

# Ratiometric fluorescence and colorimetric sensing of anion utilizing simple Schiff base derivatives

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**Abstract** Two ratiometric fluorescence and colorimetric anion sensors were designed and synthesized according to simple Schiff base reaction. Two compounds **1** and **2** were characterized by ESI–MS, elemental analyses and  $^1\text{H}$  NMR. The sensors could give fast and visible color changes from yellow to red upon presence of the strong basic anions such as acetate ion. In particular, two compounds exhibited marked blue shifts (about 136 nm) in their emission spectra, when interacting with anions. Accordingly, the compounds **1** and **2** could act as real-time ratiometric fluorescence and colorimetric sensors for anions.

**Keywords** Ratiometric fluorescence · Phenol moiety · Schiff base · Hydrazone · Colorimetric

## Introduction

As anions are ubiquitous throughout biological systems and play crucial roles in the areas of medicinal, biochemistry, and environmental chemistry, more and more attentions have been paid to the design and synthesis of the receptors

that selectively recognize specific anions such as fluoride, carboxylate, cyanide, and phosphate [1–4]. For example, phosphorylated species are biologically relevant anions and play critical roles in a variety of fundamental processes such as genetic information storage, energy transduction, signal processing, and membrane transport [5–7]. In addition, it is for sure that phosphate originating from the over use of agricultural fertilizers can also lead to eutrophication in inland waterways [8]. In particular, acetate and dicarboxylates are involved in numerous metabolic processes [9]. Organic decomposition in marine sediments can be indicated frequently by acetate production and oxidation rate [10]. The recognition and detection of anions is extremely necessary owing to that anions play so many positive and negative effects in the lives [11].

Among the methods of signaling binding events, fluorescence is becoming increasingly important as a method for chemical trace detection due to its simplicity and high sensitivity [12–14]. Accordingly, the sensors based on anion-induced changes in fluorescence appear to be particularly attractive because they offer the potential for high sensitivity at low analyte concentration [15]. Fluorescence signaling by turn-on (i.e., fluorescence enhancement) or shifts in excitation and/or emission wavelength is favored over turn-off response (i.e., fluorescence quenching), which has concomitant lower signal-to-noise ratios [16]. However, fluorescent sensors that respond to analyte binding by spectral shifts in the excitation and/or emission spectrum are superior for quantitative measurements [17]. Unfortunately, electron-rich anions interact with fluorescence sensors in the excited state will promote photo-induced electron transfer (PET), resulting fluorescence quenching of the fluorophore [18]. Accordingly, most of the fluorescence anion sensors reported in the literature are based on PET mechanism and showed turn-off responses to anions.

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It is still extremely necessary for designing new ratiometric fluorescence sensors for biologically important anions.

In general, ratiometric fluorescence sensors for anions are based on photo-induced charge transfer (PCT) [19], excimer/exciple [20], fluorescence resonance energy transfer (FRET) [21], excited-state intramolecular proton transfer (ESIPT) [22] and so forth. However, unexpectedly, these strategies have not been widely applied, in contrast to the situation in which large numbers of turn-on/off sensors have been reported frequently in recent years. Developing the ratiometric fluorescence sensors with these signaling mechanisms generally needs complicated synthetic steps, which may be the major reason for ratiometric fluorescence anion sensors reported rarely. In this paper, two simple ratiometric anion fluorescence sensors based on anion-induced inhibition of ESIPT were designed and prepared according to easy synthesis method. Significant blue shifts in emission (about 136 nm from 540 to 414 nm) were observed for two sensors as a result of inhibition of ESIPT.

## Experimental

### Apparatus and materials

$^1\text{H}$  NMR spectra were obtained on a Varian UNITY Plus-300 MHz Spectrometer. ESI-MS was performed with a MARINER apparatus. C, H, N elemental analyses were made on an elementar vario EL. UV-Vis spectra were recorded on a TU-1810 Spectrophotometer made by Beijing Puxi Tongyong apparatus company with quartz cuvette (path length = 1 cm), and fluorescence spectra were recorded on a F96 Spectrophotometer made by Shanghai Lengguang Technology Co., Ltd. The width of the slits is 10 nm.

