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Synthesis, light absorption and fluorescence properties of new thiazole analogues of the xanthene dyes

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This paper is dedicated to the memory of Professor Masaru Matsuoka.

Abstract

The synthesis of a new class of cationic dye is described, whose chromophoric system is formally related to that of the cationic xanthene dyes in which one of the aminophenyl rings is replaced by a 2-aminothiazole ring. The light absorption and fluorescence properties of a range of representative dyes of this type have been investigated. The dyes were found to absorb at shorter wavelengths than their xanthene analogues (λ_{max} 490–506 nm in methanol), in agreement with theoretical predictions, and they also showed moderate to intense fluorescence in dichloromethane solution, with Stokes shifts ranging from 33 to 50 nm.

Keywords: Thiazole cationic dyes; Xanthane analogues; Visible spectra; Fluorescnece; Fluorescent indicator

1. Introduction

The xanthene dyes, e.g. Pyronine B and Pyronine G (**1a** and **1b** respectively) have long been known, and are noted for their intense fluorescence. The 9-aryl derivatives, or rhodamines, typified by Rhodamine B (**2**) are particularly useful as laser dyes [1] and as fluorescent biolabels [2]. In general, the xanthenes are characterised by intense, narrow absorption bands with small Stokes shifts. The analogous chromeno[2,3-*d*][1,3]thiazol-4-ium system **3**, in which one of the fused benzene rings is replaced by a thiazole ring is unknown, but dyes of this type might also be expected to show similar intense light absorption and reasonable fluorescence. We now describe a synthetic route to such cationic dyes, and report on their light absorption and fluorescence characteristics.

2. Results and discussion

2.1. Synthesis of dyes

Dyes **3a**–**f** were prepared by the reaction sequence shown in Scheme 1. Thus the active methylene characteristics of the 2-aminothiazol-4(5H)-ones **4** were utilised to condense them with 3-hydroxy-4-*N*,*N*-diethylaminobenzaldehyde to

