Synthesis of colorimetric sensors for isomeric dicarboxylate anions: selective discrimination between maleate and fumarate†

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Four new colorimetric receptors (**1–4**) were synthesized and characterized. Upon addition of maleate to receptor **1** in DMSO, the appearance of the solution of receptor **1** showed a color change from dark-blue to dark-red, which can be detected by the naked eye at parts per million. Similar experiments were repeated using receptors **2–4**; the solution showed a distinct color change from blue to violet for receptor **2** and from blue-green to purple for both receptors **3** and **4**, when they are formed as complexes with maleate. The striking color changes are thought to be due to the deprotonation of the thiourea moiety of the 4-nitronaphthyl chromophore. Whereas, in the addition of fumarate to receptors **1–4**, the color of the solution changed from dark-blue to bright yellow for receptor **1** and did not induce any color change for receptors **2–4**. Thus, for a distinct color change, receptors **1–4** can act as optical chemosensors for recognition of maleate *versus* fumarate. Especially, only receptor **1** has a unique color change for the recognition of fumarate, accordingly it can be used for detection of the fumarate anion. In this research it was also found that the performance of the receptor is highly dependent on the substituent group on the phenyl ring; a stronger electron-withdrawing group resulted in a receptor with a higher binding constant with the maleate anion.

Introduction

Anions, especially dicarboxylates, play an important role in chemical and biochemical processes and their recognition and sensing by artificial chemosensors has been a focus of interest for chemists in the past decades.[1] The recognition of anionic species is generally based on electrochemical, ^{1}H NMR and fluorescent methods through changes in redox potential, chemical shift and fluorescence, respectively.[2] In recent years, a new method based on the change in color of an anionic sensor has been developed. The strategy to prepare colorimetric anion sensors is the binding site-signaling unit approach in which an appropriate chromophore is attached to a specific anion receptor.[3] These chromophores may contain electron-withdrawing groups that enhance the acidity of the anion binding subunit. Urea and thiourea subunits are currently used in the design of neutral receptors for anions, owing to their ability to act as H-bond donors,[4] and many ligands containing either one or two of these groups have been reported to be excellent sensors for dicarboxylate anions.[5] During recent years, we have been studying the synthesis of colorimetric chemosensors for dicarboxylate anions and their possible application in sensing.[6] As an extension of our previous work and in order to see how the different electron-withdrawing substituents in one of the two binding sites can influence the binding properties and modulate the spectral behaviors and color changes in host–guest systems, we have designed four novel colorimetric sensors. They were based on anthraquinone skeleton bearing thiourea groups through an ethylene spacer (Scheme 1). The host structure, featuring binding sites at the 1- and 4-positions of 1,4-diaminoanthraquinone through an ethylene spacer to form a convergent binding site, provides a feasible complexation with target species. The 4-nitronaphthyl is linked to one of the thiourea moieties. The other chromophores such as 4-trifluoromethylphenyl, 4-nitrophenyl, 4-cyanophenyl and phenyl groups are appended to the other branch of the thiourea moiety, respectively. These chromophores would provide spectral sensing character upon complexation with anions. In spite of lacking electronic conjugation between the thiourea and the anthraquionone moiety, the sensors **1–4** showed UV–vis spectral changes on complexation with anions.

Their utility in the selective colorimetric discrimination between certain organic isomers (*cis*/*trans* and *ortho*/*meta*/*para* dicarboxylates) (Chart 1) has been investigated. Differentiation of geometric isomers is, in general, a difficult task because of their rather similar chemical and physical properties. To the best of our knowledge, only few examples have been published.[6b,7] The interest in selective sensors that are able to distinguish maleate *versus* fumarate is not only related to π-diastereoisomer recognition but is also due to the different biological behavior of these anions. In fact, whereas fumarate is generated in the Krebs cycle, maleate is a well known inhibitor of this cycle and its implication in different kidney diseases has been widely described.[7b,8] Moreover, the interest to selectively discriminate between the three phthalic acid isomers (*ortho*, *meta*, and *para*) is due to the *ortho*-phthalate being a high-production-volume synthetic chemical and ubiquitous environmental contaminant. The potential health risk associated with exposure to it has been increasingly of concern.[9]

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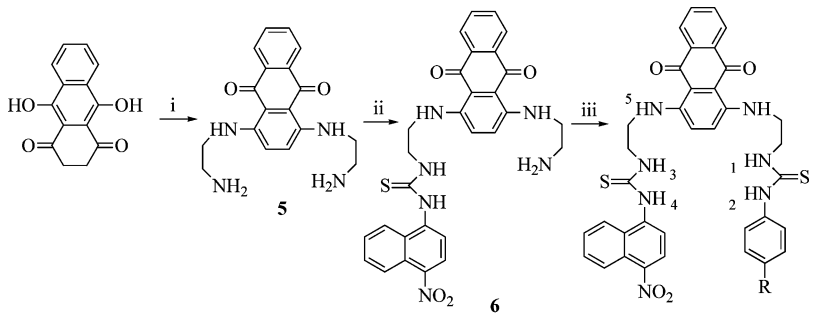


Scheme 1 *Reagents and conditions:* (i) ethylenediamine, 50 °C, 2 h, 37%; (ii) 4-nitronaphthylisothiocyanate, THF, reflux, 18 h, and (iii) 4-*R*-phenyl-isothiocyanate, THF, reflux, 18 h.

