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3-Hydroxychromone dyes exhibiting excited-state intramolecular proton transfer in water with efficient two-band fluorescence

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We demonstrate that newly synthesised 7-acetamido-3-hydroxychromones, as well as their parent 3-hydroxychromone (3HC) analogues, exhibit excited-state intramolecular proton transfer reaction in polar protic media including water, resulting in well-resolved two-band fluorescence. Among all 3HCs reported so far, the new compounds show the highest quantum yields ever reported in water (4–13%), while maintaining the sensitive ratiometric response to solvent polarity. This makes them very promising precursors for the development of two-band fluorescent labels and probes, operating in water and other protic media.

Introduction

A strong demand from the side of material science and biology for site-sensitive molecular sensors and probes with two-colour response in fluorescence has stimulated active research in this area. Very promising in this respect are organic dyes that undergo excited-state intramolecular proton transfer (ESIPT) reactions and can exhibit simultaneously the emission bands of the reactant and reaction product.[1] A typical class of ESIPT dyes are 3-hydroxyflavones (3HFs), which in different organic solvents and heterogeneous media show two well-resolved (by about 100 nm) and highly emissive bands in the fluorescence spectra. These bands are attributed to emission of the normal (N*) and tautomer (T*) excited-state species.[2,3] The unique spectroscopic properties of 3HFs has allowed their applications as prospective sensors of polarity,[4] ions[5] and electric fields,[6] and also as probes to study polymers,[7] reverse micelles,[8] lipid membranes[6b,9] and proteins.[10] The advantage of these dyes as fluorescence sensors and probes is their strong ratiometric response to interactions with the environment, provided by changes in the relative intensities of these bands. So far, all these applications refer to non-aqueous media or to aqueous microheterogeneous systems, in which these dye molecules are protected from the aqueous milieu.

There are several factors that limit the applications of 3HFs in aqueous systems. (1) 3HFs generally possess a low fluorescence quantum yield (ϕ) in water[3] and other polar solvents.[11] Moreover, the introduction of a 4′-dialkylamino group, which increases the charge transfer character of 3HF dyes in the excited state,[12] results in a dramatic quenching of their fluorescence in water.[9c] In addition, the latter compounds in polar environments show only the N* band in emission.[13] This band, being strongly solvatochromic,[4,13] is substantially shifted in polar solvents to longer wavelengths, making the T* band poorly resolvable with concomitant loss of the whole advantage of 3HFs as two-band ratiometric probes. (2) A clear understanding of the nature of the emissive forms in water is lacking, even for the parent 3HF, compound **1** (Chart 1). Due to the fact that ESIPT dyes are both strong proton donors and strong proton acceptors, water may favour the appearance of both ground-state and excited-state anionic forms and the latter can be easily mixed with the T* form in the fluorescence spectrum. Thus, some authors assigned the second (long-wavelength band) of the 3HF **1** in water to the excited-state anion,[3b] while others assigned it to the ESIPT tautomer product.[14] Thus, the key problems that have to be solved for the design and application of two-colour sensors and probes based on 3HFs operating in aqueous medium are an increase of ϕ, the

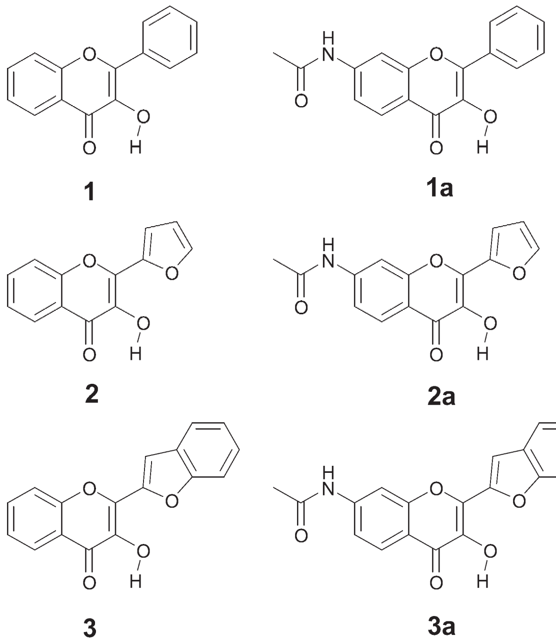

Chart 1 Formulae of the studied 3-hydroxychromones.