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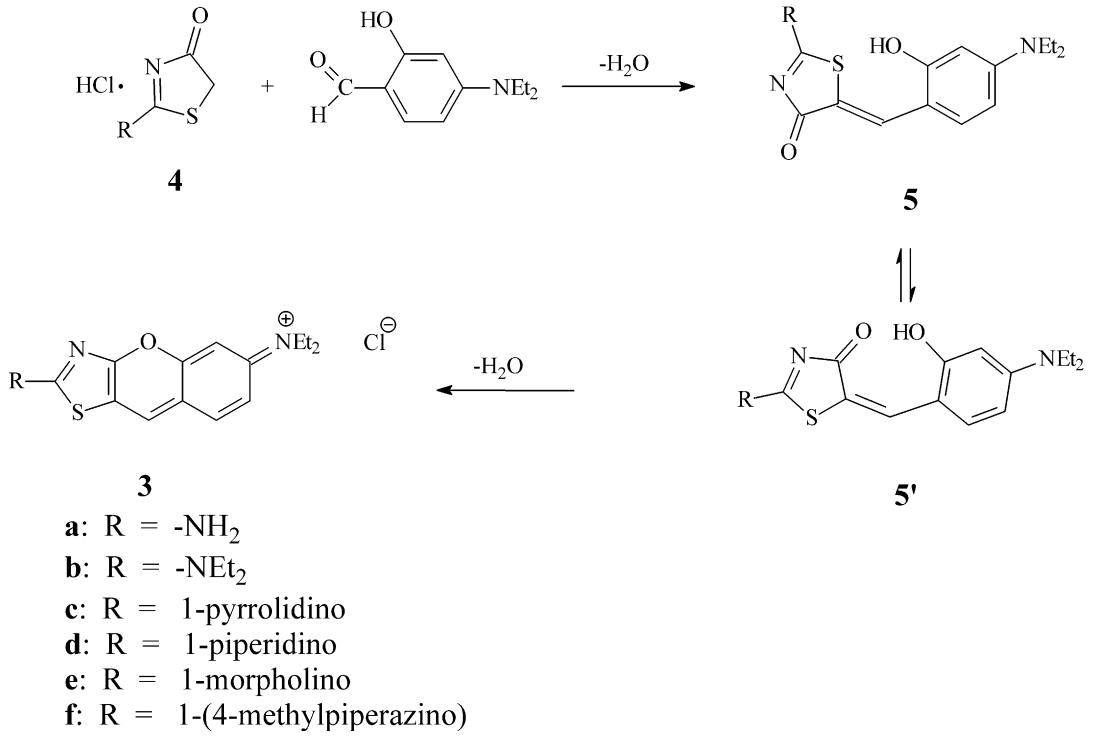
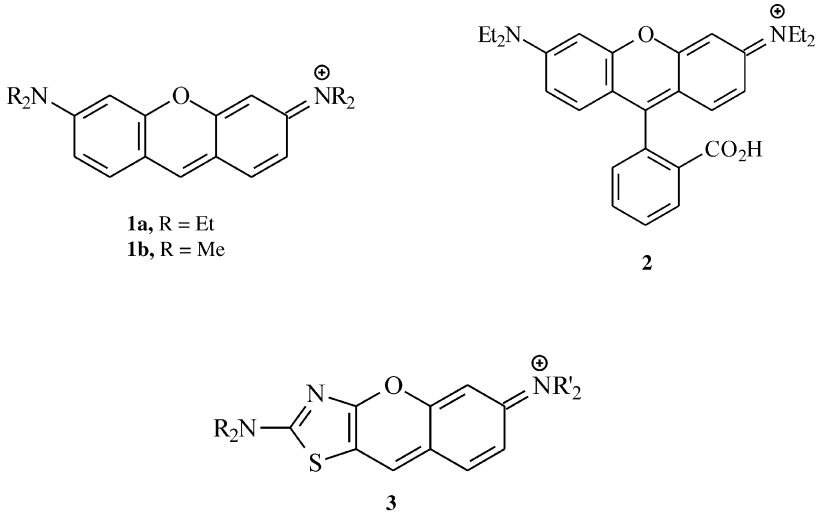


Scheme 1.



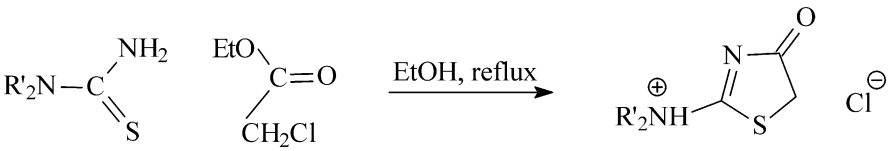
give initially the methine dyes **5**, further dehydration of which gave the desired products **3**.

The required *N*,*N*-disubstituted 2-aminothiazol-4(5H)-one hydrochlorides **4** were readily prepared by reacting the appropriate *N*,*N*-disubstituted thiourea [3] with ethyl chloroacetate (Scheme 2), and were isolated as white solids, in yields ranging from 65 to 88%.

The condensation/cyclisation sequence used to prepare **3** was most conveniently carried out in

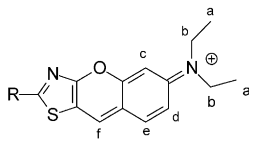
refluxing ethanol, although the reactions were relatively inefficent and the cyclised products **3** could only be isolated in poor yields (ca. 15–36%). The use of other solvents, catalysts and dehydrating media gave little if any improvement. The low efficiency of the reaction appeared to be due to the fact that the initially formed methine dye **5** tended to crystallise out during the reaction and was itself resistant to further cyclisation. As cyclisation requires the methine dye to adopt the less favourable conformation **5′**, if the more stable isomer **5** is appreciably less soluble than **5′**, then precipitation of the former will occur in competition with the desired cyclisation reaction.