All reagents for synthesis obtained commercially were used without further purification. In the titration experiments, all the anions were added in the form of tetrabutylammonium (TBA) salts, which were purchased from Alfa Aesar Chemical, stored in a vacuum desiccator containing self-indicating silica and dried fully before using. DMSO was dried with  $\text{CaH}_2$  and then distilled in reduced pressure.

### General method

All titration experiments were carried out at 298.2 K, unless otherwise mentioned. A  $5.0 \times 10^{-4}$  M solution of

the host compounds in dried DMSO and solutions of the respective anions were prepared in dried DMSO and stored under a dry atmosphere. These solutions were used for all spectroscopic studies after appropriate dilution. Then, given amount of the solution of sensors was added to the quartz cuvette and the increased amount of anions tested (0.1 M in DMSO) was added to the solution above-mentioned, whose absorbance/emission spectra were tested immediately.

$^1\text{H}$  NMR titration experiments were carried out in the DMSO- $d_6$  solution (TMS as an internal standard). A  $1.0 \times 10^{-2}$  M solution of the compound **1** in DMSO- $d_6$  was prepared. Then, the increased amount of fluoride anion solution (1.0 M in DMSO- $d_6$ ) was added to the solution above-mentioned and  $^1\text{H}$  NMR of the anion-sensor system was tested.

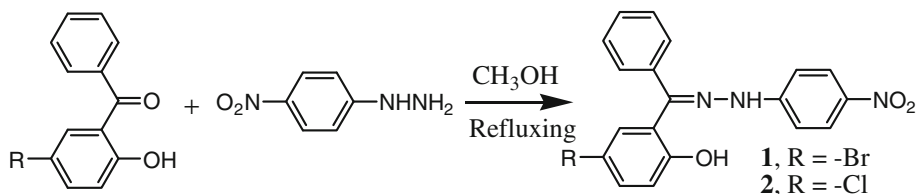
### Synthesis of the receptors

The compounds **1** and **2** were synthesized according to Scheme 1. To a solution of 2-hydroxyl-5-substituted-benzophenone (4 mmol) in hot  $\text{CH}_3\text{OH}$  (15 ml) was added 4-nitrophenylhydrazine (612 mg, 4 mmol) in methanol (15 ml). Then the mixture was heated to reflux under magnetic stirring for 4 h. After completion of the reaction, the reaction solution was cooled to room temperature and the solvent was removed to give the solid under reduced pressure. The pure products could be obtained by flash chromatography (silica,  $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH} = 100:1$  (v/v)).

### 2-hydroxyl-5-bromo-benzophenone-*p*-nitrophenylhydrazone (**1**)

(Yield = 55 %),  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz)  $\delta$ : 11.93 (s, 1H, NH), 11.72 (s, 1H, -OH), 8.18 (d,  $J = 9.3$  Hz, 2H, ArH), 7.70 (m, 5H, ArH), 7.36 (d,  $J = 7.8$  Hz, 1H, ArH), 6.98 (m, 2H, ArH), 6.83 (d,  $J = 2.4$  Hz, 1H, ArH); ESI-mass:  $\text{C}_{19}\text{H}_{14}\text{BrN}_3\text{O}_3$ ,  $m/z = 410.13$   $[\text{M}]^-$ ; elemental analysis calcd. for  $\text{C}_{19}\text{H}_{14}\text{BrN}_3\text{O}_3$  (%): C, 55.36; H, 3.42; N, 10.19 and found C, 55.60; H, 3.89; N, 10.48. IR (KBr disc)  $\nu$  3648 (s, OH); 3406 (m, N-H); 3116 (m, Ar-H); 1599 (m, -NO<sub>2</sub>); 1562; 1510 (w, C=N); 1458; 1399; 1299 (C-O); 747, 689, 536 (C-Br).