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achievement of two emission bands with comparative intensities and sufficient spectral separation between them, and providing a strong solvatochromic response in the ratio of the intensities of these bands based on a solid knowledge of the photophysical behaviour of these dyes.

Recently, we demonstrated that 2-furyl- and 2-benzofuryl-3-hydroxychromones **2** and **3** (Chart 1) exhibit significantly higher ϕ values in comparison to 3HFs, especially in polar solvents.[11,15] However, no data on the fluorescence properties of these dyes in water are available. Moreover, the addition of an electron donor (methoxy) group at the 7-position as well as fusion of a π-electron-rich furan heterocycle to the 3-hydroxychromone (3HC) system can also increase the ϕ values for dialkylamino-substituted 3HCs in organic solvents.[13,16] Still, these dialkylamino-substituted dyes are non-fluorescent in water (unpublished data). Therefore, in order to show efficient fluorescence in water the 3HC chromophore should contain a non-substituted 2-aryl group and be substituted at the 7-position with an electron-donor group. Such compounds have not been described to date.

In the present work, we have synthesised the 7-acetamido-2-aryl-3-hydroxychromones **1a**–**3a** (Chart 1) and studied their fluorescence properties in water and other polar solvents together with those of their parent compounds **1**–**3**. In comparison to the well-characterised dye **1**, dyes **2**, **3** and **1a**–**3a** show much larger fluorescence quantum yields in water. Moreover, the 7-acetamido-substituted **1a**–**3a** possess superior fluorescent properties. To our best knowledge the latter dyes are the first 3HC dyes with an efficient and well-resolved two-band emission in protic media, including water. Finally, we demonstrate that the dual emission of these dyes is connected not with excited-state deprotonation but with the ESIPT reaction.

Experimental

General

Proton NMR spectra were recorded at 250 MHz on a Bruker 300 MHz spectrometer. Mass spectra were recorded on a Kratos MS-25 mass spectrometer using the electron impact (EI) ionisation method. Absorption and fluorescence spectra were recorded on a Cary 3 Bio spectrophotometer (Varian) and Quanta Master spectrofluorometer (Photon Technology International), respectively.

All the reagents were purchased from Aldrich-Sigma Chemical Co. Solvents for synthesis were of reagent quality. For absorption and fluorescence studies the solvents were of spectroscopic grade. Water was doubly distilled and deionised. Flavone **1** was purchased from Aldrich Chemical Company. Chromones **2** and **3** were prepared as described elsewhere.[11,15]

The solutions of 3HC derivatives for absorption and fluorescence spectroscopy were used in concentrations corresponding to absorbances close to 0.1. Quantum yields ϕ of the dyes were determined with respect to a solution of **1** in toluene as the reference (ϕ = 0.29).[12b] pH titration experiments with all the studied dyes were performed in 15 mM HEPES buffer in the pH range 6.8–8.9. For dye **2a**, the titration in the extended pH range, 5.8–10.4, was performed in 15 mM phosphate–citrate–borate buffer. The studies of dye **3** in water were limited by its low solubility.

Syntheses

Chromones **1a**–**3a** were synthesised applying a recently developed modification[16] of the general two-step procedure.[17] In the first step, equimolar quantities of 4′-acetamido-2′-hydroxyacetophenone (prepared from 3-aminophenol as described elsewhere[18]) and the corresponding aldehydes were condensed into 2-propene-1-one derivatives in dry DMF using a 3-fold molar excess of sodium methoxide. After 3 h, ethanol was added to the reaction mixture, followed by an addition of a 15-fold molar excess of sodium methoxide and a 12-fold molar excess of hydrogen peroxide. Reflux for several minutes afforded chromones **1a**, **2a**, and **3a**, which were precipitated in a minimum amount of water at neutral pH, filtered and crystallised from the appropriate solvents. Under these conditions the products of hydrolysis of the amides were not detected. Synthesis of **2a** using the common procedure[17] was reported previously,[18] although neither fluorescence nor absorption data for this compound were reported.