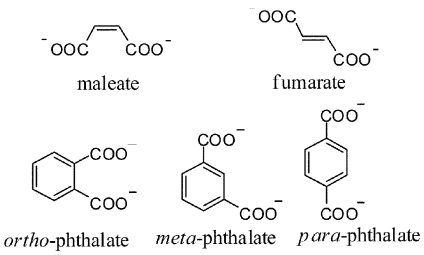


Chart 1

Results and discussion

Preparation of sensors **1–4** is depicted in Scheme 1. A synthetic intermediate, 1,4-di-(2-aminoethylamino)anthraquinone (**5**) was prepared from 2,3-dihydro-9,10-dihydroxy-1,4-anthracenedione.[10] Reaction of **5** with 0.7 equivalents of 4-nitronaphthylisothiocyanate in THF gave **6** in 42% yield. Subsequently, reaction of **6** with the corresponding isothiocyanates in THF afforded the bisthiourea derivatives **1–4** in moderate yields. All of these compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR and HRMS.

Anion binding studies

The colorimetric selective sensing ability of the receptors **1–4** with maleate and fumarate anions in DMSO was monitored by UV–vis absorption and by naked eye observation. The anions were added as tetrabutylammonium salts to the DMSO solutions of the receptors **1–4** (5×10^{-5} M). Receptor **1** displays four weak absorption bands at 385, 521, 596 and 643 nm, respectively, in DMSO. The interaction of receptor **1** with maleate anion was investigated in detail through the UV–vis spectroscopic titration, and complicated spectral behaviors were observed (Fig. 1). Upon addition of maleate to receptor **1** in DMSO, the intensity of the absorption peak at 385 nm gradually decreased, while the band at 521 nm evolved and reached its limiting value after the addition of 2.0 equivalents of maleate (Fig. 1). Interestingly, the color of the solution of receptor **1** was changed from dark-blue to dark-red, visible to the naked eye (Fig. 2). A clear isobestic point at 422 nm indicated the shifting of a well-defined binding

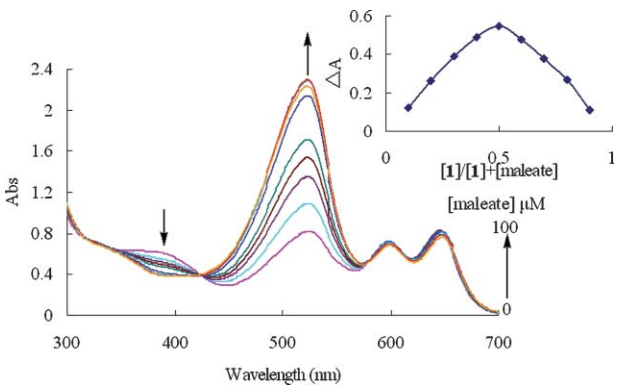


Fig. 1 A series of spectra taken over the course of the titration of a 5×10^{-5} M DMSO solution in **1** with a standard solution of maleate at 25 °C. The titration profile (insert) indicates the formation of a 1 : 1 complex.

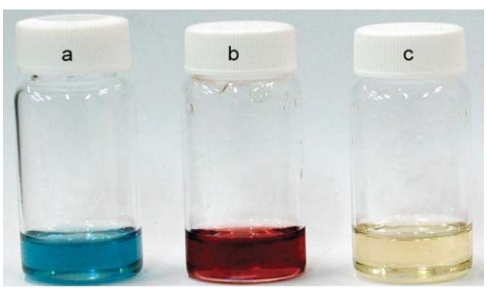


Fig. 2 Color changes of complex **1** upon addition of various anions in DMSO: (a) **1** only; (b) **1** + 2.0 equivalents of maleate; (c) **1** + 2.0 equivalents of fumarate.

equilibrium in the solution by addition of maleate. The changes in the absorbance as a function of the concentration of maleate added can be fitted to a 1 : 1 binding equilibrium model, giving the association constants shown in Table 1.[11] The band that develops at 521 nm is thought to be the monodeprotonated receptor $\mathbf{L_1}^-$ ($\mathbf{1} = \mathbf{L_1H}$), which was confirmed by the Brønsted acid–base reaction of adding 1 equivalent of strong base [*n*-Bu$_4$N]OH (*cf.* SI-1, see ESI†). The spectral behavior revealed that deprotonation of the NH

Table 1 Association constants K_a/M of receptors **1–4** with maleate and fumrate anions

