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Anion sensing by Phenazine-based urea/thiourea receptors

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ABSTRACT

The novel colorimetric receptors 2,3-bis-*N*-(9,10-diaza-anthracen-1-yl)-*N*′-phenylurea and 2,3-bis-*N*-(9,10-diaza-anthracen-1-yl)-*N*′-phenylthiourea have been prepared by the reaction of 2,3-diaminophenazine with phenylisocyanate and phenylisothiocyanate, respectively, in quantitative yields. The interaction and colorimetric sensing properties of receptor = **2** and **3** with different anions were investigated by naked eye, UV–vis and fluorescence spectroscopy in DMSO. The receptors effectively and selectively recognized biologically important F^-, CH_3COO^-, $H_2PO_4^-$ in the presence of other anions, such as Cl^-, Br^-, I^- and HSO_4^- in DMSO.

The design and synthesis of new anion binding agents is an important area of current research interest due to the growing awareness of the environmental and clinical importance of anions. The different sizes and shapes of anions are responsible for the slow growth in the development of anion binding agents as compared to cation binding agents.[1–3] The 'naked-eye' detection of anions without the use of any spectroscopic instrumentation is an important area in the design and fabrication of new anion receptors. Naked-eye or optical detectable receptors have been developed by the covalent attachment of chromogenic or fluorogenic groups. Two strategies of anion detections have been developed, one based on cationic ligands such as polyammonium, guanidium, quaternary ammonium and lewis acids and the second based on neutral ligands such as amides, ureas, thioureas, calix[4]pyrroles and related ligands.[4–10] The anion binding portion, that is, the urea and thiourea groups have been attached covalently to *o*-phenylenediamine,[11,12] calix[4]arenes,[13–15] anthraquinones,[16–20] acridone,[21] and related chromophores. The planar electron-rich heterocyclic diamine, 2,3-diaminophenazine (DAP) is an interesting chromophore with intense luminescence and significant mutagenic and genotoxic behavior. This compound is very useful in analytical chemistry for fluorimetric determinations of laccase activity[22] and in chemotherapeutics as a DNA cleavage agent.[23] Herein, we report the synthesis of two novel colorimetric anion receptors having bisurea/thiourea binding sites and phenazine as a chromogenic signaling subunit and their effective and selective 'naked-eye' differentiation between fluoride, acetate and dihydrogen phosphate in DMSO.

The reaction of *o*-phenylenediamine with ferric chloride in refluxing aqueous solution gave 2,3-diaminophenazine. Further, the reaction of 2,3-diaminophenazine with two moles of phenylisocyanate or phenylisothiocyanate in DMF/THF (4:1,v/v) gave 2,3-bis-*N*-(9,10-diaza-anthracen-1-yl)-*N*′-phenylurea **2** and 2,3-bis-*N*-(9,10-diaza-anthracen-1-yl)-*N*′-phenylthiourea **3**, respectively (Scheme 1). The spectroscopic analysis results were consistent with the proposed structures of the receptors. FT-IR spectra of receptors **2** and **3** showed broad –NH bands at 3340–3380 cm^{-1}. Further, a strong band at 1635 cm^{-1} in the IR spectrum of **2** was assigned to C=O stretching.

The naked-eye detection experiment was carried out initially in DMSO by addition of the corresponding anion (5×10^{-6}–5×10^{-2} M) to solutions of receptors **2** and **3** (5×10^{-5} M). The addition of tetrabutyl ammonium fluoride anions into DMSO solutions of **2** and **3** resulted in yellow to red color changes, due to the appearance of a broad new band centered at 512 nm and 516 nm in the UV–vis spectra of receptors **2** and **3**, respectively, suggesting that fluoride interacts with the receptors **2** and **3** more strongly due to its higher electronegativity and smaller size compared to the other halides. Moreover, the color change of thiourea **3** was much more sensitive to F^- than that of urea **2**. One tenth equivalent of F^- was enough to induce an observable color change from yellow to pink in **3**. Further addition of F^- changed the color of the solution from pink to dark red. The most remarkable effect was the selective color change induced in **2** and **3** towards dihydrogen phosphate and acetate. Receptor **2** gave a well-defined orange color on addition of $H_2PO_4^-$ and AcO^- (5×10^{-4} M). In contrast, the solution of thiourea **3** in the presence of dihydrogen phosphate and acetate turned red at a low concentration (5×10^{-5} M) (Fig. 1). The color changes are most probably due to the formation of hydrogen

