A novel coumarin-based switching-on fluorescent and colorimetric sensor for F−

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ABSTRACT

A novel turn-on fluorescent and colorimetric sensor, N-(4′-nitrophenyl)-2-oxo-6-(phenylazo)-2H-chromene-3-carbohydrazide (**1**), for fluoride in dimethyl sulfoxide (DMSO) was designed and synthesized. The binding ability evaluated by UV–vis and fluorescence titration experiments reveals that **1** can selectively recognize fluoride. In particular, addition of F− to the DMSO solution of **1** resulted in an enhancement in fluorescence intensity at 338 and 352 nm, which can provide a way of 'naked-eye' detection for fluorides. The spectral change of **1** is due to the anion-induced increase of the charge density in and the rigidity of the host molecule. Furthermore, the binding mode with F− was investigated by ^{1}H NMR experiments.

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1. Introduction

Since anions are ubiquitous and play important roles in many biological and chemical systems, there is an increasing interest in the design and development of receptors that selectively recognize and sense specific anions through visible and optical feedback [1,2]. Among the range of biologically important anions, fluoride is of particular interest owing to its serious effects in the human body. The scarcity of fluoride in the human body can lead to dental caries [3] and osteoporosis [4]. However, excess fluoride can result in fluorosis [5–7], which produces a chalky, cloudy, or opaque appearance of the tooth enamel. In severe fluorosis, the enamel becomes soft, crumbly, and darkly stained. This diversity of its function, both beneficial and otherwise, leads to the important emphasis on the detection of fluoride ion. And, of course, it is significant to design and synthesize the sensitive sensors for fluoride.

Of particular interest in this regard are chromogenic and fluorogenic chemosensors for anions. In particular, the fluorogenic chemosensors are attracting increasing attention for their high sensitivity, convenience, fast on-site evaluation, and low cost. The design of fluorescent chemosensors is mainly based on photo-induced electron/energy transfer (PET) [8], intramolecular charge transfer [9], excimer/exciplex formation [10], fluorescence resonance energy transfer [11], an increase of the rigidity of the host

molecules [12], and so on. Although plenty of effective fluorescent sensors have been successfully developed for sensing anions, most of them are fluorescence quenching (switching-off) sensors [13–15], which is disadvantageous for a high signal outputs. As for the sensors that exhibit fluorescence enhancement (switching-on), they have many merits, for example, they can extend the detection limit and sensitivity, and will bind to anions. Therefore, we should think more of the construction of a turn-on fluorescent sensor that is selective and sensitive to specific analyte [16,17].

Commonly, a typical anion chemosensor is the compound coupling of at least two units: a fluorophore and a binding unit [2]. In this paper, with the conception of designing turn-on fluorescent receptors for fluoride, a novel anion sensor was designed and synthesized by the combination of coumarin derivative [18–20] (as the fluorophore) and p-nitrophenylhydrazine (as the anion-binding unit) (Scheme 1). As expected, receptor **1** showed fluorescence enhancement upon the addition of anions through introducing hydrogen bonding. Moreover, among several common anions, receptor **1** can selectively recognize fluoride.

2. Experimental section

2.1. Reagents

Most of the starting materials were obtained commercially and all reagents and solvents used were of analytical grade. All anions, in the form of tetrabutylammonium salts, were purchased from

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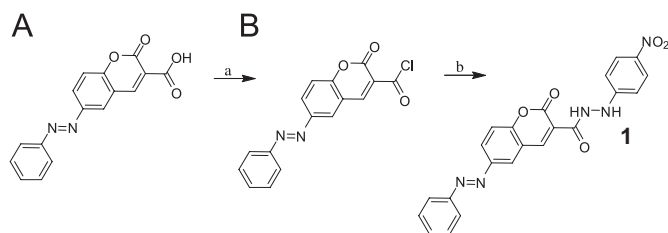
Scheme 1. . Synthesis of receptor **1**. Note: Reagent and conditions: (a) R: SOCl$_2$, S: benzene, 20 min, reflux (b) R: *p*-nitrophenylhydrazine, C: pyridine, S: CH$_2$Cl$_2$, 6 h, stirring.