Anion	Receptor	K/M[a]	R[b]
Maleate[c]	**1**	$(6.85 \pm 0.03) \times 10^3$	0.9974
	2	$(1.14 \pm 0.02) \times 10^4$	0.9958
	3	$(7.57 \pm 0.03) \times 10^3$	0.9943
	4	$(6.12 \pm 0.02) \times 10^3$	0.9927
Fumrate[c]	**1**	$(1.43 \pm 0.03) \times 10^3$	0.9943
	2	$(1.52 \pm 0.02) \times 10^3$	0.9988
	3	$(1.47 \pm 0.03) \times 10^3$	0.9947
	4	$(1.38 \pm 0.02) \times 10^3$	0.9957

[a] The data were calculated from UV–vis titrations in DMSO. [b] The data values of R were obtained by the results of nonlinear curve fitting. [c] The anions were used as their tetrabutylammonium salts.

fragment by maleate is responsible for the drastic color change, as a result of a charge transfer interaction between the nitrogen atom of the thiourea unit and the electron deficient 4-nitronaphthyl moiety. Such a deprotonation was related to the acidity of the H-bond donor site.[12] The deprotonation of receptor **1** with maleate was corroborated by ^{1}H NMR titration experiments carried out in DMSO-d_6 (Fig. 3). It was found that the proton signal of N–H_4 (δ = 10.14 ppm), which is closer to the 4-nitronaphthyl group (signals of N–H protons were assigned by referring to the 2D NOSEY spectrum of **1**) (*cf.* SI-2, see ESI†), underwent downfield shifts with increasing maleate concentration. The N–H_4 peak disappeared after addition of 1.0 equivalent of maleate, whilst a new signal was observed at δ = 20.16 ppm. This suggests the formation of a [**HM**]$^-$ (**M** = maleate anion) species.[13] The monodeprotonation is also signaled by the significant upfield shift of the protons of the naphthyl group.[14] Such an effect derives from

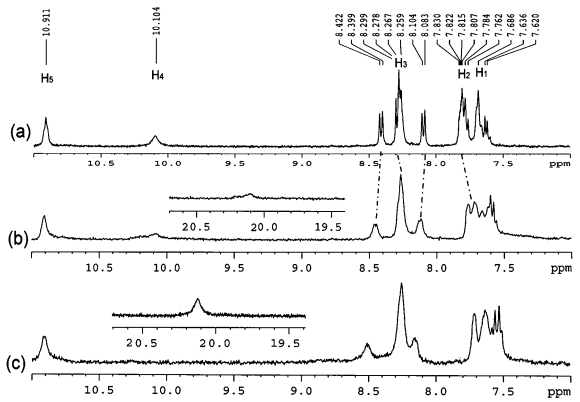


Fig. 3 ^{1}HNMR (400 Hz) spectra of sensor **1** (10 mM) in DMSO-d_6 upon addition of various quantities of maleate: (a) 0 eq.; (b) 0.5 eq.; (c) 1.0 eq.

the through-bond propagation onto the naphthyl framework of the electronic charge generated on N–H deprotonation. In addition, the other signals of thioureas (N–H) were also found to undergo downfield shifts when maleate was added. The results implied that once the complex is formed by the receptor **1** with maleate, the monodeprotonation of the receptor occurs. To provide support for the supposition, the intermolecular N–H–O hydrogen bonded distances were calculated at the HF/6-31G (d) level using *ab initio* calculations (Fig. 4). Four protons of thioureas are directed toward anion ligands but each hydrogen-bond distance is different as shown in Table 2. Among them, only the proton (H_4) that is connected to the 4-nitronaphthyl group has a much shorter distance to the carboxylic group than a typical hydrogen-bond distance, which ranges between 1.86 and 2.16 Å.[15]

In contrast, a similar experiment was carried out with fumarate anion and a different UV–vis spectral behavior was observed. Upon addition of fumarate anion, the intensity of the absorption band at 385 nm was slightly increased while the band at 521 nm was gradually decreased and blue-shifted to 472 nm, and a tailing absorption at 400 nm appeared (Fig. 5). The blue shift was probably due to recognition of fumarate, the anion induced twisting of the two thiourea moieties out of the plane of the 4-nitronaphthyl and 4-trifluoromethylphenyl chromophores, respectively. During the process, the most pronounced effect is the fumarate anion induced color change from dark-blue to bright-yellow (Fig. 2). The dramatic color change might be the first reported example for the recognition of fumarate by a colorimetric sensor. In order to investigate the notion that N–H deprotonation effects were or

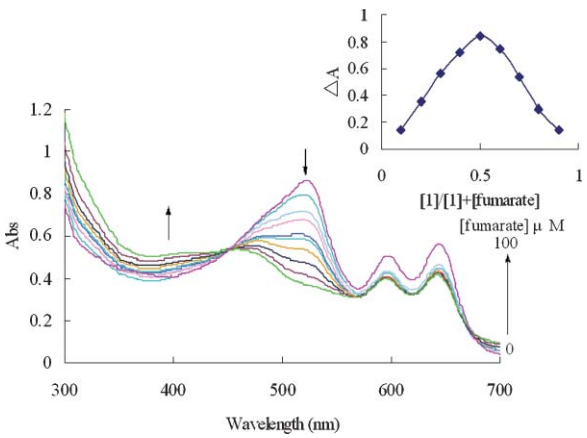


Fig. 5 A series of spectra taken over the course of the titration of a 5×10^{-5} M DMSO solution in **1** with a standard solution of fumarate at 25 °C. The titration profile (insert) indicates the formation of a 1 : 1 complex.