**Scheme 1** General synthetic routes to the target compounds **1** and **2**



2-hydroxyl-5-chloro-benzophenone-*p*-nitrophenylhydrazone (**2**)

(Yield = 56 %),  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 300 MHz)  $\delta$ : 11.68 (s, 1H, -NH), 8.18 (d,  $J = 9.3$  Hz, 2H, ArH), 7.68 (m, 5H, ArH), 7.36 (d,  $J = 7.8$  Hz, 2H, ArH), 6.94 (m, 2H, ArH), 6.70 (d,  $J = 2.4$  Hz, 1H, ArH); ESI-mass:  $\text{C}_{19}\text{H}_{14}\text{ClN}_3\text{O}_3$ ,  $m/z = 366.11$   $[\text{M}]^-$ ; elemental analysis calcd. for  $\text{C}_{19}\text{H}_{14}\text{ClN}_3\text{O}_3$  (%): C, 62.05; H, 3.84; N, 11.43; and found C, 62.20; H, 3.89; N, 11.48. IR (KBr disc):  $\nu$  ( $\text{cm}^{-1}$ ) 3626 (s, O-H); 3426 (m, N-H), 3112 (m, Ar-H), 1600 (m, -NO<sub>2</sub>), 1562, 1507 (w, C=N), 1458, 1404, 1299 (C-O), 751, 693, 542 (C-Cl).