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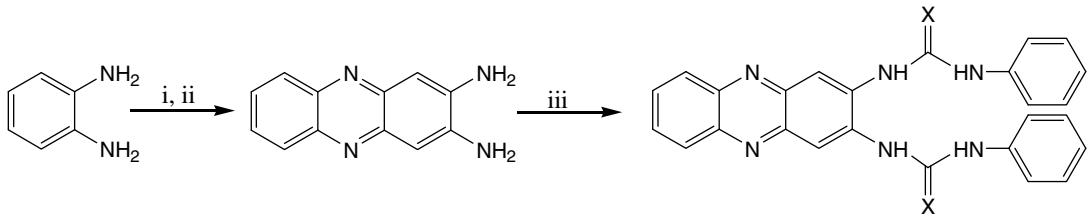

1

2, X = O (76 %)
3, X = S (53 %)

Scheme 1. The synthesis of receptors **2** and **3**. Reagents and conditions: (i) anhydrous ferric chloride, H_2O, rt, 12 h; (ii) concentrated aq ammonia, 15 min, rt, 23%; (iii) phenyl isocyanate or phenylisothiocyanate (excess), DMF/THF (4:1, v/v), reflux, 48 h.

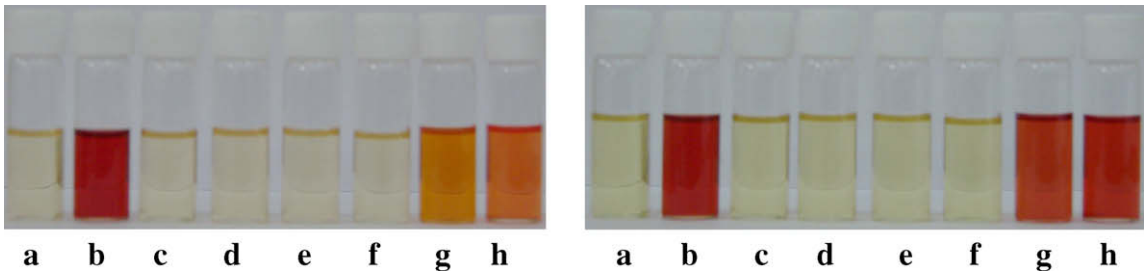

Figure 1. Left panel: color changes of receptor **2** (5.0×10^{-5} M) in DMSO upon addition of tetrabutylammonium anions (5.0×10^{-2} M); (a) = free receptor, (b) = fluoride, (c) = chloride, (d) = bromide, (e) = iodide, (f) = hydrogen sulfate, (g) = dihydrogen phosphate, (h) = acetate; right panel: color changes of receptor **3** (5.0×10^{-5} M) in DMSO upon addition of tetrabutylammonium anions (5.0×10^{-3} M); (a) = free receptor, (b) = fluoride, (c) = chloride, (d) = bromide, (e) = iodide, (f) = hydrogen sulfate, (g) = dihydrogen phosphate, (h) = acetate.

bond interactions between the urea or thiourea groups and the corresponding anions.[24,25] Both the receptors were found to be insensitive to addition of a large excess (5×10^{-2} M) of chloride, bromide, iodide and hydrogen sulfate. The anion sensing ability is reflected in quantitative terms in the UV–vis absorption spectra of receptors **2** and **3**. The UV–vis spectra of urea **2** and thiourea **3** changed dramatically on addition of fluoride, dihydrogen phosphate and acetate anions. Figure 2 shows the absorption spectra of **2** and **3** in the presence of fluoride anions. On addition of fluoride anions in DMSO (5.0×10^{-4} M), the characteristic absorption peaks of **2** and **3** at 400 nm decreased gradually with a strong red shift and a new peaks at 512 nm and 516 nm were produced, respectively. At the same time a clear isobestic point at 440 nm for receptor **2** and three clear isobestic points at 295 nm, 345 nm and 440 nm were observed for **3** (Fig. 2). The absorption intensity of $\mathbf{3}\cdot\mathbf{F}^-$ complex at 516 nm of was greater than the intensity of $\mathbf{2}\cdot\mathbf{F}^-$ complex at 512 nm, when less than 2 equiv of F^- was added. This result shows that F^- binds more tightly with thiourea **3** than with urea **2**, indicating the formation of stronger hydrogen bonds between the acidic N-H groups of thiourea **3** and F^-.