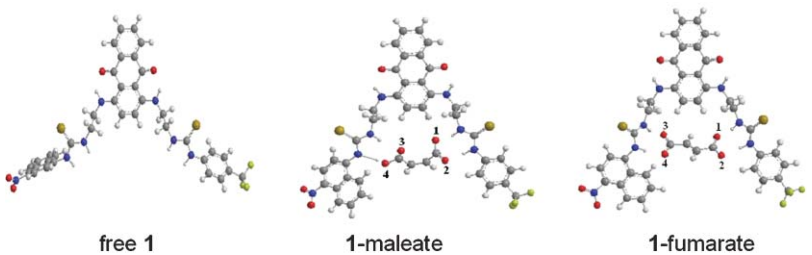
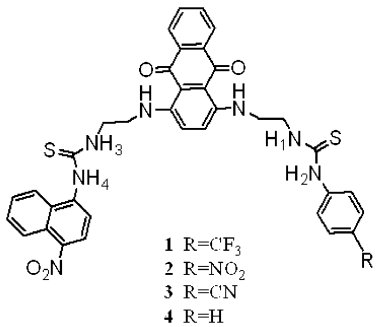


Fig. 4 Optimized geometries from *ab initio* HF/6-31G(D) calculations.

Table 2 Receptor distances[a] of NH···L$^-$ hydrogen bonds from *ab initio* HF/6–31G(D) calculations



Receptor	Compound	H(1)···L$^-$	H(2)···L$^-$	H(3)···L$^-$	H(4)···L$^-$
Maleate[b]	**1**	1.9555(O1)	1.8585(O2)	2.1555(O3)	0.9715(O4)
	2	1.9385(O1)	1.8385(O2)	2.1595(O3)	0.9715(O4)
	3	1.9455(O1)	1.8475(O2)	2.1575(O3)	0.9715(O4)
	4	1.9625(O1)	1.8755(O2)	2.1465(O3)	0.9705(O4)
Fumrate[b]	**1**	1.7805(O1)	1.8765(O2)	1.7815(O3)	1.9145(O4)
	2	1.7635(O1)	1.8815(O2)	1.7875(O3)	1.8885(O4)
	3	1.7705(O1)	1.8935(O2)	1.7865(O3)	1.8845(O4)
	4	1.7935(O1)	1.9435(O2)	1.7805(O3)	1.8805(O4)

[a] The unit of computed distances is Å. [b] Four oxygen atoms (O1, O2, O3 and O4) of the guest form hydrogen bonds with the receptors where O1 is hydrogen-bonded to H1 and O2 to H2 and O3 to H3 and O4 to H4.

were not contributing to the anion-induced effects, the ^{1}H NMR spectral analyses were carried out in DMSO-d_6. A notable feature of these titrations is that the proton signals of thioureas underwent less downfield shifts when **1** formed a complex with fumarate. After the addition of 1 equivalent of fumarate, the signal of N–H_4 was found to shift from 10.14 to 10.83 ppm (Fig. 6). This relative small downfield shift indicates that the complex is formed through multiple hydrogen bonds and is inconsistent with a deprotonation process between receptor and fumarate. This rationale was also supported by the *ab initio* calculation that showed the proton (H_4) that is connected to the 4-nitronaphthyl group has a typical hydrogen-bond distance to the carboxylic group (Table 2). Judging from the UV–vis titrations, the binding of fumarate allowed the Job's plot method[11] (as shown in the inset of Fig. 5) to be used in the determination of the binding stoichiometry, which was found to be a 1 : 1 host–guest complexation. The association constant was calculated and is shown in Table 1. The comparison of the UV–vis absorption spectra of the complex **1** upon addition of either maleate or fumarate anions is shown in Fig. 7. Apparently, the receptor **1** has demonstrated higher sensitivity and selectivity recognition for maleate over fumarate in DMSO.

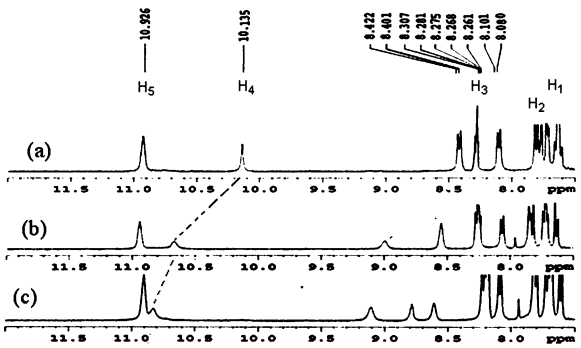


Fig. 6 ^{1}HNMR (400 Hz) spectra of **1** (10 mM) in DMSO-d_6 upon addition of various quantities of fumarate: (a) 0 eq.; (b) 0.5 eq.; (c) 1.0 eq.