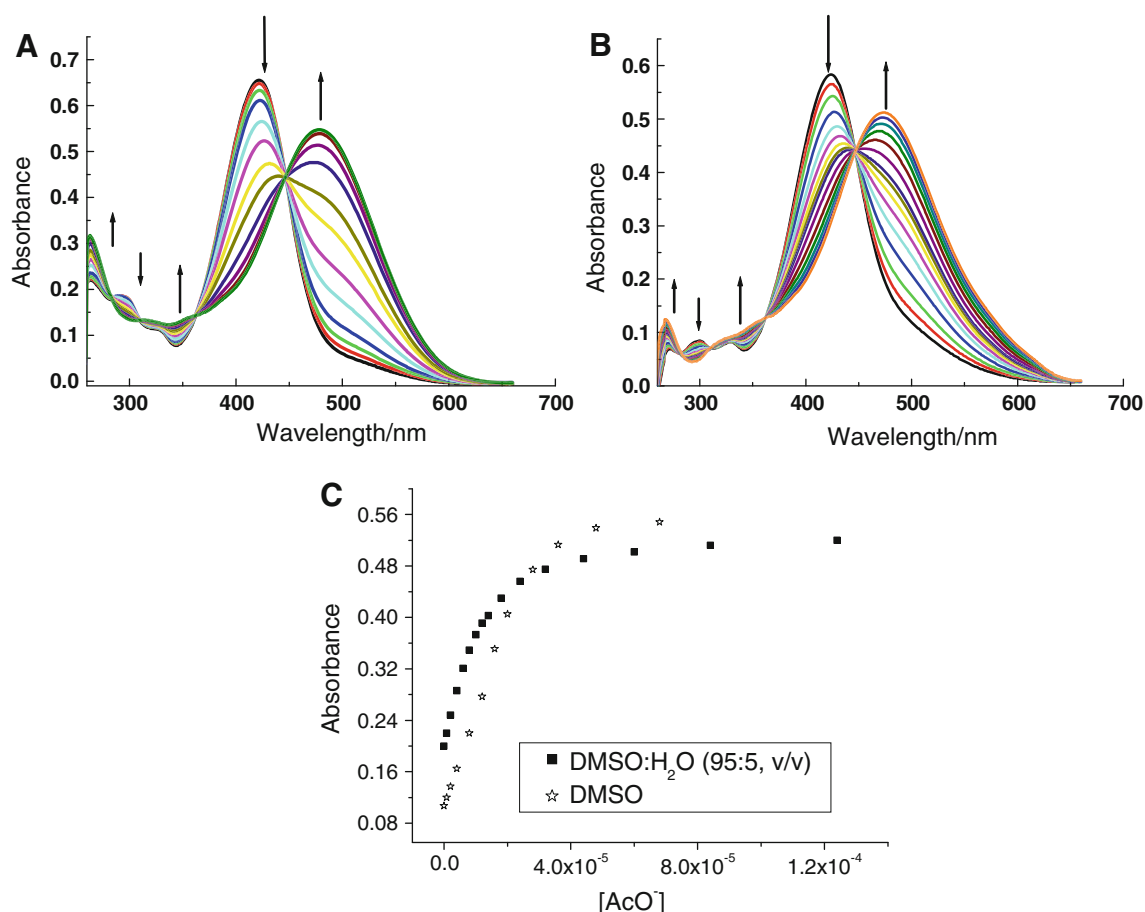
## Results and discussion

### UV-Vis spectral titrations

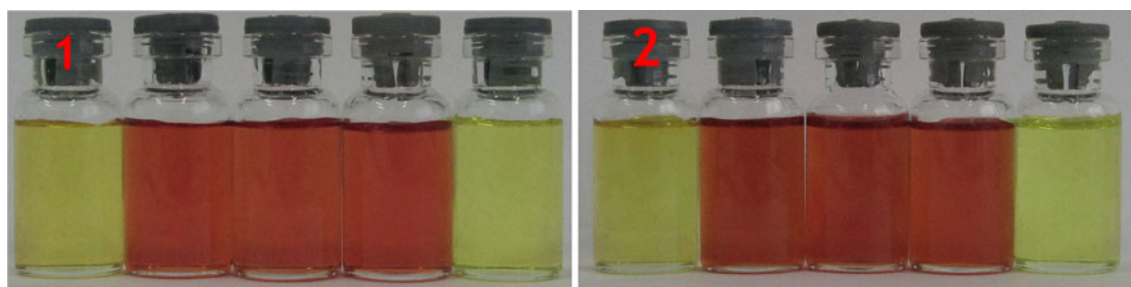
The binding ability of the sensor **1** ( $2 \times 10^{-5}$  M) was firstly studied using UV-Vis spectral titrations in DMSO.

Figure 1a showed spectral changes of **1** in the absence and the presence of acetate ion in DMSO solution. Obviously, the free **1** exhibited a main absorbance band centered at 420 nm, being ascribed to  $\pi$ - $\pi^*$  transition of *p*-nitrophenylhydrazone moiety [23]. The presence of  $\text{AcO}^-$  resulted in absorbance band at 420 nm decreasing gradually and a new absorbance band at 480 nm appeared. As the sensor **1** interacted with acetate ion by hydrogen bonds and even deprotonated, the electron density in binding sites of the sensor **1** significantly increased, which would promote the charge transfer between the electron-rich binding sites and the electron-deficient nitro moiety in neutral/deprotonated form. As a result, a new absorption band at 480 nm appeared and the visible color changes from yellow to red were simultaneously observed (see Fig. 2). The compound **2** exhibited similar spectral responses and color changes responses to anions in solution with the compound **1** owing to similar molecule structures (Fig. 3).