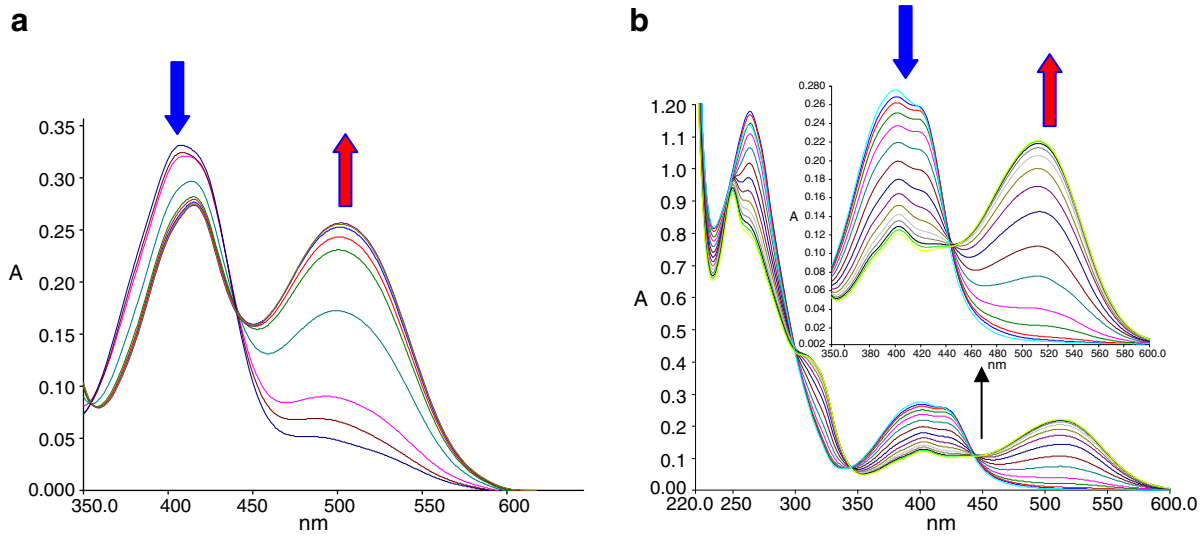

Figure 2. UV–vis spectral changes observed for **2** and **3** in DMSO at room temperature. Left: UV–vis titrations of urea **2** (5.0×10^{-4} M) upon addition of tetrabutylammonium fluoride (from 0.0, 5.0×10^{-5} to 5.0×10^{-4} M) right: UV–vis titrations of thiourea **3** (5.0×10^{-4} M) upon addition of tetrabutylammonium fluoride (from 0.0, 5.0×10^{-5} to 7.5×10^{-4} M) (inset expanded region from 350 nm to 600 nm).

Further the UV–vis spectral change of urea **2** on addition of dihydrogen phosphate was significantly different from that with fluoride. The λ_{max} slowly moved from 400 to 425 nm, when 10 equiv of dihydrogen phosphate ions was added. Addition of more than 10 equiv of phosphate did not result in any further spectral changes. This behavior of urea **2** was also reflected in the naked eye detection with a yellow to orange color change. However, the spectral changes of thiourea **3** in response to dihydrogen phosphate and acetate were similar to those with fluoride addition. The addition of other halides and hydrogen sulfate ions did not cause any spectral changes with both urea **2** and thiourea **3**. The UV–vis spectral changes of thiourea **3** in the presence and absence of different anions are depicted in Figure 3. Fluorescence spectroscopy studies were also carried out in order to evaluate the ability of the receptors to operate as a fluorescent anion sensor. A 2.5 ml solution of 2,3-bis-*N*-(9,10-diaza-anthracen-1-yl)-*N'*-phenylurea (**2**, 2.0×10^{-4} M) in DMSO in a quartz cell was titrated with an increasing volume of tetrabutylammonium fluoride (Fig. 4). The fluorescence spectra of **2** showed a characteristic peak at 610 nm. A large quenching (>85%) in the intensity of the 610 nm band was observed on addition of 1.0 equiv of F^- anions indicating that on formation of the hydrogen-bonded complex between F^- and **2**, the excited state was modified considerably leading to the quenching of fluorescence. On continuous addition of F^- to a solution of **2** the peak was red shifted to 645 nm. A clear isobestic point was observed at 625 nm. The changes observed in the fluorescence spectra on adding more than 1 equiv of F^- aliquots to **2** were insignificant, which is in good agreement with the results of the UV–vis titration.

