A novel colorimetric and fluorescent sensor for fluoride and pyrophosphate based on fluorenone signaling units

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ABSTRACT

In this study, a novel chromogenic receptor, 1-(naphthalen-1-yl)-3-(9-oxo-9H-fluoren-1-yl)urea (1), utilizing fluorenone and naphthalene moieties as signaling groups was designed and synthesized. The interaction and colorimetric sensing properties of receptor 1 with different anions were investigated by the naked eye, as well as UV-visible and fluorescence spectroscopy. The addition of 100 equiv. of fluoride and pyrophosphate as tetrabutylammonium salts to 1.25 x 10^-6 M CH3CN:DMSO (9:1, v/v) solution mixture of receptor 1 produced a wine-red color. The oxoanions and a variety of other anions were not capable of producing any significant color change with receptor 1 under similar experimental conditions. The corresponding UV-vis measurements showed a bathochromic shift of the 295 nm band of receptor 1 to ~500 nm for fluoride and pyrophosphate. Fluorescence emission changes indicate clearly that receptor 1 behaves like an ideal photo-induced electron transfer (PET) sensor upon complexation with anions. The limit of detection (LOD) of the sensor 1 is calculated to be ca. 250 and 110 nM for F^- and HP2O7^3-, respectively. The ^1H NMR titration studies shed further light on their mode of binding with receptor 1. The quantum mechanical calculations through time dependent density functional theory (TD-DFT) using basis set B3P86/TZVP support our experimental findings with a good agreement.

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1. Introduction

Due to many possible applications in analytical chemistry and biomedical research, a considerable amount of research attention has focused on the design of receptors with the ability to selectively bind cation, neutral and anionic species [1–3]. In particular, the design and synthesis of receptors capable of binding anionic guests is of critical importance, due to its potential applications in chemical, environmental, and biological processes [4]. Among the various anions, fluoride (F^-) and pyrophosphate (HP2O7^3-) are the most important because of their various roles in medicinal field [5] and cellular processes [6], respectively. For this obvious reason, various research groups are focusing on the detection and discrimination of these biological entities. There have been a variety of reports thus far concerning fluoride [7] and pyrophosphate [8] selective receptors using various spectroscopic detection methods.

The broad application of anions necessitates the development of easily synthesized receptors that can recognize and sense the anions at very low levels. Due to its simplicity and high sensitivity, fluorescence is becoming increasingly important as a method for chemical trace detection [9]. Receptors based on anion-induced changes in fluorescence appear to be particularly attractive because they offer the potential for high sensitivity at low analyte concentration [10]. Fluorosensors for a large number of biotic and abiotic analytes have been designed in the past decade by appending a fluorescent fragment to the envisaged receptor framework: in all cases, an efficient mechanism has to be provided for either quenching or reactivating fluorescence, following substrate recognition. Fluorescent anion receptors utilizing photo-induced electron transfer (PET) [11], intramolecular charge transfer (ICT) [12], excited-state proton transfer [13], metal-to-ligand charge transfer [14], excimer/exciplex formation [15], and competitive binding [16] mechanisms have all been recently developed.

The fluorenone family compounds are base materials for the production of dyes and optical brightening agents. Fluorenone have been used extensively as catalyst precursors for electro-catalytixoeoxidation [17], inhibition of DNA tumor viruses [18], light-emitting materials [19], etc., but have rarely been used as chemical sensors. 9-Fluorenone has been investigated as an attractive element in organic solar cells and display devices. This has led us to design and synthesize a fluorescent sensor containing a fluorenone framework that is particularly suitable for environmental and biological applications. In this paper, we describe a novel chromofluorogenic receptor, 1-(naphthalen-1-yl)-3-(9-oxo-9H-fluoren-1-yl)urea (1), which contains fluorenone as a chromophore/fluorphore and urea moiety as a

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binding unit for developing a colorimetric sensor for F⁻ and HP₂O₇⁻. Sensor 1 selectively detects anions such as F⁻ and HP₂O₇⁻ in CH₃CN: DMSO (9:1, v/v) solution via both colorimetric and fluorimetric methods. The anion binding property of sensor 1 was assessed by monitoring the change of fluorescence emission intensity and using ¹H NMR titration techniques after the addition of anions in the form of tetrabutylammonium salts.