In order to isolate the cationic dye, the precipitated **5** was first removed from the reaction mixture by filtration, and the filtrates were then diluted with water to deposit other water insoluble impurities, which could be filtered off. The resultant clear solution of **3** as its chloride salt was then treated with sodium perchlorate, when the perchlorate salt of **3** was deposited. After isolation by filtration, further purification was then effected by recrystallisation. The purity of the products was confirmed by TLC, and structural characterisation was carried put by mass spectrometry and NMR spectroscopy. Relevant data are summarised in Table 1.



Scheme 2.

Table 1
Characterisation data for dyes **3**



3

Dye	Mass spectrum	^{1}H NMR spectra ($CDCl_3$)		
		N–CH_2CH_3	Aromatic H	*R*
3a	(FAB; 3-NBA) *m/z* 274 (M^+), $C_{14}H_{16}N_3OS$ requires 274.	1.15 (t, 6H-a, *J* = 7.0) 3.54 (q, 4H-b, *J* = 7.0)	7.10 (s, 1H-c); 7.13 (d, 1H-d, *J* = 9.2); 7.81 (d, 1H-e, *J* = 9.2); 8.73 (s, 1H-f)	NH_2 10.34 (s, 2H)
3b	(ES^+) *m/z* 330 (M^+), $C_{18}H_{24}N_3OS$ requires 330.	1.18 (t, 3H-a, *J* = 7.0) 1.27 (t, 3H-a, *J* = 7.0) 3.44–3.60 (m, 4H-b)	6.77 (s,1H-c); 6.97 (d, 1H-d, *J* = 9.0); 7.83 (d, 1H-e, *J* = 9.0); 8.92 (s, 1H-f)	NCH_2CH_3 1.36–1.46 (m, 6H) 3.62 (q, 2H, *J* = 7.2) 3.89 (q, 2H, *J* = 7.2)
3c	(ES^+) *m/z* 328 (M^+), $C_{18}H_{22}N_3OS$ requires 328	1.27 (t, 6H-a, *J* = 7.2) 3.51 (q, 4H-b, *J* = 7.2)	6.76 (s, 1H-c); 6.97 (d, 1H-d, *J* = 9.2); 7.82 (d, 1H-e, *J* = 9.2); 8.88 (s, 1H-f)	Pyrrolidino 2.18–2.30 (m, 4H) 3.63 (t, 2H, *J* = 6.8) 3.96 (t, 2H, *J* = 6.8)
3d	(FAB; 3-NBA) *m/z* 342 (M^+), $C_{19}H_{24}N_3OS$ requires 342.	1.27 (t, 6H-a, *J* = 7.2) 3.50 (q, 4H-b, *J* = 7.2),	6.76 (s, 1H-c); 6.96 (d, 1H-d, *J* = 9.2); 7.81 (d, 1H-e, *J* = 9.2); 8.89 (s, 1H-f)	Piperidino 1.83 (m, 6H) 3.66–3.68 (m, 2H), 4.09 (m^a, 2H)
3e	(ES^-) *m/z* 344 (M^+), $C_{18}H_{22}N_3O_2S$ requires 344	1.15 (t, 6H-a, *J* = 6.9) 3.55 (q, 4H-b, *J* = 6.9)	7.11 (s, 1H-c); 7.17 (d, 1H-d, *J* = 9.2); 7.83 (d, 1H-e, *J* = 9.2); 8.87 (s, 1H-f)	Morpholino 3.81 (m, 4H) 4.04 (m, 4H)
3f	(FAB; 3-NBA) *m/z* 357 (M^+), $C_{19}H_{25}N_4OS$ requires 357.	1.18 (t, 6H-a, *J* = 6.9) 3.55 (q, 4H-b, *J* = 6.9)	7.09 (s, 1H-c); 7.15 (d, 1H-d, *J* = 9.2); 7.82 (d, 1H-e, *J* = 9.2); 8.85 (s, 1H-f)	*N*-Methylpiperazino 2.28 (s, 3H) 3.72 (m, 4H) 4.04 (m, 4H)