Sigma-Aldrich Chemical Co., stored in a desiccator under vacuum containing self-indicating silica, and used without any further purification. Dimethyl sulfoxide (DMSO) was distilled in vacuo after being dried with CaSO$_4$. Tetra-n-butylammonium salts (such as (*n*-C$_4$H$_9$)$_4$NF, (*n*-C$_4$H$_9$)$_4$NCl, (*n*-C$_4$H$_9$)$_4$NBr, (*n*-C$_4$H$_9$)$_4$NI, (*n*-C$_4$H$_9$)$_4$NAcO and (*n*-C$_4$H$_9$)$_4$NH$_2$PO$_4$) were dried for 24 h in vacuum with P$_2$O$_5$ at 333 K before use. C, H, and N elemental analyses were made on an elementar vario EL.

2.2. General methods

^{1}H NMR spectra were recorded on a Varian UNITY Plus-400 MHz spectrometer. ESI-MS was performed with a Mariner apparatus. UV–vis spectroscopy titrations were made on a Shimadzu UV2450 spectrophotometer at 298.2 ± 0.1 K. A series of DMSO solutions having the same host concentration and different anion concentrations were prepared. Fluorescent spectra were recorded on a Shimadzu RF-5301PC spectrophotometer at 298.2 ± 0.1 K and the width of the slits used is 5.

2.3. Synthesis of 2-oxo-6-(phenylazo)-chromene-3-carbonyl-p-nitrobenzhydrazine (1)

1 was synthesized in two steps (Scheme 1) starting from 2-oxo-6-(phenylazo)-chromene-3-carboxylic acid (**A**) that was prepared by the literature procedure [21]. Synthesis of other compounds is described below.

2.3.1. 2-oxo-6-(phenylazo)-chromene-3-carbonyl chloride (B)
A mixture of (**A**) (0.74 g, 2.5 mmol) and SOCl$_2$ (5 ml) in benzene (20 ml) was refluxed for 20 min. Then, the solution was concentrated to dryness under reduced pressure. The slight yellow residue was dissolved in 25 ml dry CH$_2$Cl$_2$ for use in the next step.

2.3.2. 2-oxo-6-(phenyldiazenyl)-chromene-3-carbonyl-p-nitrobenzhydrazide (1)
The solution of **B** in CH$_2$Cl$_2$ (25 ml) obtained from the former step was added slowly at 0 °C to a solution of *p*-nitrophenylhydrazine (0.38 g, 2.50 mmol) and pyridine (0.4 ml, 5.00 mmol) in CH$_2$Cl$_2$ (20 ml). The reaction mixture was stirred for 12 h, the formed precipitate was filtered off and washed five times with CH$_2$Cl$_2$ (30 ml). This procedure yielded 0.84 g (72%) of pure product after drying in vacuo. ^{1}H NMR(400 MHz; DMSO-d$_6$; Me$_4$Si) 10.5 (1H, s, NH), 9.5 (1H, s, NH), 9.0 (1H, s, Ph), 8.6 (1H, s, Ph), 8.3 (1H, s, Ph), 8.1 (2H, d, Ph, *J* = 7.6), 7.9 (3H, dd, Ph, *J* = 8.1), 7.7 (1H, s, Ph), 7.6 (3H, m, Ph, *J* = 8.5), and 6.9 (1H, d, Ph, *J* = 8.3). ^{13}C NMR(DMSO-d$_6$): δ 171.21, 164.34, 162.17, 157.56, 157.45, 154.23, 152.38, 151.24, 150.12, 150.03, 147.12, 147.03, 146.11, 146.01, 138.29, 138.26, 132.14, 132.05, 128.34, 128.33, 125.73, and 121.96. Elemental analysis: Calc. for C$_{22}$H$_{15}$N$_5$O$_5$: C, 61.54; H, 3.52; N, 16.31; Found: C, 61.63; H, 3.39; N, 16.32. ESI-MS (*m/z*): 429.10 (M+H, calcd. 429).

3. Results and discussion

3.1. UV–vis anion titration studies

Firstly, the color of solution of **1** changed to madder red from yellow after the addition of fluoride (F$^-$), which suggested the appearance of strong binding. However, the addition of acetate (AcO$^-$), dihydrogenphosphate (H$_2$PO$_4^-$), chloride (Cl$^-$), bromide (Br$^-$), or iodine (I$^-$) resulted in a slight change in color (Fig. 1). Thus, the 'naked-eye' detection of F$^-$ was made possible.