2. Experimental section

2.1. General

All of the anions were purchased in the form of tetrabutylammonium salts from Sigma–Aldrich. Solvents such as dimethylsulphoxide (DMSO) and acetonitrile (CH₃CN) were purified prior to use. Spectroscopic-grade solvents were used for the titration studies. Commercial-grade chemicals and solvents were used as such unless otherwise specified. Silica Gel G was used for thin-layer chromatography (TLC). 1-Amino-9H-fluorenone, 1-naphthyl isocyanate, and other reagents and solvents were purchased from Sigma–Aldrich and used as such.

All the experiments were conducted at room temperature. All new compounds were fully characterized via standard spectroscopic techniques. Microanalyses were conducted on a Perkin Elmer 2400 Series II CHNS/O Analyzer. Infrared spectra were recorded on a JASCO FTIR-6300 spectrometer. Electronic absorption spectra were recorded with an Agilent 8453 spectrophotometer. ¹H NMR and ¹³C NMR spectra were generated on a Bruker 400 FTNMR spectrometer. ¹H NMR and ¹³C NMR spectra were characterized by two peaks (315 and a shoulder at 395 nm) (Supplementary material, Fig. A1).

2.2. Synthesis of 1-(naphthalen-1-yl)-3-(9-oxo-9H-fluoren-1-yl)urea (1)

1-Naphthylisocyanate (0.23 mL; 1.625 mmol) was added to a dry 10 mL acetonitrile solution of 1-amino-9H-fluorenone (0.050 g; 1.30 mmol) at room temperature. The reaction mixture was stirred overnight (~12 h). The completion of the reaction was monitored by TLC. Column chromatography was used to purify the product (90:10; v/v) mixture and used for the detection experiments. Titration experiments were conducted by measuring the changes in fluorescence emission that occurred upon the addition of anions to the degassed CH₃CN: DMSO (9:1, v/v) solution of 1 (5 μM). For all receptor 1 measurements, excitation was at 315 nm; both excitation and emission slit widths were 3 nm for detection and 5 nm for titration experiments. The initial volume of receptor 1 was 2 mL. Every titration was repeated at least twice until consistent values were obtained.

2.5. ¹H NMR studies

The solution of receptor 1 (0.001 M in DMSO-d₆) was titrated by adding known quantities of concentrated solution of anions (0.004 M) in the form of tetrabutylammonium salts. The chemical shift changes of the -NH protons of the urea moiety in the receptor were monitored. All titrations were repeated at least twice to obtain consistent values. DMSO-d₆ was purchased from Sigma–Aldrich and dried over molecular sieves (4 Å) prior to use. The anion salts were dried for at least a day in dynamic vacuum, prior to the experiments.

3. Results and discussion

3.1. Design and synthesis of probe 1

In molecular recognition chemistry, the cavity size and binding sites play a critical role in determining the sensitivity and specificity of the sensor. The conscious modification in these parameters can result in interesting supra-systems. In general, most positively charged anion receptors have amide, pyrrole, urea, and ammonium or guanidinium groups as binding sites, which form N-H–A⁻ hydrogen bonds [20]. As they offer appropriate binding sites for the guests and stabilize the complexes via non-covalent interactions such as hydrogen and ionic bonding, urea-based anion sensors are generally considered to be of utmost importance [21]. Reaction of 1:1 molar ratio of 1-amino-9H-fluoren-9-one and 1-naphthyl isocyanate in dry CH₃CN yielded receptor 1 as pale yellow microcrystals (Scheme 1). The structure of receptor 1 was elucidated by elemental analyses, NMR (¹H and ¹³C), and mass analysis (Supplementary material, Figs. A2 and A3).