2.2. Light absorption properties

The cyclised dyes **3** can be regarded as xanthene dye analogues, in which one of the aminophenyl rings of the xanthene system has been replaced by a 2-aminothiazole ring. The two classes of chromophore are thus *iso-π-electronic*. Compared to the symmetrical bis-*N*,*N*-diethylaminoxanthene dye. Pyronin B (**1a**), the thiazole dyes are significantly more hypsochromic. Thus, for example, whereas Pyronin B perchlorate has a λ_{max} value of 553 nm in ethanol [4], the bis-*N*,*N*-diethylaminothiazole analogue **3b** has $\lambda_{max} = 502$ nm in the same solvent. As can be seen from the data of Table 2, dyes **3a**–**f** are red in colour, with λ_{max} values in the range 490 – 506 nm in methanol, whereas corresponding xanthenes are generally purple to violet.

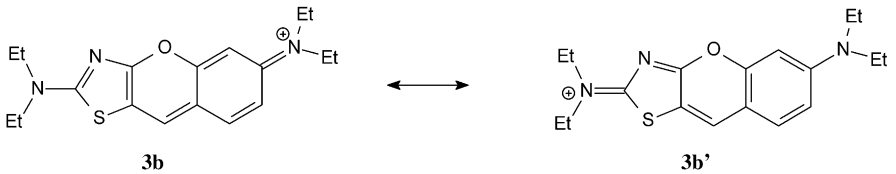
It is well known that the light absorption properties of substituted derivatives of di- and tri-arylmethane cationic dyes, can be interpreted successfully by perturbational MO theory, as summarised, for example, by *Dewar's rules* [5]. The xanthene dyes are amenable to this approach, as they have a similar high degree of electronic symmetry to the diarylmethane dyes. However, Dewar's rules specifically refer to π-electron systems that are directly analogous to the odd-alternant hydrocarbons, i.e. conjugated systems that do not contain any odd-numbered rings. Thus the thiazole analogues **3**, which contain five-membered rings, are inappropriate for application of these rules. However, simple resonance theory can be applied qualitatively. Thus in the thiazole **3b**, the two principal resonance forms are **3b** and **3b′** (Scheme 3), and unlike the two corresponding resonance forms of Pyronine B, which are identical, these are of unequal energy and will make an unequal contribution to the hybrid state. According to resonance theory, this should lead to a hypsochromic shift of the first absorption band, as observed in practice.

Table 2
Visible absorption spectroscopic properties of dyes **3a**–**f**

Dye	λ_{max} (nm) (DCM)	ε_{max} ($lmol^{-1}$ cm^{-1}) (DCM)	λ_{max} (nm) (MeOH)	λ_{max} (nm) (acetone)
3a	511	47,100[a]	490	492
3b	523	59,300	499	501
3c	521	67,100	499	500
3d	522	62,800	499	501
3e	526	67,000	506	504
3f	525	43,400[a]	504	502

[a] Containing 5% methanol to aid dissolution.

The thiazole xanthene analogues were examined by the PPP-MO method, using parameters that have previously been derived for related dye classes. The positively charged terminal nitrogen atoms in cationic diarylmethane dyes are assigned lower VSIP (valence state ionisation potential) and larger EA (electron affinity) values than analogous uncharged atoms, as they are constrained to a state of sp^2-hybridisation, with maximum overlap between the nitrogen lone pair orbital and the π-orbital system of the remainder of the chromogen [6]. The resultant increase in effective electron releasing capacity of the amino nitrogen has to be reflected in a lower VSIP value for the nitrogen atom. It has been shown that the nitrogen atom parameters which provide the best correlation with the experimental results for the xanthene analogue of Michler's Hydrol Blue, i.e. Pyronine G (**1b**), were VSIP = 12.5 eV and EA = 9.5 eV, with values of VSIP = 40.0 eV and EA = 14.5 eV for the bridging oxygen atom [7]. These *N*,*N*-dimethylamino nitrogen values were modified empirically for our *N*,*N*-diethylamino thiazole analogue **3b** in order to take into account the slightly greater electron releasing capacity of the *N*,*N*-diethylamino group, giving values of



3b **3b'**

Scheme 3.