Then, the anion-binding properties were studied by carrying out quantitative analysis by UV–vis titration of the receptor in dry DMSO solution using standard tetrabutylammonium salts of AcO$^-$, F$^-$, H$_2$PO$_4^-$, Cl$^-$, Br$^-$, and I$^-$ at 298.2 ± 0.1 K. As shown in Fig. 2, the solution of **1** (1.0 × 10^{-5} M) has the original absorption peaks at 318 and 342 nm. Significantly, these two original absorption peaks were decreasing, whereas a new peak at 396 nm appeared and increased by F$^-$ titration, accompanying the formation of an isosbestic point at 372 nm. Moreover, the peak at 503 nm developed at the same time and the solution turned to
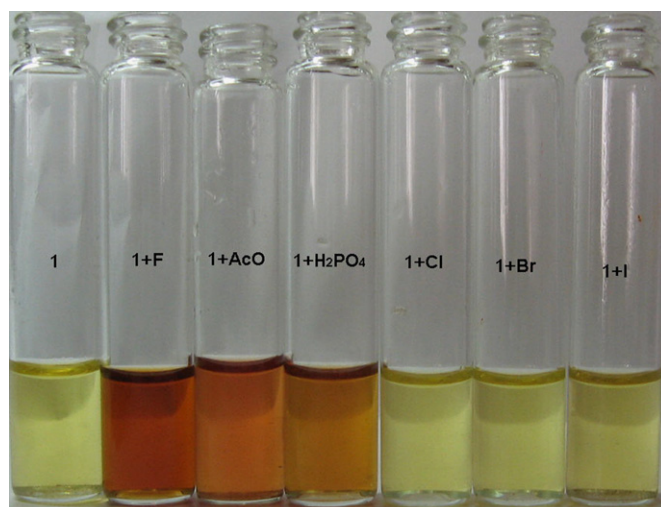

Fig. 1. Color changes of receptor **1** in DMSO (5.0 × 10^{-4} M) in the absence and presence of 2 equiv. of anions (from the left to the right: **1** only, **1**+F$^-$, **1**+AcO$^-$, **1**+H$_2$PO$_4^-$, **1**+Cl$^-$, **1**+Br$^-$ and **1**+I$^-$).
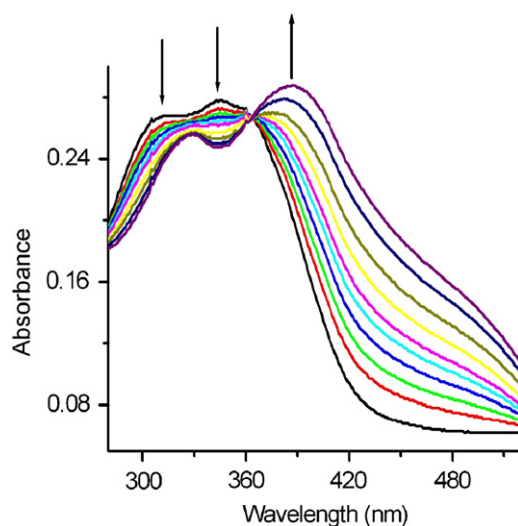

Fig. 2. Evolution of the UV–vis spectrum of receptor **1** (1.0 × 10^{-5} M in DMSO) during the titration with tetrabutylammoniun (TBA) F$^-$.

madder red from yellow. By the same kind of visual inspection, the addition of AcO$^-$ and H$_2$PO$_4^-$ did lead to a similar spectral change, but the changes were minor. However, as the Cl$^-$, Br$^-$ and I$^-$ were titrated into **1**, the spectra were hardly changed even the anions added were excessive.

The continuous variation method was used to determine the stoichiometric ratios of the receptor to the fluoride anion guest. In Fig. 3, Job plot [22] of **1** and F$^-$ in DMSO shows the maximum at a molar fraction of 0.5. This result indicates that **1** binds fluoride anion guest with a 1:1 ratio. Moreover, similar results can also be obtained for other anions (AcO$^-$ and H$_2$PO$_4^-$).

For a complex of 1:1 stoichiometry, the relation in Eq. (1) could be derived easily, where X is the absorption intensity, and C_H or C_G is the concentration of the host or the anion guest, correspondingly [23].

$$X = X_0 + (X_{lim} - X_0)\{C_H + C_G + 1/K_{ass}$$
$$- [(C_H + C_G + 1/K_{ass})^2 - 4C_H C_G]^{1/2}\}/2C_H \quad (1)$$

The affinity constants (K_{ass}) of receptor **1** for anionic species are calculated and listed in Table 1.