3.2. Colorimetric naked-eye detection

The colorimetric anion sensing ability of receptor 1 was studied by some visual color changes observed upon mixing with various anionic guests (1:100 equiv. ratio) in the form of tetrabutylammonium salts in CH₃CN:DMSO (9:1, v/v) mixture. It was observed that the host solution undergoes significant color changes from pale yellow (395 nm) to wine red (~500 nm) upon addition of F⁻ and HP₂O₇⁻ (Fig. 1A). This is associated with deprotonation of more acidic urea proton and the subsequent charge transfer phenomenon [22,23]. As the receptor bound to}

the anions, hydrogen bonds were constructed to form stable complexes, and the electron density in the supramolecular system was increased. It promotes the charge transfer phenomenon between the electron-rich anion-bound urea moiety and the electron-deficient fluorenone center of receptor 1 in neutral/deprotonated form, thus causing a color change in the host solution [24]. On the other hand, the exposure of receptor 1 to solutions of Cl\(^-\), Br\(^-\), I\(^-\), CH\(_3\)COO\(^-\), HSO\(_4\)\(^-\), H\(_2\)PO\(_4\)\(^-\), and NO\(_3\)\(^-\), led to no conspicuous changes in color, and no significant changes in absorption spectra were observed (Fig. 1C).

3.3. Fluorescence detection

The photophysical response of receptor 1 toward the addition of anions was assessed in CH\(_3\)CN:DMSO (9:1, v/v) mixture (Fig. 2). Among the examined anions, receptor 1 evidences strong fluorescence quenching effects with I\(^-\). It is also important to note that F\(^-\), CH\(_3\)COO\(^-\) and HP\(_2\)O\(_7\)\(^3-\) evidenced a slightly red-shifted fluorescence quenching and Cl\(^-\), Br\(^-\), and H\(_2\)PO\(_4\)\(^-\) evidenced enhancements in fluorescence. This unique fluorescence behaviour implied that the naphthalene moiety might be directly involved in the bonding interaction with the ions along with the formation of complexes [25].

There have been quite a few reports concerning fluorescent chemosensors for anions bearing benzylic amine [26] or urea groups [27], which are based on a photo-induced electron transfer (PET) mechanism. Our fluorenone-based receptor should, in principle, also evidence ideal PET behavior upon anion recognition.

3.4. \(^1\)H NMR titrations

As the visible changes were observed with only F\(^-\) and HP\(_2\)O\(_7\)\(^3-\) ions, the \(^1\)H NMR titrations of receptor 1 were conducted with only these anions by their concomitant additions as tetrabutylammonium salts to the 1×10\(^{-3}\) M solution of the receptor 1 in DMSO-d\(_6\). The aromatic signals of the receptor 1 broadened and -NH signals (9.86 ppm and 9.71 ppm) disappeared upon addition of 0.5 equiv. F\(^-\) and HP\(_2\)O\(_7\)\(^3-\) (Supplementary material, Fig. A4). This implies the deprotonation of one amide proton and the interaction of these anions with the other amide proton of 1 via intermolecular hydrogen bonding [28]. Further
additions of $F^-$ and $\text{HP}_2\text{O}_7^{3-}$ induce slightly shifts upfield of the phenyl protons from 8.00 and 7.28 ppm to 7.93 and 7.18 ppm, respectively, thereby indicating the increase of the electron density on the phenyl ring owing to a bond-propagation effect [29]. This disappearance of $\text{-NH}$ took place at 0.5 equiv. for $F^-$ and $\text{HP}_2\text{O}_7^{3-}$, which may be understood in terms of the following equilibria:

$$LH + X^- \leftrightarrow [L \cdot H \cdot X]^-$$  \hspace{1cm} (1)

$$[L \cdot H \cdot X]^- \leftrightarrow L^- + HX$$  \hspace{1cm} (2)

$$[L \cdot H \cdot X]^- + X^- \leftrightarrow L^- + [HX_2]^-$$  \hspace{1cm} (3)

However, it should be noted that we observed no signal for $[HX_2]^{-}$ in its $^1\text{H}$ NMR spectral titrations even up to $\delta$ 20 ppm, probably as the result of its instability in highly polar solvents such as DMSO [30].