VSIP = 12.0 eV and EA = 9.0 eV. Using these parameters, the absorption spectra of the *N*,*N*-dimethylamino dye 1b and the thiazole dye **3b** were calculated by the PPP method. The experimental and calculated λ_{max} values for these two dyes are compared in Fig. 1. Fig. 1 also shows the calculated ground state charge distribution for the two dyes, and Fig. 2 compares the changes in π-electron charge density for their visible electronic transitions.

As can be seen from Fig. 1, the xanthene dye **1b** shows a high degree of electronic symmetry in its ground state. In contrast, the thiazole **3b** has a much lower degree of electronic symmetry in its ground state, with a significantly greater positive charge on the thiazole amino group nitrogen than on the phenyl amino group nitrogen. This implies that resonance form **3b′** makes a greater contribution to the hybrid ground state than **3b** (Scheme 3). This is to be expected in view of the greater aromaticity of the benzene ring compared to the thiazole ring. The π-electron density changes are also informative (Fig. 2), and in the case of Pyronin G, there is little change in π-electron density at any position accompanying electronic excitation, with the sole exception of the bridging carbon atom, where there is a large increase. However, in the case of **3b**, light absorption to give the first excited singlet state results in a significant measure of electron density loss from the *N*,*N*-diethylamino group attached to the benzene ring, with a concomitant build up of electron density in the thiazole ring. It is noteworthy that the nitrogen atom of the diethylamino group attached to the thiazole ring shows practically no change in electron density in the excited state. This suggests that substituents attached to this nitrogen will have only a small effect on the λ_{max} value of the dye, and helps to explain why the dyes **3a**–**f** show little variation in their λ_{max} values (for example, the values fall within the range 511–526 nm in DCM).

2.3. Fluorescence properties

The fluorescence properties of dyes **3** in the solvents dichloromethane and methanol are summarised in Table 3. Approximate fluorescence quantum yields were measured relative to Rhodamine 6G, which has an absolute fluorescence quantum yield close to unity [8]. Thus the fluorescence intensity of each dye solution at its emission maximum was measured relative to that of Rhodamine 6G, keeping the solution absorbance values constant (ca. 0.1).

It can be seen from Table 4 that in DCM the fluorescence quantum efficiencies are relatively high, but are not as high as that of Rhodamine 6G, and the values range from 0.25 to 0.78. The most efficient dye is **3c**, which has a pyrrolidino group in the thiazole ring. The low quantum efficiency (0.40) of the *N*-piperazinyl dye **3f** can be attributed to some measure of intramolecular photoelectron transfer (PET) quenching from the

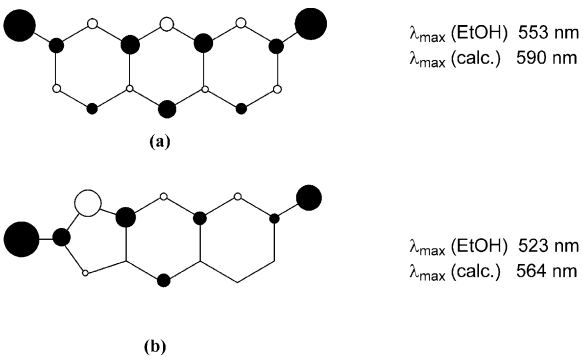


Fig. 1. Comparison of the ground state charge distribution for (a) pyronine G (**1b**), and (b) dye **3b** (filled circles and open circles represent a net positive charge and net negative charge, respectively).