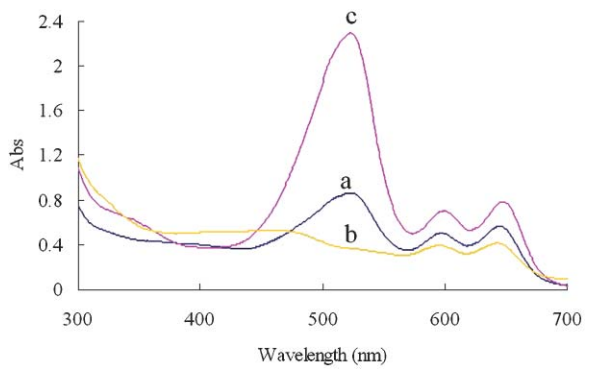


Fig. 7 UV–vis spectra change of **1** operated in DMSO (5.0×10^{-5}M) after addition of 2.0 equivalents of anions: (a) **1** only; (b) **1** + fumarate; (c) **1** + maleate.

In order to investigate how the different substituents on the phenyl ring of the other branch of the thiourea group can influence the anion binding and sensing properties, the 4-trifluoromethylphenyl group was replaced by a more electron withdrawing group, a 4-nitrophenyl group; the receptor **2** was examined. Among the sensors illustrated in Scheme 1, compound **2** is expected to show the strongest binding with maleate, due to the enhanced acidity of the thiourea protons. With progressive addition of maleate to receptor **2**, the intensity of the absorption peak at 360 nm was gradually decreased and a new band at 523 nm concomitantly evolved and reached its limiting value after the addition of 2.0 equivalents of maleate. A clear isobestic point at

423 nm was observed (Fig. 9). Such a significant change of the UV–vis spectra of receptor **2** upon titration with maleate can be attributed to the deprotonation of the thiourea proton, similar to that mentioned above. The monodeprotonated receptor $\mathbf{L_2}^-$ ($\mathbf{2} = \mathbf{L_2H}$), is responsible for the absorption at 523 nm. This was similarly confirmed by the reaction of adding strong base [*n*-Bu_4N]OH (*cf.* SI-3, see ESI†) and also corroborated by ^1H NMR titration of receptor **2** with maleate in which the peak of $[\mathbf{HM}]^-$ (**M** = maleate anion) appeared at 20.22 ppm (*cf.* SI-4, see ESI†). These changes are accompanied by a different color change from a blue solution to a violet color (Fig. 8). Judging from the UV–vis titrations, the Job's plot method showed the formation of a 1 : 1 stoichiochemistry complex of **2** with maleate (as shown in the inset of Fig. 9). The association constant was calculated[11] and listed in Table 1. To elucidate the interaction between the maleate anion and the receptor **2**, the *ab initio* calculation of the [**2** · maleate] complex was taken. It clearly shows only the proton (H_4) has a much shorter distance to the carboxylic group than a typical hydrogen-bond distance (Table 2). Based on these results it can be concluded that both the N–H_4 monodeprotonation on the 4-nitronaphthyl thiourea unit and the hydrogen-bond induced π-delocalization on the other 4-nitrophenyl thiourea moiety are believed to be responsible for signaling the binding event.

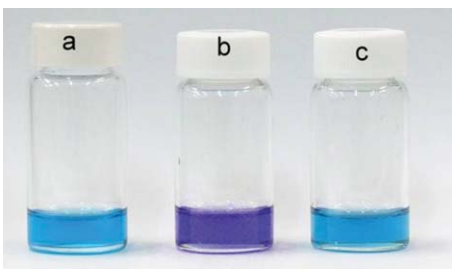


Fig. 8 Color changes of complex **2** upon addition of various anions in DMSO: (a) **2** only; (b) **2** + 2.0 equiv. of maleate; (c) **2** + 2.0 equiv. of fumarate.

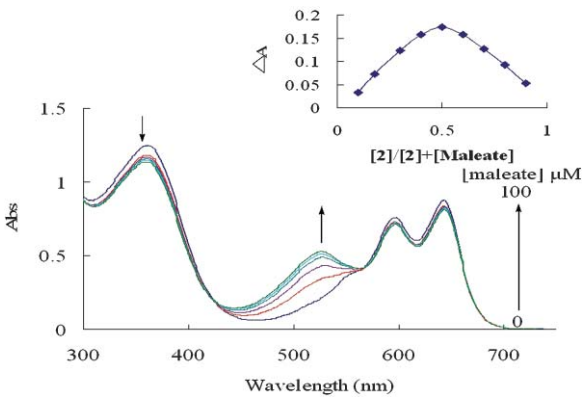


Fig. 9 A series of spectra taken over the course of the titration of a 5×10^{-5} M DMSO solution in **2** with a standard solution of maleate at 25 °C. The titration profile (insert) indicates the formation of a 1 : 1 complex.

