Such a phenomenon was recorded when applying the particular substituent *N*-phenyl on the maleimide ring, thus demonstrating that the large conjugated framework contributed to the bathochromic shift of 3-indolyl-4-indazolyl maleimide compounds. Solutions of **sensor a-c** exhibited large Stokes shift of 107, 95, and 102 nm, respectively. Moreover, *N*-phenyl-substituted **sensor a** manifested greater Stokes shift on the maleimide ring compared with **sensor b** and **c**. Table 1 shows the greatest Stokes shift of **sensor a** at wavelength of 107 nm, with excitation and emission wavelength of 459 and 566 nm, respectively. These



Fig. 2 Absorption spectra (a) and fluorescence emission spectra (b) of compounds sensor a-c $(1 \times 10^{-5} \text{ mol } L^{-1})$ in toluene (UV-2550, PMT voltage: 700 V)

results demonstrate that compounds with relatively large conjugated framework were associated with larger Stokes shifts. Meanwhile, the $\Phi_{\rm f}$ value of **sensor a–c** was analyzed, being calculated to be 38, 27, and 42%, respectively.

DFT calculations to rationalize the excitation and emission spectra

Density functional theory (DFT) quantum calculations were carried out using the optimized geometry of each molecule. The ground-state geometry was optimized in gas phase at B3LYP/6-31G level of theory [40, 41]. According to the results of the DFT calculations, the molecular system of **sensor c** showed nearly planar structure (Fig. 3), enhancing the extent of conjugation. As a result, **sensor c** exhibited the highest fluorescence quantum yield among all the compounds. The absorption and emission spectra were calculated using time-dependent DFT (TD-DFT). Note that the values of the experimental absorption and emission spectra were close to the calculation results (Table 2).

Fluorescence spectral studies of sensors a-c with Ag⁺

The fluorescence characteristics of **sensor a–c** were investigated in THF after addition of several metal cations (Ag⁺, Li⁺, Cu²⁺, Zn²⁺, Hg²⁺, Ni²⁺, Al³⁺, Fe³⁺, and Cr³⁺) by fluorescence titration. The fluorescence response of **sensor a–c** $(1.0 \times 10^{-5} \text{ mol/L})$ to the above-mentioned metal cations (10 eq) is illustrated in Fig. 4. Notably, the fluorescence emission spectrum ($\lambda_{em} = 540 \text{ nm}$) of **sensor b** displayed a bathochromic shift from 540 to 592 nm in presence of Ag⁺, but did not show any obvious changes in presence of other metal cations. This can be attributed to formation of metal–organic coordinating clathrate between **sensor b** and Ag⁺. However, no significant changes in the fluorescence emission spectrum of **sensor a** and **c** was seen (substituted with *N*-alkyl and *N*-phenyl on maleimide ring) even in the presence of any metal cation. Therefore, the coordinating position between Ag⁺



Fig. 3 Optimized geometry of compounds sensor a-c in different orientations

Compound	λ_{abs}^{a} (nm, cal)	$\lambda_{abs}^{\ \ b}$ (nm, exp)	$f_{\rm abs}{}^{\rm c}$	λ_{em}^{a} (nm, cal)	λ_{em}^{b} (nm, exp)	$f_{\rm em}{}^{\rm c}$
Sensor a	468	454	0.1250	570	566	0.1511
Sensor b	458	437	0.0675	547	543	0.2541
Sensor c	436	444	0.0774	535	553	0.0918

Table 2 Calculation results for sensors a-c

^aTheoretical calculations using B3LYP/6-31G basis set without solution

^bIn toluene solvent

^cOscillator strength coefficients

and **sensor b** could be located in the imidogen radical of maleimide ring of **sensor b**.

Moreover, the possible mode of interaction between **sensor b** and Ag^+ was determined from the experimental results, as confirmed by the ion titration method and ¹H NMR spectroscopy. These observations indicated that **sensor b** could be used as a chemosensor for Ag^+ .

Stoichiometry and sensing mechanism of Ag⁺

Figure 5 shows a peak in the absorption spectrum of **sensor b** at wavelength of about 445 nm. Furthermore, a 15-nm bathochromic shift in the absorption peak from 445 to 461 nm was observed when $Ag^+ (2.0 \times 10^{-6}-2.0 \times 10^{-5} \text{ mol/L})$ was gradually added to the solution of **sensor b** $(1 \times 10^{-5} \text{ mol/L})$. The fluorescence spectrum of **sensor b** showed a maximum emission peak at nearly 540 nm, associated with a 52-nm bathochromic shift from 540 to 592 nm as $Ag^+ (2.0 \times 10^{-6}-2.0 \times 10^{-5} \text{ mol/L})$ was gradually added. Both the maximum absorption wavelength and fluorescence emission wavelength displayed negligible changes



Fig. 4 Fluorescence spectra of **sensor a** (**a**), **sensor b** (**b**), and **sensor c** (**c**) $(1.0 \times 10^{-5} \text{ mol/L})$ in presence of different metal ions (10 eq) in THF/H₂O (200:1, v/v) (PMT voltage: 700 V), and **d sensor b** $(1.0 \times 10^{-5} \text{ mol/L})$ in presence of different metal ions (10 eq) in THF/H₂O (200:1, v/v) under UV lamp (365 nm)