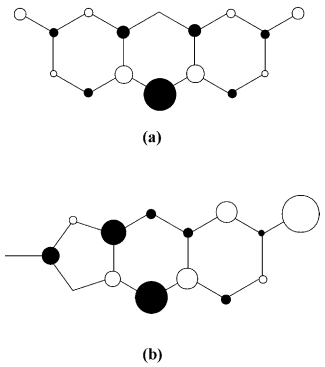


Fig. 2. Comparison of the π-electron density changes for the visible absorption transition of (a) pyronine G (**1b**) and (b) dye **3b** (open circles and filled circles represent a decrease and increase in electron density, respectively).

basic *N*-methyl residue. Stokes shifts of dyes **3** in DCM were relatively small, as expected for such rigid structures, but were slightly higher than those observed for the true xanthenes. With the exception of **3a,** which had a large Stokes shift (50 nm) the values were consistent, falling in the range 33–38 nm.

In polar protic solvents such as methanol, the quantum yield was dramatically lowered, which suggests that intermolecular hydrogen bonding interactions are important in reducing the lifetime of the fluorescent excited singlet state. It is interesting to note that the Stokes shifts of the dyes **3b**–**3f** are almost doubled in methanol, and range from 60 to 66 nm. The value for the weakly fluorescent dye **3a,** however, only increases from 50 nm in DCM to 71 nm in methanol.

It was found that the light absorption and emission properties of **3a** were pH dependent, and this may be attributed to the ability of the primary amino group in this dye to undergo deprotonation under basic conditions, giving the neutral imino dye **6**, as shown in Scheme 4.

The cationic charge on **3a** significantly lowers the pK_a value of the primary amino group, and deprotonation to give the neutral imino dye **6** occurs under mildly basic conditions. Thus addition of a small amount of triethylamine gave a pronounced colour change (red to yellow), and the fluorescence maximum shifted to shorter wavelengths accordingly. The relevant spectral data for DCM solutions of **3a** and**6** are summarised in Table 4.

On addition of the triethylamine (as a dilute solution in DCM) to the solution of **3a** in DCM, the absorption (and excitation) maximum moved some 77 nm to shorter wavelengths, and the absorbance value decreased by ca. 50%. The fluorescence maximum was displaced by 46 nm to shorter wavelengths, resulting in a much larger Stokes shift for the deprotonated dye **6** than for the cationic dye **3a** (81 and 50 nm, respectively). Most surprisingly, with excitation at the appropriate excitation maximum, the fluorescence intensity of the solution of **6** was more than twice that of **3a**. If one takes into account the fact that the absorbance of the solution of **6** at its excitation wavelength was only about one half that of **3a** at its excitation wavelength, then this suggests that the fluorescence quantum efficiency of **6** is ca. 3–4 times higher than that of **3a,** i.e. the fluorescence quantum yield for **6** should be ca. 0.75 or higher. This is an unusually high value for a dye with such a large Stokes shift. A more detailed investigation of the effect of pH on the fluorescence properties of **3a** was therefore carried out.

A solution of **3a** in distilled water was adjusted to ca. pH 3 by addition of acetic acid, resulting in an absorbance value of 0.47 at λ_{max} in a 1 cm cell. Dilute Na_2CO_3 solution was then added in small amounts, and the pH, absorbance and fluorescence intensity values noted. Fig. 3 shows

Table 4
Fluorescence excitation and emission data for **3a** and **6** in dichloromethane

Dye	λ_{max} (ex) (nm)	Abs[a]	λ_{max} (em) (nm)	Intensity[b]	Stokes shift (nm)
3a	511	0.096	561	45.5	50
6[c]	434	0.056	515	98.9	81

[a] Absorbance at λ_{max}.
[b] Fluorescence intensity at λ_{max}(em), arbitrary units; neutral solution in DCM.
[c] Addition of small amount of Et_3N to the solution of **3a**.