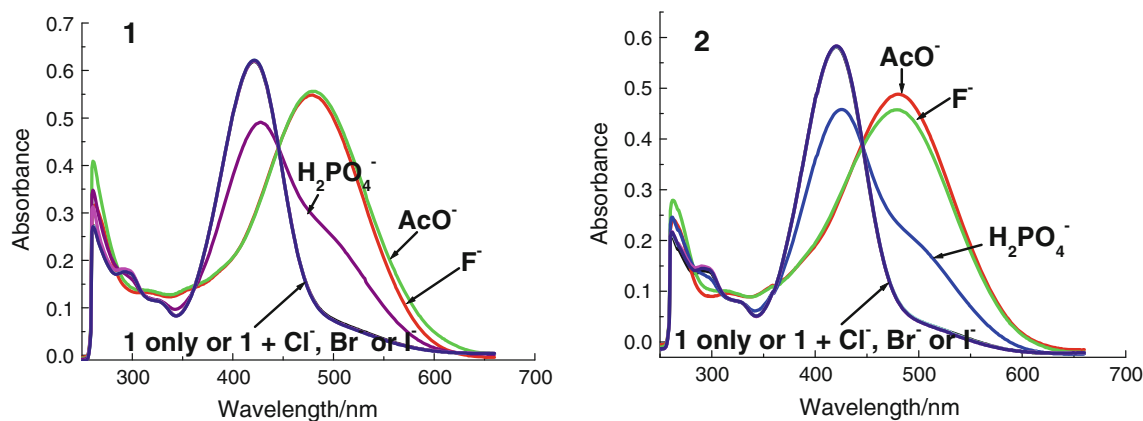
The anion sensing ability of two simple sensors **1** and **2** was also evaluated in aqueous solution (DMSO:H<sub>2</sub>O (95:5, v/v)). As Fig. 1b, S8 and S9 in the Supporting information



**Fig. 1** UV-Vis changes of the sensor **1** ( $2 \times 10^{-5}$  M, DMSO (a) and DMSO:H<sub>2</sub>O (95:5, v/v) aqueous solution (b)) in the absence and presence of  $\text{AcO}^-$  and (c): absorbance at 480 nm versus the concentration of  $\text{AcO}^-$  added in DMSO or DMSO:H<sub>2</sub>O (95:5, v/v)



**Fig. 2** Color changes of the sensors **1** and **2** ( $2 \times 10^{-5}$  M, DMSO) in the absence and presence of different anions (from left to right: the host only,  $\text{AcO}^-$ ,  $\text{F}^-$ ,  $\text{H}_2\text{PO}_4^-$  and miscellaneous anions including  $\text{Cl}^-$ ,  $\text{Br}^-$  and  $\text{I}^-$ , respectively)

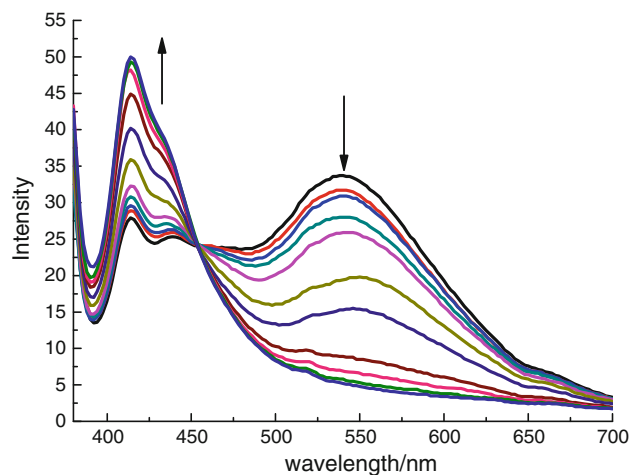


**Fig. 3** UV-vis changes of the sensors **1** and **2** ( $2 \times 10^{-5}$  M, DMSO) in the absence and presence of different anions tested

demonstrated, presence of anionic ions resulted in significant changes in UV-Vis spectra of **1** and **2** in DMSO:H<sub>2</sub>O (95:5, v/v) solution. The results showed that the anion sensors **1** and **2** could work even in the competitive medium.