Obviously, the recognition function of **1** is selective and strongest for F$^-$. The reason may be that receptor **1** has the cavity that is proper to F$^-$, which is a spherical anion, and hence can match the receptor better than trigonal and tetrahedral anions. Furthermore, F$^-$ is an atomic anion, which means that it can form a five-membered chelate ring with the two NH binding sites, which is more stable than the seven-membered chelate ring formed by AcO$^-$ or H$_2$PO$_4^-$. Also, the basicity of F$^-$ is stronger than AcO$^-$ and H$_2$PO$_4^-$. Finally, considering the difference in electronegativity, the ability of F$^-$ binding to H is much stronger than Cl$^-$, Br$^-$, and I$^-$.

3.2. Fluorescence anion titration studies

To corroborate well with those findings obtained during the UV–vis titrations described above, the fluorescence titration
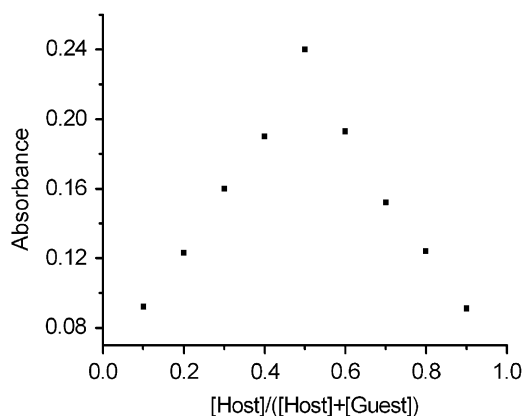

Fig. 3. The stoichiometry analysis of complex **1**·F$^-$ by Job plot analysis.

Table 1
Affinity constants of receptor **1** with anions at 298.2 ± 0.1 K in DMSO.

Anion (M^{-1})	AcO$^-$	F$^-$	H$_2$PO$_4^-$	Cl$^-$	Br$^-$	I$^-$
lg K^a_{ass}	3.40 ± 0.17	4.95 ± 0.21	2.81 ± 0.14	ND[b]	ND[b]	ND[b]
lg K^c_{ass}	3.38 ± 0.19	5.01 ± 0.24	2.82 ± 0.11	ND[b]	ND[b]	ND[b]

[a] The affinity constants determined by UV–vis.
[b] ND = cannot be determined.
[c] The affinity constants determined by fluorescence.

experiments were carried out. Fig. 4 showed the changes in the intensity of fluorescence emission in DMSO with the increase of fluoride ion concentration. Receptor **1** (1.0×10^{-5} M) displayed two weak fluorescence emission bands at 338 and 352 nm, respectively, when excited at $\lambda = 293$ nm. As expected, the analytical results of the fluorescence titration were very consistent with those findings of the UV–vis titration, which can be seen from Table 1. The presence of F$^-$ gave birth to an enhancement in fluorescence intensity of the solution of **1**. Similarly, AcO$^-$ and H$_2$PO$_4^-$ also can induce enhancements in fluorescence but the extent of that is lower. However, the Br$^-$, Cl$^-$, and I$^-$ in DMSO do not affect the emission spectra, even when present in excess (>300 equiv.). We believe that this is the outcome of a conjunct operation from the difference in size of binding sites and the direct result of the basicity of anions.

It is believed that anion-hydrogen bonding with the receptor will change the photophysical properties of fluorophore because the complexed anion increased the rigidity of the host molecules. Thus, on adding F$^-$, AcO$^-$, or H$_2$PO$_4^-$ to the solution of **1** in DMSO, the anion complex is formed between receptor **1** and the special anion through hydrogen bonding. On the one hand, the complex of anion can increase the charge density of the host, leading to the quenching of emission intensity [24]. However, on the other hand, as a consequence of anion coordination, the rigidity of the formed complex increases, making the nonradiative decay from the excited state less probable; consequently, the emission intensity increases, and the tendency of enhancement is stronger than that of quenching. Thus, the whole exhibition of emission intensity is enhanced.