On the contrary, upon addition of different concentrations of fumarate to the solution of receptor **2**, the initial weak shoulder peak at 552 nm was blue-shifted to 527 nm with a small decrease in the intensity of the peak at 360 nm (*cf.* SI-5, see ESI†). However, in this process, no noticeable color change was observed and the solution remained blue (Fig. 8). Thus, it indicates that the receptor **2** is weakly binding or not interacting significantly with fumarate in this solvent medium. The interaction of receptor **2** with fumarate was corroborated by ^1H NMR titration experiments. It was found that when receptor **2** formed a complex with fumarate, the proton signal of N–H_4 underwent downfield shifts with increasing fumarate concentration from 10.28 to 11.50 ppm (*cf.* SI-6, see ESI†). By the same token, this relative small downfield shift indicates the formation of weak hydrogen bonding instead of deprotonation between fumarate and receptor. This is also supported by the *ab initio* calculation (Table 2). Since receptor **2** has a unique color change and higher selectivity for maleate than fumarate, it can act as an optical chemosensor for recognition of maleate *versus* fumatate. The different color observed with maleate and fumarate can be related to the receptor stereochemistry that gives rise to different geometries depending on the anion stereochemistry. Thus, the maleate anion with its *cis* configuration perfectly fits into the complex inducing a conformation change in the receptor. By contrast, the fumarate anion with a *trans* disposition of carboxylate moieties does not induce changes in the ligand conformation and only a small increase of the UV–vis absorption is observed. The proposed conformational structure for the complex formed between receptor **2** and the maleate anion is shown in Fig. 10. Besides that, the basicity of the anion may also play an important role for recognition. Since the maleate dianion is more basic than the fumarate dianion (maleic acid, pK_{a1} : 5.0, pK_{a2} : 18.8; fumaric acid, pK_{a1} : 9.0, pK_{a2} : 11.0 in DMSO),[16] thus the deprotonation of N–H_4 will occur preferentially for the maleate anion.

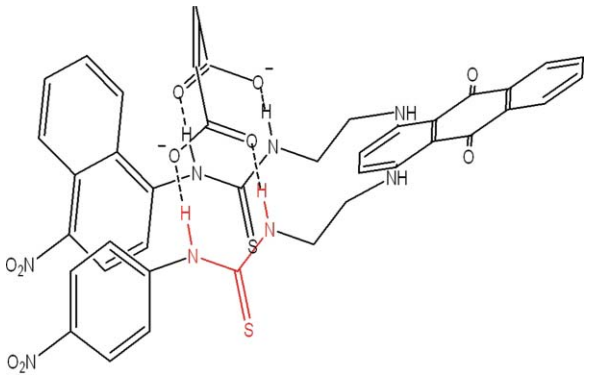


Fig. 10 Possible binding model of **2** with maleate anion.

A weaker or a non-electron-withdrawing substituent on the phenyl moiety will decrease the acidity of the thiourea protons. This is illustrated by the receptor **3** (R = CN) and the receptor **4** (R = H). The UV–vis absorption spectral profiles of **3** and **4** with addition of maleate anion are similar to that of **1** with maleate anion (*cf.* SI-7 and SI-8, see ESI†). It appeared that both the receptors **3** and **4** have the same propensity; deprotonation of the NH group occurred upon addition of maleate anion. This is similarly proved by the titration of **3** and **4** with [*n*-Bu_4N]OH and by ^1H NMR titration experiments, respectively. During the titration process, the color of the solution changes from an initial

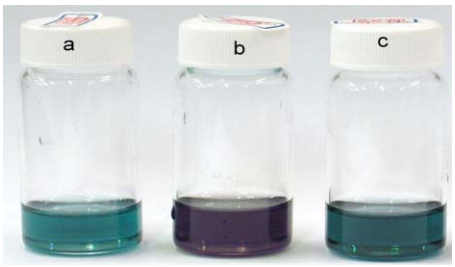


Fig. 11 Color changes of complex **3** upon addition of various anions in DMSO: (a) **3** only; (b) **3** + 2.0 equiv. of maleate; (c) **3** + 2.0 equiv. of fumarate.

blue-green color to a purple color (Fig. 11). An apparent 1 : 1 binding constant of each receptor was determined and listed in Table 1.

Consistent with the result of receptor **2**, titration of **3** or **4** with fumarate anion also gave an unnoticeable color change. The color of the solution still remains the original blue-green color (Fig. 11). The weak hydrogen-bonding between **3** or **4** with fumarate was corroborated by the ^{1}H NMR titration experiments and the hydrogen-bonded distances were reflected in the *ab initio* calculations (Table 2). Due to the unique color change and higher selectivity for maleate than fumarate anion of **3** and **4**, they can be used as optical chemosensors for recognition of maleate *versus* fumarate.

The binding of **1–4** receptors with three aromatic isomeric dicarboxylate anions (*ortho*/*meta*/*para*-phthalate) were also studied. Unfortunately, no distinct color change was observed (*cf.* S1–12–S1–14, see ESI†). Therefore, the chromogenic reagents **1–4** cannot be used for discrimination between these three aromatic isomers.