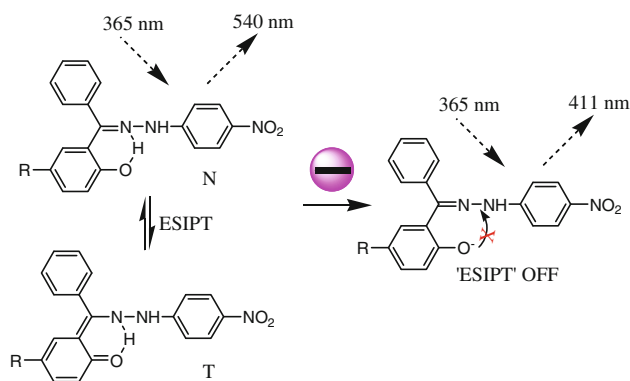
#### Fluorescence titrations

The fluorescence responses of the sensors **1** and **2** to anions were assessed by observing emission spectra with excitation at 365 nm. Just as Fig. 4 displayed, the sensor **1** gave a strong tautomer emission at 540 nm (Form T) and an extremely weak normal emission band at 414 nm (Form N). In the compound **1**, there is one hydroxyl group and therefore, the strong emission of **1** might result from ESIPT, which accounted for an emission at 540 nm. Upon addition of acetate ion, there were significant decreases in intensity of emission at 540 nm and increases in the intensity of emission at 411 nm. The result could be rationalized on basis of inhibition of ESIPT [22, 25]. The more acidic hydroxyl proton would be deprotonated upon exposure to acetate ion and hence, ESIPT process was inhibited, leading to complete disappearance of tautomer emission at 540 nm (see Scheme 2). This finding could be confirmed by the geometry optimized structure of **2**- $\text{AcO}^-$



**Fig. 4** Fluorescence changes of the sensor **1** ( $2 \times 10^{-5}$  M, DMSO) in the absence and presence of  $\text{AcO}^-$

according to the density functional theory using B3LYP/6-31G(d). It could be seen from Fig. 5 that shows that the strong hydrogen-bond formed between the moieties ( $-\text{NH}$  and  $-\text{OH}$ ) and the acetate oxygen with bond length of 1.64 and 2.01 Å, respectively [26]. The presence of other anions tested with similar basicity with acetate such as fluoride and dihydrogen phosphate caused similar emission



**Scheme 2** The possible signaling mechanism account for fluorescence responses of the compounds **1** and **2**

changes. However, the weak basic anions such as  $\text{Cl}^-$ ,  $\text{Br}^-$  and  $\text{I}^-$  did not inhibit ES IPT effectively and no obvious fluorescence changes were seen (see Fig. 6). Similar ratiometric fluorescence responses to anions tested were observed for the sensor **2**, just as Fig. 6 and S6 in supporting information showed.

During UV–Vis spectral titrations, there were well-defined isosbestic points at 283, 311, 363 and 447 nm, respectively, which indicated that there existed only one type of **1**- $\text{AcO}^-$  complex [24]. Association constants of compounds **1** and **2** for anionic species, which were shown in Table 1, were determined by nonlinear fitting analyses of the titration curves (480 nm) according to the equation derived from the 1:1 host–guest complexation. The titration profile at 480 nm (see Fig. 1c as examples or S2 and S4 in the Supporting information) supported the formation of a