Table 3
Fluorescence properties of dyes **3a**–**f**

Dye	λ_{max}(ex) (nm) (DCM)	λ_{max} (em) (nm) (DCM)	Quantum yield[a] (DCM)	λ_{max} (ex) (nm) (MeOH)	λ_{max} (em) (nm) (MeOH)	Quantum yield[a] (MeOH)
3a	511	561	0.25	490	561	0.09
3b	523	557	0.67	499	561	0.18
3c	521	554	0.78	499	562	0.19
3d	522	560	0.66	499	563	0.20
3e	526	562	0.57	502	568	0.16
3f	525	562	0.40	504	564	0.10

[a] Relative to Rhodamine 6G in dichloromethane.

the pH dependence of the absorbance value at the λ_{max} of the solution, and Fig. 4 shows the pH dependence of the fluorescence intensity. In the latter case, monitoring was at the appropriate fluorescence and excitation maxima for each pH value.

The pH transition range for the conversion of **3a–6** was found to be ca. 8.0–8.5 both by absorbance and fluorescence measurements. Over this range there was a rapid decrease in the absorbance of the solution with increasing pH, and a more pronounced increase in fluorescence intensity. The fluorescence intensity did not increase further beyond pH 9. It is possible that **3a** could be a useful sensitive fluorescent pH indicator for the pH range 8–9.

3. Experimental

3.1. General

UV-Visible spectra were recorded on a Perkin-Elmer Lambda 15 spectrophotometer, and fluorescence spectra on a Perkin-Elmer LS9 luminescence spectrometer. ^{1}H NMR spectra were recorded on a Brooker 400 MHz spectrometer. Electrospray mass spectra were determined on a Quattro II instrument, using methanol–water 1:1 solvent, and FAB spectra were measured on an Autospec spectrometer (caesium ion bombardment) with a 3-nitrobenzyl alcohol matrix. Melting points are uncorrected.

3.2. Dye synthesis

3.2.1. General procedure for the preparation of N,N-disubstituted 2-aminothiazol-4(5H)-one hydrochlorides (**4b–f**)

A solution of the appropriate *N*,*N*-disubstituted thiourea (0.5–1.0 g) and an equivalent proportion of ethyl chloroacetate in ethanol (10–20 cm^3) was refluxed for 3 h. After cooling, the product was precipitated by slow addition of diethyl ether. The solid was filtered off, washed with ethanol and dried. The purity of the product was confirmed by t.l.c. Yields and characterisation data are summarised in Table 5.

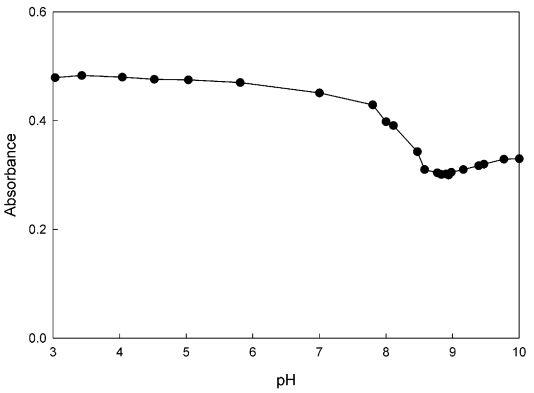


Fig. 3. Change in the absorbance at of an aqueous solution of **3a** at λ_{max} as a function of pH.

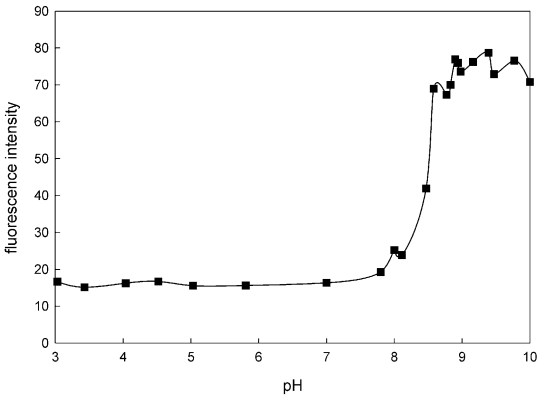
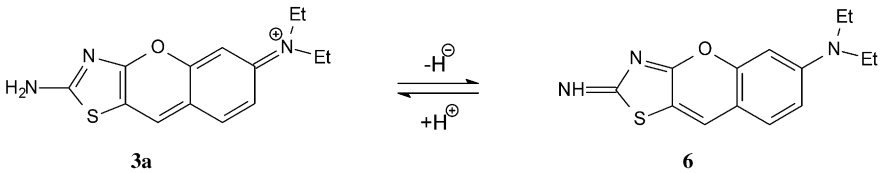