All the titration curves gave a satisfactory fit to a 1:1 binding model as confirmed by the continuous variation method. The selectivity trends in the binding affinities of the anions for **2** and **3** followed the order of $F^- > AcO^- > H_2PO_4^- \gg Br^- > Cl^- > HSO_4^- > I^-$. The observed binding sequence was not completely consistent with the anion basicity. The quantitative titration experiments allowed us to determine the apparent binding constants of urea **2** and thiourea **3** with different anions as described in the literature.[12] The respective K_{ass} values are summarized in Table 1.

In summary, we have developed the new structurally simple sensors **2** and **3** having bis-urea/thiourea binding sites anchored to phenazine signaling subunits that not only allow for the easy colorimetric detection of F^- and $H_2PO_4^-$ ions, but also are amenable to 'color tuning' depending upon the type and amount of anions. These receptors may find use in various sensing applications as well as in other situations, such as anion transport and purification.

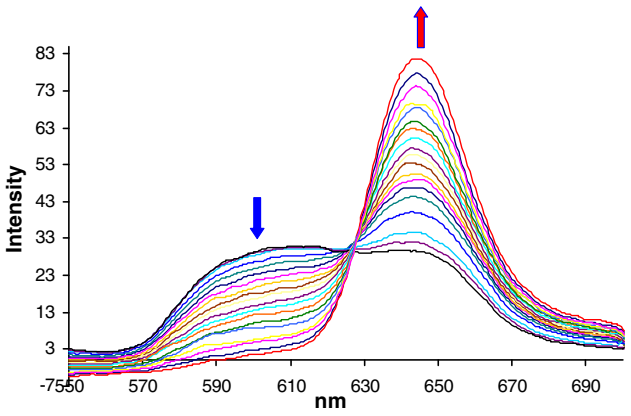

Figure 4. Fluorescence spectra of **2** (5.0×10^{-4} M) in the presence of *n*-$Bu_4N^+F^-$ (from 0.0, 5.0×10^{-5} to 7.5×10^{-4} M) in DMSO at room temperature; λ_{ex} = 450 nm.

Table 1
Association constants (Kass)[a] for 2 and 3 (M^{-1}) and anionic substrates both at 5.0×10^{-4} M in DMSO solution at 25 °C[b]

Receptor	Association constant K_{ass} (M^{-1})						
	F^-	Cl^-	Br^-	I^-	HSO_4^-	$H_2PO_4^-$	AcO^-
2	5.6×10^4	n.d[c]	75	n.d.[c]	n.d.[c]	4.8×10^3	9.5×10^3
3	9.1×10^5	n.d.[c]	350	n.d.[c]	n.d.[c]	4.1×10^5	6.2×10^5

[a] Determined from absorption spectroscopic titrations.
[b] All errors are ± 10%.
[c] Changes in the UV–vis spectra were insufficient to calculate the binding constants.

Synthesis of 2,3-bis-N-(9,10-diaza-anthracen-1-yl)-N'-phenylurea/thiourea: Phenylisocyanate (273.7 mg, 2.3 mmol) was added dropwise to a solution of 2,3-diaminophenazine (420 mg, 2 mmol) in dry DMF and THF (4:1, v/v, 25 ml) with stirring under inert atmosphere at reflux at 80 °C for 48 h. The reaction mixture was cooled at room temperature and the solvent was removed under reduced pressure leaving 5 ml of the solvent. Crushed ice was poured into the reaction mixture and precipitated was filtered. After several