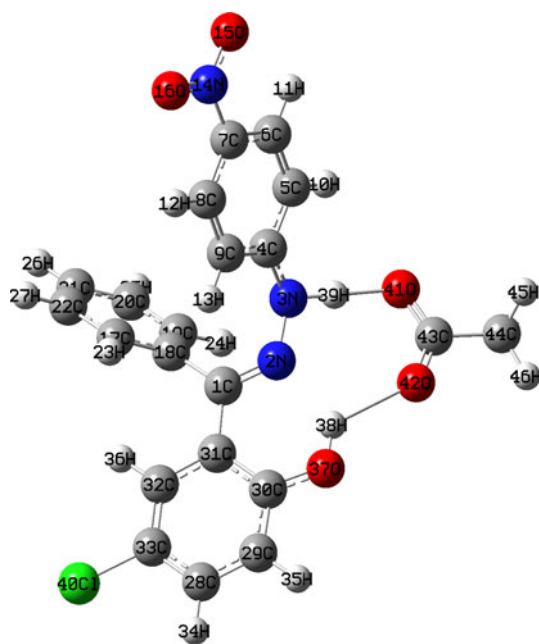
1:1 stoichiometric, **1**- $\text{AcO}^-$  species with an association constant  $K_{\text{ass}}$  of  $1.4 \times 10^5 \text{ M}^{-1}$ . Obviously, the sensors gave higher affinity for acetate ion than the other anions tested possibly due to basicity of acetate ion and shape complementarity between the hosts and  $\text{AcO}^-$ . It should be noteworthy that generally, association constants of the host–guest systems obtained from fluorescence data are bigger than those of the host–guest systems obtained from UV–Vis spectral data. The results could result from the fact that the acidity of anions binding sites of the two sensors in excited state are stronger than that anions binding sites of the two sensors in ground state. Accordingly, the sensors in excited state showed greater affinity for anions. Electronic effects of the substituents such as chlorine atom and bromine atom in the sensors **1** and **2** are similar and thus the sensors have similar anion binding ability (see Table 1).

### $^1\text{H}$ NMR titrations

To corroborate well with the result obtained from spectra titration,  $^1\text{H}$  NMR titrations were carried out in  $\text{DMSO}-d_6$  (see Fig. 7). Obviously shown in Fig. 7, the free **1** showed two peaks at 11.93 and 11.72 ppm, which could be ascribed to  $-\text{OH}$  and  $-\text{NH}$ , respectively. When  $\text{AcO}^-$  ions were introduced, the signals at 11.93 and 11.72 ppm disappeared, indicating the  $-\text{OH}$  and  $-\text{NH}$  moieties of the compound **1** acted as anion binding sites and exhibited deprotonation upon interacting with strong basic anions. Accordingly, the significant red shifts in absorption and color changes resulted from deprotonation of the anion binding sites [27]. The ES IPT between  $-\text{OH}$  and nitrogen atom of  $-\text{C}=\text{N}-$  moiety was inhibited and as a result, the emission band at 540 nm resulting from ES IPT disappeared, inducing ratiometric fluorescence changes of **1**. Similarly, the  $^1\text{H}$  NMR titrations of the sensor **2** with  $\text{AcO}^-$  (see S7 in Supporting information) also supported the results obtained in the UV–Vis and fluorescence titrations.