3.5. Fluorescence titrations

To investigate the selectivity and the sensitivity of 1 towards biologically important anions such as $F^-$ and $\text{HP}_2\text{O}_7^{3-}$, we conducted a series of fluorescence titrations in degassed CH$_3$CN:DMSO (9:1, v/v) mixture. Receptor 1 evidenced a strong emission centered at 380 nm with three shoulder emission bands at 278, 315 and 543 nm, respectively, upon excitation at 315 nm. The fluorescence emission of 1 was effectively quenched upon the addition of $F^-$ and $\text{HP}_2\text{O}_7^{3-}$ (Fig. 3). To account for such fluorescence quenching, the PET mechanism was exploited. As has been previously shown, the fluorescence of the anion PET chemosensor was generally ‘switched off’ rather than ‘switched on’ upon ion sensing, unlike the majority of PET sensors. In the case of 1, the excited state of the fluorophore was not quenched, or was quenched only to a minimal extent, by electron transfer (ET) from the receptor to the fluorophore prior to the sensor–anion interactions. However, upon interaction with anions, the reduction potential of sensor 1 was increased; in other words, electron transfer from the electron-rich amide moiety bonded with the anion to the electron-deficient fluorenone moiety became more feasible (Scheme 2). Upon further addition of $F^-$ and $\text{HP}_2\text{O}_7^{3-}$, it appeared that the deprotonated species, which was more electron-rich than the hydrogen-bonded complex with anion, activated the PET process more efficiently and evidenced more profound quenching [31]. From these changes, the binding constants ($\log K_a$) were determined for $F^-$ and $\text{HP}_2\text{O}_7^{3-}$ [32].

Fig. 3. Fluorescence emission spectral changes of receptor 1 (5 μM) with increasing concentrations of (A) TBAF (0–50 equiv.) and (B) TBAPPi (0–25 equiv.) in CH$_3$CN:DMSO (90:10, v/v) mixture (excitation=315 nm; slit width=5 nm). Fluorescence intensity changes upon addition of equivalents of (C) fluoride and (D) pyrophosphate in the CH$_3$CN:DMSO (9:1, v/v) mixture. (Inset) Job plot analysis for 1$^-F$ and 1$^-\text{HP}_2\text{O}_7^{3-}$ complexes in CH$_3$CN:DMSO (90:10, v/v).

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(Table 1), and titration curves were best fit to 1:2 host-guest stoichiometry (Fig. 3C and D, inset). The plot of fluorescence emission intensity changes of 1 \([{{I_o} - {I_i}}]/{I_o}\) against inverse concentration of anion \([1/\text{[Anion]}]\) gives a linear plot with acceptable regression value (Fig. 4), which indicates the uniform quenching upon increasing the concentration of anion, where \(I_o\) is intensity at absence of anion and \(I_i\) is the intensity at each addition of anion concentration. Limit of detection (LOD) was calculated by using the equation, \(\text{LOD} = \frac{3.3 \times \text{SD}}{S}\), where, SD is the standard deviation of the response and \(S\) is the slope of the calibration curve. Fig. 4 was used to estimate the LOD of sensor 1 which is calculated to be ca. 250 and 110 nM for F\(^-\) and \(\text{HP}_2\text{O}_7\)\(^-\), respectively. The fluorescence titration experiment was repeated twice to determine the reproducibility of sensor 1. The titration results show the precision has acceptable value with less than 5% error.

3.6. Competitive binding

To ensure a strong binding pattern, a competitive binding study of host 1 with F\(^-\) in the presence of \(\text{HP}_2\text{O}_7\)\(^-\) and \(\text{Cl}^-\) was conducted. The fluorescence emission changes clearly show that these anions are not competitive with each other and that host 1 binds more tightly with \(\text{HP}_2\text{O}_7\)\(^-\) than does F\(^-\); which is justified by the appearance of the corresponding fluorescence emission peaks. As is demonstrated in Fig. 5, the fluorescence intensity of 1 was not completely recovered when \(\text{HP}_2\text{O}_7\)\(^-\) (1:1 ratio) was added to the solution of host 1 with F\(^-\) (1:25 ratio) and \(\text{Cl}^-\).