Conclusions

In conclusion, a class of easily prepared sensitive colorimetric receptors was synthesized, and the recognition of isomeric dicarboxylate anions was also studied. Among them, receptors **1–4** show good sensitivity and selectivity for discrimination of maleate *versus* fumarate by drastic color changes. Thus, receptors **1–4** can be used as optical chemosensors for recognition of maleate *versus* fumarate anion. Among them, the receptor **1** has also a unique color change for recognition of fumarate, accordingly it can be used for detection of the fumarate anion. It was also found that the performance of the sensor is highly dependent on the substituent on the phenyl ring; a stronger electron-withdrawing group (*i.e.* NO_2 > CN > CF_3 > H) resulted in a sensor with a higher binding constant with maleate. From the results of the titrations with [*n*-Bu_4N]OH, ^{1}H NMR titration experiments and the *ab initio* calculations, it was clearly demonstrated that a NH deprotonation occurred for the receptors in the presence of the maleate anion and on the other hand, only a multiple hydrogen-bonded complex was formed in the presence of the fumarate anion. Further work will continue to develop the practical colorimetric sensors for recognition of isomeric dicarboxylates that are effective in aqueous solution.

Experimental

General

The chemical reagents were purchased from Acros or Aldrich Corporation and utilized as received, unless indicated otherwise. All solvents were purified by standard procedures. Melting points were measured on a Yanaco MP-S3 melting-point apparatus. The infrared spectra were performed on a Perkin Elmer System 2000 FT-IR spectrophotometer. UV–Vis spectra were measured on a Cary 300 spectrometer. All NMR spectra were measured on a Bruker spectrometer at 400 (^{1}H) and 100 MHz (^{13}C) with DMSO-d_6 as solvent. High-resolution mass spectra were measured with a Finnigan/Thermo Quest MAT 95XL instrument.

Preparation of tetrabutylammonium salts

To a stirred solution of a dicarboxylic acid (2.5 mmol) in dry methanol (5 mL), 2.0 equiv. of a 1.0 M solution of tetrabutylammonium hydroxide in methanol (5 mL) was added. The resulting mixture was stirred for 2 h at room temperature. The solvent was evaporated *in vacuo* and dried over P_2O_5. The resulting tetrabutylammonium salt was stored under anhydrous conditions before use.

1-(2-Aminoethylamino)-4-(4-nitronaphthylthiourelyene-ethene-amino)anthraquinone (6)

To a stirred solution of 1,4-di-(2-aminoethylamino)anthraquinone[8] (0.30 g, 0.90 mmol) in THF (50 mL), 4-nitronaphthylisothiocyanate (0.14 g, 0.60 mmol) in THF (50 mL) was added at room temperature. The resulting mixture was stirred and heated to reflux for 18 h. After cooling to room temperature, the solution was concentrated in vacuum. The crude product was washed with CH_2Cl_2 several times to afford the pure **6**. Yield: 0.22 g (42%), as a blue solid. mp: 125–128 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.73–3.76 (m, 8H), 7.64–7.72 (m, 2H), 7.74–7.84 (m, 4H), 8.09 (d, 2H, J = 8.4 Hz), 8.22–8.32 (m, 6H), 8.42 (d, 2H, J = 8.4 Hz), 10.93 (br s, 2H). ^{13}C NMR (DMSO-d_6): δ 30.8, 40.7, 44.2, 108.8, 122.5, 122.8, 123.8, 124.7, 125.5, 125.8, 127.6, 129.4, 130.1, 132.8, 134.1, 141.1, 143.5, 146.3, 181.2, 182.1. FT-IR (KBr): 3293, 3068, 2930, 2853, 2331, 1560, 1506, 1311, 1265, 1168, 1045, 1025, 830, 769, 718, 601 cm^{-1}. UV (DMSO): 372 nm (ε = 10 270), 596 nm (ε = 17 200), 641 nm (ε = 19 175). HRMS (FAB) calcd for $C_{29}H_{26}N_6O_4S$ [M$^+$] 554.1736; found 554.1731.

1-(4-Nitronaphthylthiourelyene-ethene-amino)-4-(4-trifluoromethylphenyl-thiourelyene-ethene-amino)anthraquinone (1)

To a stirred solution of **6** (0.30 g, 0.54 mmol) in THF (50 mL), 4-trifluoromethylphenylisothiocyanate (0.22 g, 1.08 mmol) in THF (50 mL) was added at room temperature. The resulting mixture was stirred and heated to reflux for 22 h. After cooling to room temperature, the solution was concentrated in vacuum. The crude product was washed with CH_2Cl_2 several times to afford the pure **1**. Yield: 0.15 g (37%), mp: 195–196 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.70–3.82 (m, 8H), 7.63–7.67 (m, 2H), 7.70–7.76 (m, 2H), 7.80–7.83 (m, 4H), 8.08 (d, 2H, J = 8.4 Hz), 8.26–8.30 (m, 6H), 8.40 (d, 2H, J = 8.8 Hz), 10.10 (br s, 2H), 10.90 (br s, 2H). ^{13}C NMR (DMSO-d_6): δ 40.4, 44.0, 108.6, 122.1, 122.6, 123.6, 124.5,

125.3, 125.6, 127.3, 129.2, 129.7, 132.4, 133.7, 140.6, 143.1, 146.1, 180.7, 181.8. FT-IR (KBr): 3269, 2332, 1518, 1323, 1267, 1166, 1065, 1021, 831, 767, 729, 669 cm^{-1}. UV (DMSO): 521 nm ($\varepsilon =$ 21 344), 596 nm ($\varepsilon = 10\,253$), 643 nm ($\varepsilon = 10\,626$). HRMS (FAB) calcd for $C_{37}H_{30}F_3N_7O_4S_2$ [M^+] 757.1744; found 757.1756.