3.3. ^{1}H NMR anion titration studies

Proton NMR titration experiments were conducted to further investigate the interaction of **1** with F$^-$ in DMSO-d$_6$. It was noticed in Fig. 5 that original signals of carbonylhydrazine –NH$_a$ and –NH$_b$ (marked in Scheme 2) protons, appearing at 10.5 and 9.5 ppm, respectively, were moved to downfield with a change of about $\Delta\delta = 0.8$ and 1.2 ppm, respectively, when 2 equiv. F$^-$ was added. This suggests that F$^-$ is combined with receptor **1** by hydrogen bonding. On the other hand, the aromatic protons elsewhere have upfield shifts due to the NH-anion hydrogen bond formation, which increases the electron density of the phenyl ring and produces a consistent result as the fluorescence anion titration studies have observed. Above all, these results corroborated that a
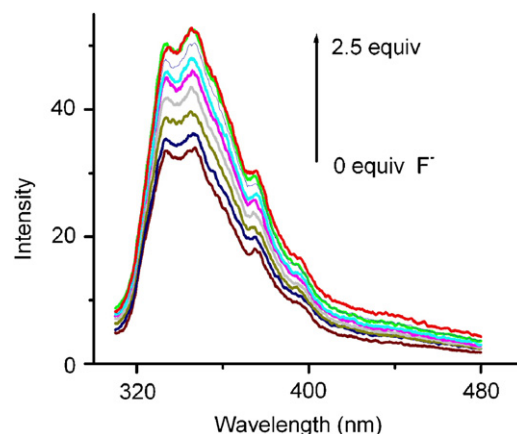

Fig. 4. Fluorescence emission (excitation wavelength is 273 nm) changes of receptor **1** (1.0×10^{-5} M in DMSO) upon the addition of tetrabutylammoniun (TBA) F$^-$.
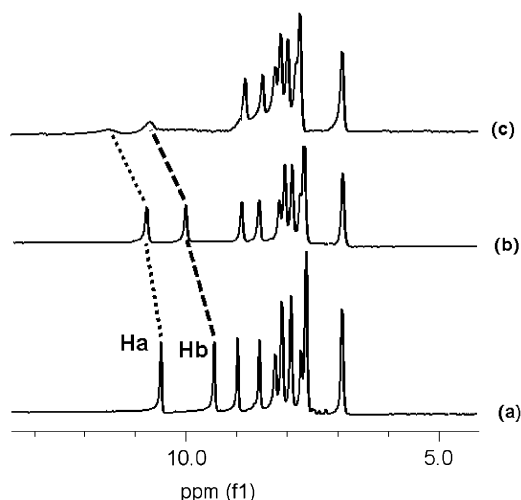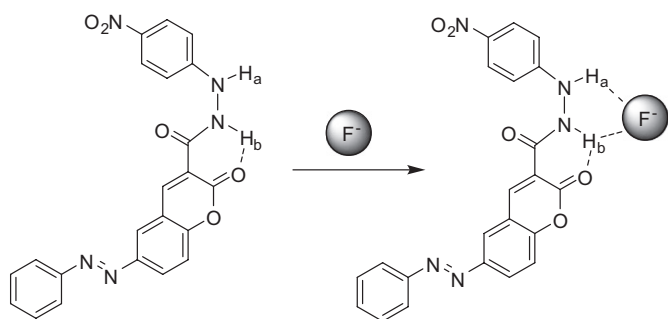
Fig. 5. ^{1}H NMR spectra of receptor **1** in DMSO-d_6 (1×10^{-3} M) upon the addition of molar equiv. of F$^-$: (a) 0, (b) 0.5 equiv. and (c) 2 equiv.

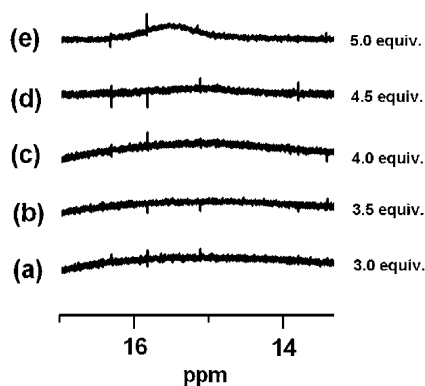**Scheme 2.** The proposed binding mode (small quantities of fluoride) of **1** in solution.

Fig. 6. Downfield part of ^{1}H NMR spectra of receptor **1** in DMSO-d_6 (1×10^{-3} M) upon the addition of molar equiv.of F$^-$: (a) 3.0, (b) 3.5 equiv., (c) 4.0 equiv., (d) 4.5 equiv. and (e) 5.0 equiv.