Fig. 4. Change in fluorescence intensity of an aqueous solution of **3a** as a function of pH. Fluorescence intensity measured at the appropriate excitation and emission maxima for each pH value.



Scheme 4.

Table 5
Yields and characterisation data *N*,*N*-disubstituted 2-amino-thiazol-4(5H)-one hydrochlorides **4b–f**

Compound	Formula	Yield (%)	M.p. (°C)	Mass spectrum (EI) (found, *m*/*z*)
4b	$C_7H_{12}N_2SO$	80	168–172	173(M + H)
4c	$C_7H_{10}N_2SO$	88	158–162	171(M + H)
4d	$C_8H_{12}N_2SO$	62	143–147	185(M + H)
4e	$C_7H_{10}N_2O_2S$	65	174–178	187(M + H)
4f	$C_8H_{13}N_3OS$	80	245–249	200(M + H)

Table 6
Yields, recrystallisation solvent and m.p. data for dyes **3a–f**

Dye	Yield (%)	Recrystallisation solvent	M.p./°C
3a	16	MeOH	270–272
3b	20	MeOH/pet.ether	180–186
3c	24	MeOH	265–267
3d	36	MeOH	230–233
3e	22	MeOH	237–241
3f	15	MeOH	198–200

*3.2.2. General procedure for synthesis of the thiazole dye perchlorates (**3a–f**)*

The appropriate 2-aminothiazol-4(5H)-one hydrochloride **4** (5 mmol) and *N*,*N*-diethylamino-salicylaldehyde (0.96 g, 5 mmol) were dissolved in ethanol (20 cm^3) and the solution was refluxed for 12 h. After cooling, the deposited yellow solid was filtered off and the filtrates collected. These were diluted with 30 cm^3 of water, stirred for 30 min and filtered to remove a further yellow deposit. Sodium perchlorate (1.0 g) was then added to the clear orange filtrates, and after stirring for 2 h, the red precipitate was extracted into dichloro-methane. The organic layer was isolated and dried, and the solvent was removed under vacuum. The solid residue was recrystallised to give the perchlorate salt of **3** in pure form, as indicated by t.l.c. and ^{1}H NMR spectroscopy. Recrystallisation solvent, yields and melting points are summarised in Table 6. In all cases the product was characterised by mass spectrometry and ^{1}H NMR spectroscopy, and relevant data are collated in Table 1.

4. Conclusions

Replacing one of the aminophenyl rings in the cationic xanthene dye chromophoric system by a 2-amino-substituted thiazole ring produces the iso-π-electronic system **3**. These new dyes absorb at somewhat shorter wavelengths than analogous xanthene dyes (e.g. Pyronin B), and for example, in methanol are red in colour, compared to the purple to violet colours of the xanthenes. Like their xanthene analogues, dyes **3** have high molar absorption coefficients and are fluorescent. Fluorescence quantum yields range from ca. 0.25–0.78 in dichloromethane, and decrease markedly in protic solvents (0.1–0.2 in methanol). The primary 2-aminothioazole dye **3a** is readily deprotonated in aqueous solution at ca. pH 8. This is accompanied by a hypsochromic shift of both the absorption and fluorescence maxima, and by an unexpectedly large increase in fluores-cence efficiency.

Acknowledgements

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