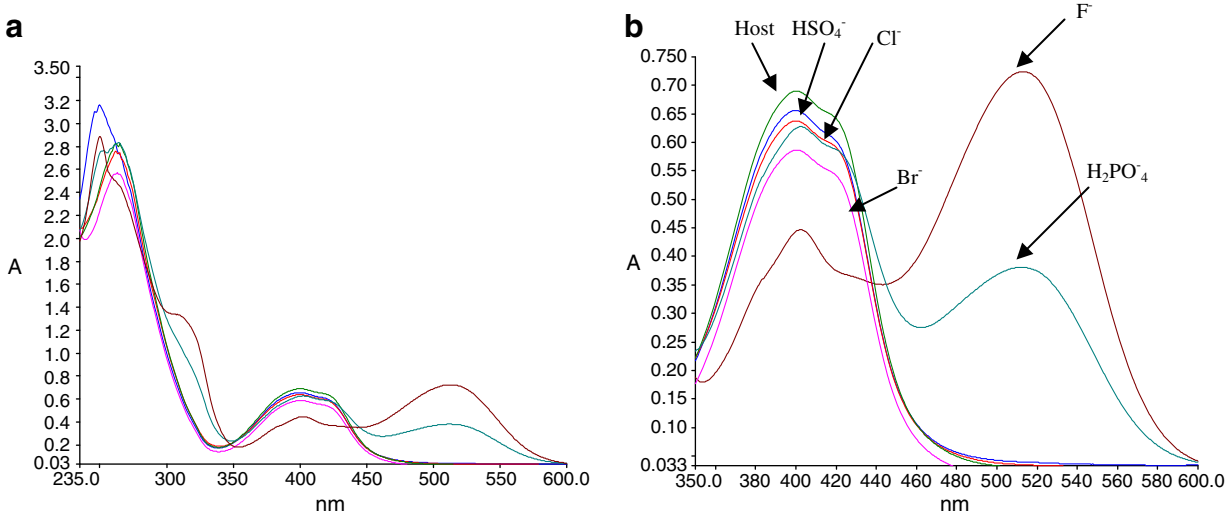

Figure 3. (a) Absorption spectra of **3** (5×10^{-4} M) in the presence and absence of different anions (5.0×10^{-4} M) in DMSO. (b) Expanded region from 350 nm to 600 nm.

washings with THF and acetonitrile (5 × 10 ml), the desired compound **2** was crystallized by hot methanol/DMSO to obtain as pale yellow crystals.

Data for receptor ***2***: yield: 76%, mp: 238 °C, UV–vis: λ_{max} (5×10^{-5} M, DMSO)/nm: 275 (1.32), 408 (0.378), IR (KBr): ν_{max}/cm^{-1} 3340 (–NH), 1635 (C═O), 1552, 1438, 1353, 1221, 750; ^{1}H NMR: δ_H (300 MHz; DMSO-d_6; Me_4Si): 9.27 (2H, s, NH), 8.62 (2H, s, NH), 8.58 (2H, s, 1,4-$CH_{phenazine}$), 8.12 (2H, d, J = 7.5 Hz, 5,8-$CH_{phenazine}$), 7.79 (2H, m, 6,7-$CH_{phenazine}$), 7.42 (4H, d, J = 8.7 Hz, *o*-phenyl), 7.29 (4H, m, *m*-phenyl), 6.91 (2H, m, *p*-phenyl); ^{13}C NMR: δ (75 MHz; DMSO-d_6): 151.9, 143.9, 141.8, 140.8, 140.2, 139.7, 129.1, 128.8, 128.5, 127.3, 116.5; HR-MS for $C_{26}H_{20}N_6O_2Na$: calcd: 471.1540, found: 471.1535.

The compound **3** was prepared by using above procedure using 2,3-diaminophenazine (420 mg, 2 mmol) and phenylisothiocyanate (310.5 mg, 2.3 mmol) with stirring under inert atmosphere at reflux at 80 °C for 50 h. Compound **3** was crystallized by hot methanol/DMSO.

Data for receptor **3**: yield: 53%; mp: >300 °C; UV–vis: λ_{max} (5×10^{-5} M, DMSO)/nm: 265 (1.23), 400 (0.293); IR(KBr): ν_{max}/cm^{-1} 3377 (NH), 1610, 1566, 1498, 1313, 1226, 753; ^{1}H NMR: δ_H (300 MHz; DMSO-d_6; Me_4Si): 10.35 (2H, s, NH), 9.42 (2H, s, NH), 9.01 (2H, s, 1,4-$CH_{phenazine}$), 8.82 (2H, d, J = 7.4 Hz, 5,8-$CH_{phenazine}$), 8.19 (2H, m, 6,7-$CH_{phenazine}$), 7.75 (4H, d, J = 8.7 Hz, *o*-phenyl), 7.58 (4H, m, *m*-phenyl), 7.21 (2H, m, *p*-phenyl). ^{13}C NMR: δ (75 MHz; DMSO-d_6): 179.1, 144.1, 142.3, 140.2, 139.2, 138.5, 128.7, 128.6, 128.2, 127.9, 118.3; HR-MS for $C_{26}H_{20}N_6S_2Na$: calcd: 503.1083, found: 503.1080.

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