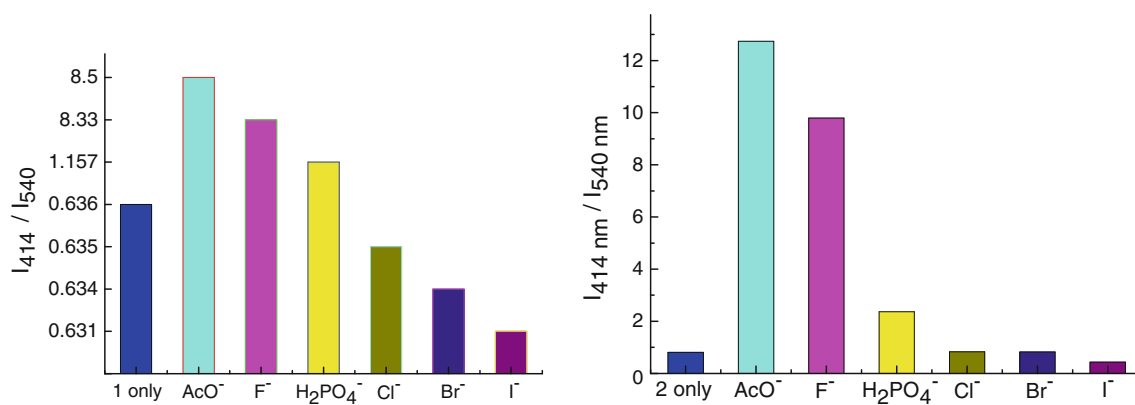
### Conclusion

To conclude, we designed and prepared two simple ratiometric fluorescence and colorimetric sensors based on Schiff base derivatives for anions. In dry  $\text{DMSO}$  solution, the sensors could sense anions with strong basicity by the ‘naked eye’ without resorting to any expensive equipment. Importantly, the sensors exhibited ratiometric fluorescence responses to anions and visible blue shifts in emission spectra (about 136 nm from 540 to 414 nm) were observed. Such blue shifts in emission spectra could be attributed to anion-induced inhibition of ES IPT. The strategy of design of ratiometric fluorescence sensor for anion based on inhibition of ES IPT is expected to extend



**Fig. 5** Optimized structure (B3LYP/6-31G) of **2**- $\text{AcO}^-$





**Fig. 6** Fluorescence changes of the sensors **1** and **2** ( $2 \times 10^{-5}$  M, DMSO) in the absence and presence of different anions tested

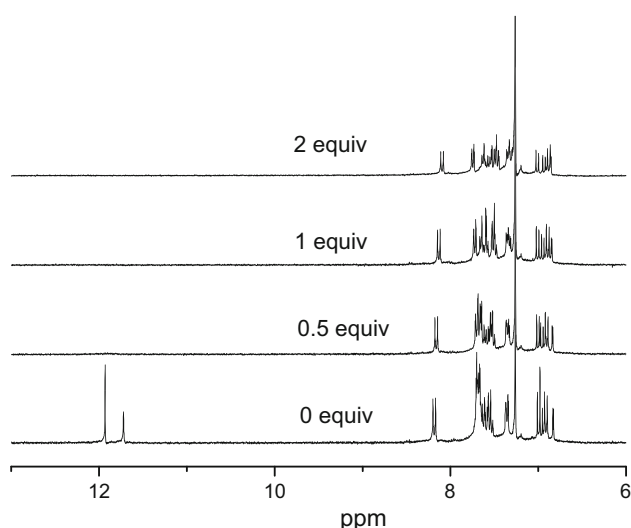
**Table 1** Association constants  $K_{\text{ass}}$  ( $\text{M}^{-1}$ ) between the receptors (**1** and **2**) and anions in DMSO

	Association constants <sup>a</sup>		Association constants <sup>b</sup>	
	<b>1</b>	<b>2</b>	<b>1</b>	<b>2</b>
AcO <sup>-</sup>	$1.4 \times 10^5$	$1.0 \times 10^5$	$2.1 \times 10^5$	$9.6 \times 10^4$
F <sup>-</sup>	$4.5 \times 10^3$	$3.3 \times 10^3$	$5.2 \times 10^3$	$1.6 \times 10^4$
H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	$1.0 \times 10^4$	$1.0 \times 10^4$	$1.5 \times 10^4$	$8.9 \times 10^3$
Cl <sup>-</sup>	ND	ND	ND	ND
Br <sup>-</sup>	ND	ND	ND	ND
I <sup>-</sup>	ND	ND	ND	ND

ND, The association constant could not be determined

<sup>a</sup> The association constants were determined by the UV–Vis spectra

<sup>b</sup> The association constants were determined by the fluorescence spectra



**Fig. 7** <sup>1</sup>H NMR spectra of sensor **1** in DMSO-*d*<sub>6</sub> ( $1 \times 10^{-2}$  M) on addition of acetate ions

the development of ratiometric fluorescent sensors for biologically inorganic anions.

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- for carbon cycling acetate cycling in the water column of the Cariaco Basin: seasonal and vertical variability and implication for carbon cycling. *Limnol. Oceanogr.* **47**, 1119–1128 (2002)
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