3.7. Theoretical studies

We further investigated the optimized geometries of the free receptor and the receptor–anion complexes using B3P86 density functional theory [33–35] with TZVP basis set [36] (Fig. 6). At the respective optimized geometries, time-dependent density functional theory (TD-DFT) calculations in the presence of CH\(_3\)CN with various solvation models implemented in Gaussian 09 program suite were carried out in order to obtain the UV-visible spectra of anions. The theoretical results for absorption maximum band with highest oscillator strength and next highest wavelength were summarized in the Table A1. The simulated UV-visible spectra of host molecules are well matched with the experimental ones. TD-DFT calculations revealed that the origin of the newly developed spectral band upon complexation of 1 with F\(^-\) and \(\text{HP}_2\text{O}_7\)\(^-\) ions around 500 nm is due to the contributions from intramolecular transitions (Supporting material, Fig. A5). The theoretical UV/Vis red shift behaviour of 1 upon binding with F\(^-\) ion and differentiation of 1 for F\(^-\) and \(\text{Cl}^-\) are compatible with that of experiment in a qualitative manner. The method used for these calculations described herein, although not accurate, helps us to predict the n-conjugation length, which may lead to a favourable intramolecular charge transfer in the 1 upon addition of anions.

4. Conclusion

In summary, we have developed a simple "on-off" signal ensemble system 1 for the selective detection of F\(^-\)and \(\text{HP}_2\text{O}_7\)\(^-\), based on the fluorone moiety. Owing to the rigidity of the fluorone skeleton, it may form structurally well-defined complexes with anions, which are additionally stabilized by strong hydrogen bonding stemming from the central ketone group of the fluorone. The system provides chromogenic and fluorogenic dual signals by displaying (i) a wine-red

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**Table 1** Binding constant for 1–anion complexes.

<table>
<thead>
<tr>
<th>Anion</th>
<th>(K_a) [M(^{-1})](^a)</th>
<th>(K_1)</th>
<th>(K_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F(^-)</td>
<td>(3.30 \times 10^4)</td>
<td>1.90 \times 10^0</td>
<td>1.30 \times 10^0</td>
</tr>
<tr>
<td>(\text{HP}_2\text{O}_7)(^-)</td>
<td>(3.20 \times 10^5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Anions used are in the form of tetrabutylammonium salts.

\(^b\) The association constants \(K_a\) [M\(^{-1}\)] were measured using the fluorescence titration (25 °C). All fluorescence emissions were measured in dry CH\(_3\)CN:DMSO (90:10, v/v) at room temperature and repeated 2 or 3 times. Using data from different wavelengths yielded, on all occasions, the same binding constants with 10% error.
color and (ii) an immediate fluorescence response from an initially pale yellow solution, upon exposure to $F^-$ and $\text{HP}_2\text{O}_7^{3-}$. The response time was only a few seconds in CH$_3$CN:DMSO (9:1, v/v) mixture, and we were able to readily follow this phenomenon by either absorption or emission changes. Furthermore, the binding phenomenon can be readily monitored via fluorescence quenching effects. The easily prepared sensor system synthesized herein may prove to be an ideal chemodosimeter for the detection and determination of $F^-$ and $\text{HP}_2\text{O}_7^{3-}$ in organic solution, and could lead to the development of a convenient and reliable detection method for $F^-$ and $\text{HP}_2\text{O}_7^{3-}$ in practical and commercial applications. The theoretical calculations and the $^1$H NMR titration analysis are in good agreement with chemical shift changes. Potential practical applications of this class of fluorone-based receptors are currently being explored in our laboratory; the findings of these studies will be reported as they become available.

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Appendix A. Supplementary data

Characterization data (UV-visible, $^1$H NMR & $^{13}$C NMR) for 1, and partial $^1$H NMR titration spectrum and theoretical calculation methods are described. Supplementary data associated with this article can be found in the online version. Supplementary materials related to this article can be found online at http://dx.doi.org/10.1016/j.microc.2012.04.012.

References

[32] C.R. Cooper, T.D. James, Synthesis and evaluation of D-glucosamine-selective fluorescent sensors, J. Chem. Soc. Perkin Trans. 1 (2000) 963. All fluorescence emissions were measured in DMSO at room temperature and repeated for 2 or 3 times. Using data from different wavelengths gave, on all occasions, same binding constants with 10% error. Determined using the equation: $\log [\text{K}]=\log [\text{anion}]/\log [\text{K}]=\log [\text{anion}]/\log [\text{K}]$.

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