complex has been formed between receptor **1** and F$^-$. Interestingly, upon the addition of 5.0 equiv. of F$^-$, a new signal at 15.8 ppm appeared clearly, which was ascribed to the FHF$^-$ dimmer (Fig. 6). The formation of FHF$^-$ was due to the excessive fluoride quantities inducing proton transfer from **1** to F$^-$. These results reveal that the binding process includes two steps: (i) hydrogen bonding interactions (for small quantities of fluoride) and (ii) proton transfer between the receptor and the coordinated
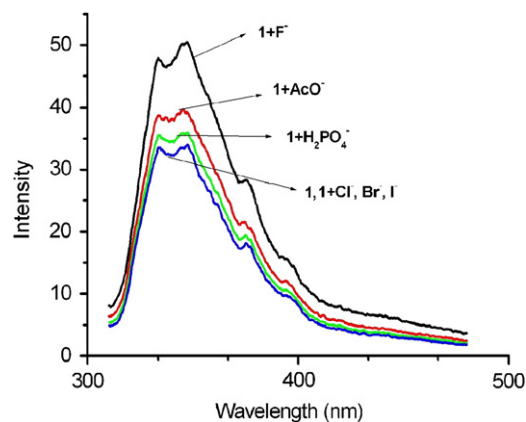

Fig. 7. Fluorescence emission changes of receptor **1** in DMSO (1.0×10^{-5} M) upon the addition of 2 equiv. different anions.
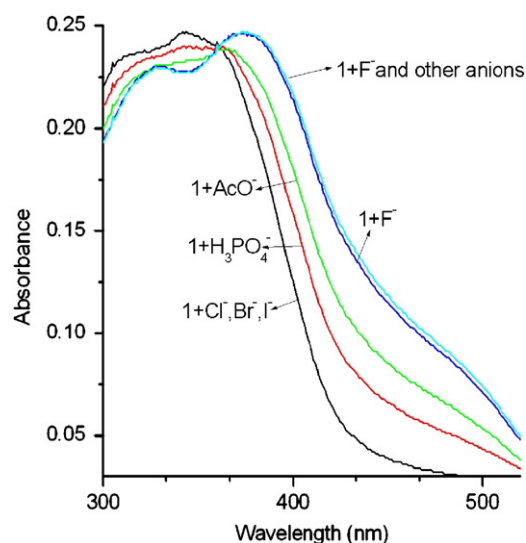

Fig. 8. UV–vis spectra spectra of **1** (1×10^{-5} M) in DMSO in the presence of 2 equiv. of F$^-$ ion and miscellaneous anions including F$^-$, H$_2$PO$_4^-$, Cl$^-$, Br$^-$, and I$^-$.

anion (for high quantities of fluoride). Correspondingly, the binding mode of **1** with F$^-$ was proposed and is given in Scheme 2.

3.4. Analytical application

In order to examine the potential application of the sensor in analytical chemistry, receptor **1** was applied in the detection of biologically important anions such as the fluoride ion. The color of the sensor solution is changed from yellow to red upon the addition of 2 equiv. of either AcO$^-$, H$_2$PO$_4^-$ or F$^-$. However, the red color induced by fluoride is deeper while by H$_2$PO$_4^-$ or AcO$^-$ ions it is lighter, and this difference can be easily distinguished by the naked eye (see Fig. 1). Using the method of non-linear least square fitting the data from UV–vis and fluorescence titrations were analyzed and the obtained affinity constants are summarized in Table 1. Obviously, as seen in Table 1, fluoride could give a stronger complexation than the other anions. Furthermore, the sensor that exhibits fluorescence enhancement (switching-on) has many merits, for example, expending detection limit and sensitivity. Guided by these factors, we recorded the fluorescence spectra changes (Fig. 7) of **1** operated in DMSO (1.0×10^{-5} M) after the addition of 2 equiv. of anions, which provided convinced evidence that **1** was an excellent sensor for F$^-$. Finally, in the presence of

miscellaneous competitive anions, the presence of the F$^-$ ion still results in a similar absorption (see Fig. 8), which demonstrates that the increases of absorbance resulting from the addition of the F$^-$ ion will not be influenced by the subsequent addition of miscellaneous anions.

All these indicate that the selectivity of **1** for the F$^-$ ion over other competitive anions is remarkably high and sensor **1** will be found as a convenient way of application in the practical detection of fluoride anions.

4. Conclusion

In summary, a novel fluorescent anion receptor based on coumarin could recognize fluoride among the anions investigated. Varying from the fluorescent receptors reported, receptor **1** displayed positive responses but not negative responses toward anions, which is advantageous in terms of the detection limit and sensitivity. Although these merits can be achieved in the system investigated, in a complex matrix, they could be hardly achievable, which also sets a challenging subject that will be conquered in the successive research.

Acknowledgments

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