Fig. 5 Absorption (a) and fluorescence (b) response of **sensor b** $(1 \times 10^{-5} \text{ mol/L})$ exposed to Ag⁺ at various concentrations: 2.0×10^{-6} , 4.0×10^{-6} , 6.0×10^{-6} , 8.0×10^{-6} , 1.0×10^{-5} , 1.2×10^{-5} , 1.4×10^{-5} , 1.6×10^{-5} , 1.8×10^{-5} , and $2.0 \times 10^{-5} \text{ mol/L}$ (from left to right). Spectra measured in THF/H₂O (200:1, v/v) solution (PMT voltage: 700 V)



Fig. 6 Job's plot for determining the stoichiometry for sensor b ($\lambda_{abs} = 457$ nm) and Ag⁺ in THF/H₂O (200:1, v/v). Total concentration = 1.0×10^{-4} mol/L

when more Ag^+ was titrated. Therefore, **sensor b** could be an effective ratiometric fluorescent sensor for Ag^+ .

A Job's plot was constructed by fluorescence titration to determine the binding stoichiometric ratio between **sensor b** and Ag^+ and ascertain the sensing mechanism. As seen in Fig. 6, the Job's plot (with the vertical axis representing fluorescence intensity at 592 nm) showed an inflection point at 0.5 mol fraction of **sensor b**, indicating formation of a 1:1 metal–ligand complex between **sensor b** and Ag^+ . As a result, it can be speculated that the coordination between Ag^+ and **sensor b** resulted from the imidogen and the carbonyl of maleimide ring, suggesting that N and O atoms were the binding sites (Scheme 2).

The Ag⁺ sensing mechanism was further investigated using ¹H and ¹³C NMR titration. Figure 7 compares the ¹H NMR spectra of **sensor b** with and without Ag⁺. The results show that **sensor b** in mixed solvent [dimethylsulfoxide (DMSO)- $d_6/H_2O = 200:1$] displayed the proton signals of imidogen radical at 11.20 ppm, which disappeared in presence of Ag⁺ (2 eq). Figure 8 compares the ¹³C NMR spectra of **sensor b** with and without Ag⁺. The chemical shifts of the carbon atom on carbonyl were approximately 172 ppm, while that of the carbon atom on nitrogen methyl was approximately 35 ppm. However, in presence of Ag⁺, the



Scheme 2 Response mechanism of sensor b to Ag⁺



Fig. 7 ¹H NMR spectra of sensor b and sensor b with Ag⁺ (2 eq) in DMSO-*d*₆/H₂O (200:1, v/v)

chemical shift of the carbon atom on carbonyl changed to about 110 ppm, while that of the carbon atom on nitrogen methyl changed to about 20 ppm. The change in the chemical shifts of the carbon atom on carbonyl was greater than that of the carbon atom on nitrogen methyl, demonstrating that Ag^+ exerted a prominent influence on the carbonyl of **sensor b**. These observations indicate ionization of the imidogen radical, generally supporting the sensing mechanism proposed above.

Selectivity to analyte compared with competing metal ions

High selectivity to the analyte compared with competing metal ions is a key factor influencing the utilization of a fluorescent sensor for a certain metal ion. Therefore, the fluorescence intensity ratio ($I_{592 \text{ nm}}/I_{540 \text{ nm}}$) of **sensor b** ($\lambda_{abs} = 457 \text{ nm}$) was determined in presence of various metal ions, including Li⁺, Cu²⁺, Zn²⁺, Hg²⁺, Ni²⁺, Al³⁺, Fe³⁺, and Cr³⁺ and Ag⁺ (2 eq, respectively). Dramatic growth in the bars can be seen when Ag⁺ was introduced into **sensor b** in THF (Fig. 9). These results clearly demonstrate that such a chemosensor could be applied for selective detection of Ag⁺ without interference from other metal ions.



Fig. 8 13 C NMR spectra of sensor b and sensor b with Ag⁺ (2 eq) in DMSO- d_6/H_2O (200:1, v/v)

Fig. 9 Fluorescence intensity ratio $(I_{592 \text{ nm}}/I_{540 \text{ nm}})$ of **sensor b** $(1 \times 10^{-5} \text{ mol/L})$ in THF. Red bars indicate the ratio *I592* nm/*I540* nm of **sensor b** in presence of different metal ions (2 eq), while blue bars represent the ratio *I592* nm/*I540* nm of **sensor b** in presence of different metal ions and Ag⁺ (2 eq) (with mean and standard deviation computed using data from three parallel experiments)



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Conclusions

A novel, selective ratiometric fluorescent **sensor b** for detection of silver ions and its counterparts were developed based on 3-indolyl-4-indazolyl maleimide. Photophysical measurements on the maleimide compounds showed that they exhibit conspicuous fluorescence in solution. **Sensor b** showed excellent selectivity and sensitivity for Ag^+ compared with other competing metal ions. An obvious bathochromic shift (52 nm) of the fluorescence emission was observed on addition of silver ion. Therefore, **sensor b** is appropriate for application in biomedical and environmental detection.

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