1-(4-Nitronaphthylthiourelyene-ethene-amino)-4-(4-nitrophenylthiourelyene-ethene-amino)anthraquinone (2)

A similar procedure to the synthesis of **1** was carried out using 4-nitrophenylisothiocyanate in place of 4-trifluoromethylphenylisothiocyanate. Yield: 0.16 g (41%), mp: 197–199 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.74–3.76 (m, 8H), 7.69–7.71 (m, 2H), 7.75–7.82 (m, 7H), 8.09–8.15 (m, 4H), 8.25–8.31 (m, 3H), 8.54 (br s, 2H), 10.28 (br s, 2H), 10.91 (br s, 2H). ^{13}C NMR (DMSO-d_6): δ 40.6, 40.8, 44.0, 108.9, 109.0, 114.7, 120.9, 122.4, 123.9, 124.7, 125.9, 127.6, 129.9, 132.6, 134.0, 142.1, 143.4, 146.2, 180.8, 181.0, 181.1, 182.2. FT-IR (KBr): 3263, 3053, 2935, 2858, 2341, 1639, 1593, 1568, 1506, 1322, 1250 cm^{-1}. UV (DMSO): 360 nm ($\varepsilon =$ 24 925), 596 nm ($\varepsilon =$ 15 195), 643 nm ($\varepsilon =$ 17 544). HRMS (FAB) calcd for $C_{36}H_{30}N_8O_6S_2$ [M^+] 734.1730; found 734.1719.

1-(4-Nitronaphthylthiourelyene-ethene-amino)-4-(4-cyanophenylthiourelyene-ethene-amino)anthraquinone(3)

A similar procedure to the synthesis of **1** was carried out using 4-cyanophenylisothiocyanate in place of 4-trifluoromethylphenylisothiocyanate. Yield: 0.15 g (36%), mp: 197–198 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.73–3.76 (m, 8H), 7.66–7.68 (m, 2H), 7.75–7.82 (m, 6H), 8.11 (d, 2H, $J = 8.4$ Hz), 8.26–8.30 (m, 6H), 8.42 (d, 2H, $J = 8.8$ Hz), 10.14 (br s, 2H), 10.91 (br s, 2H). ^{13}C NMR (DMSO-d_6): δ 39.6, 39.8, 44.0, 108.8, 122.5, 122.8, 123.8, 124.7, 125.5, 125.8, 127.6, 129.4, 130.1, 132.8, 134.1, 141.1, 143.5, 146.3, 181.2, 182.1. FT-IR (KBr): 3295, 3064, 2928, 2854, 2335, 1570, 1510, 1310, 1264, 1170 cm^{-1}. UV (DMSO): 524 nm ($\varepsilon =$ 10 320), 596 nm ($\varepsilon =$ 11 160), 643 nm ($\varepsilon =$ 13 540). HRMS (FAB) calcd for $C_{37}H_{30}N_8O_4S_2$ [M^+] 714.8153; found 714.8157.

1-(4-Nitronaphthylthiourelyene-ethene-amino)-4-(phenylthiourelyene-ethene-amino)anthraquinone (4)

A similar procedure to the synthesis of **1** was carried out using phthylisothiocyanate in place of 4-trifluoromethylphenylisothiocyanate. Yield: 0.23 g (59%), mp: 206–207 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.72–3.75 (m, 8H), 7.60–7.64 (m, 1H), 7.67–7.73 (m, 2H), 7.76–7.86 (m, 6H), 8.10 (d, 2H, $J = 8.4$ Hz), 8.26–8.34 (m, 6H), 8.41 (d, 2H, $J = 8.4$ Hz), 10.13 (br s, 2H), 10.92 (br s, 2H). ^{13}C NMR (DMSO-d_6): δ 30.9, 40.8, 44.3, 108.9, 122.4, 122.9, 123.9, 124.8, 125.6, 125.9, 127.7, 129.5, 130.0, 132.7, 134.0, 141.0, 143.4, 146.4, 181.1, 182.2. FT-IR (KBr): 3293, 3068, 2930, 2853, 2330, 1639, 1568, 1506, 1311, 1265, 1168, 1045, 1025, 830 cm^{-1}. UV (DMSO): 382 nm ($\varepsilon =$ 10 438), 523 nm ($\varepsilon =$ 8768), 596 nm ($\varepsilon =$ 17 290), 643 nm ($\varepsilon =$ 19 294). HRMS (FAB) calcd for $C_{36}H_{31}N_7O_4S_2$ [M^+] 689.1789; found 689.1792.

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