***N*-(3-Hydroxyflavon-7-yl)acetamide (1a).** Crystallised from 1-butanol; yield 19%; mp 233 °C. UV in acetonitrile: ε(340 nm) = 20,700 l mol^{-1} cm^{-1}, ε(324 nm) = 22,400 l mol^{-1} cm^{-1}. ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ 2.14 (3H, s), 7.38–7.60 (4H, m), 8.04 (1H, d, J = 8.6 Hz), 8.10–8.25 (3H, m), 10.58 (1H, s). MS (EI): m/z 295.1 (M$^+$).

***N*-(2-Furan-2-yl-3-hydroxychromon-7-yl)acetamide (2a).** Crystallised from 1-butanol; yield 36% mp ≥ 280 °C (decomp.). UV in acetonitrile: ε(351 nm) = 24,900 l mol^{-1} cm^{-1}, ε(325 nm) = 20,600 l mol^{-1} cm^{-1}. ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ 2.13 (3H, s), 6.78 (1H, m), 7.24 (1H, d, J = 3.3 Hz), 7.40 (1H, d, J = 8.7 Hz), 7.99–8.04 (2H, m), 8.16 (1H, s), 10.55 (1H, s). MS (EI): m/z 285.1 (M$^+$).

***N*-(2-Benzo[*b*]furan-2-yl-3-hydroxychromon-7-yl)acetamide (3a).** Crystallised from 1-butanol; yield 18%; mp ≥ 280 °C (decomp.). UV in acetonitrile: ε(377 nm) = 29,300 l mol^{-1} cm^{-1}, ε(360 nm) = 29,800 l mol^{-1} cm^{-1}. ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ 2.16 (3H, s), 7.30–7.50 (4H, m), 7.72 (1H, s), 7.81 (1H, d, J = 8.7 Hz), 8.05 (1H, d, J = 8.7 Hz), 8.27 (1H, s), 10.59 (1H, s). MS (EI): m/z 335.1 (M$^+$).

Results and discussion

The studies of fluorescence spectra in different polar solvents demonstrate that the new chromones **1a**–**3a** preserve the two-band emission spectra inherent to their parent compounds **1**–**3** (Fig. 1). Their characteristic feature is the solvent-dependent dual emission in different organic solvents (Fig. 1), which is due to the ESIPT reaction.[1,2] For different 3HCs studied in a broad range of organic solvents it is well-established that the short-wavelength band belongs to emission of the initially excited N* state, while the long-wavelength band corresponds to emission of the T* state, which is a product of ESIPT.[2,3,13–15]

All the studied 3HCs demonstrate well-resolved dual emission in water (Fig. 2). So far, among the dyes of the 3HC family the dual emission in water was reported only for the simplest 3HF, **1**,[3,14] and this result gave rise to a discussion regarding the origin of its long-wavelength band in water: was the emission due to the excited-state anion[3b] or to the ESIPT product, the T* tautomer?[14] In other words, does the 3-OH proton transfer to the 4-carbonyl, as in other studied solvents, or to water molecules due to their high proton-accepting ability? To respond to this question, we studied the effects of pH on spectroscopic properties.

For all the studied dyes spectrophotometric pH titrations in water showed that an increase of pH from 7 to 9 results in the appearance and increase in intensity of the long-wavelength band around 420 nm and a decrease of the short-wavelength band located at 340–360 nm, along with the observation of an isosbestic point. For the case of dye **2a** the titration experiment is illustrated in Fig. 3. The isosbestic point for this dye is observed at 388 nm. Evidently, the observed transformation of the absorption spectra is due to ground-state dissociation of the neutral form of the dye with an absorption maximum at 364 nm, which results in the formation of the anion with its

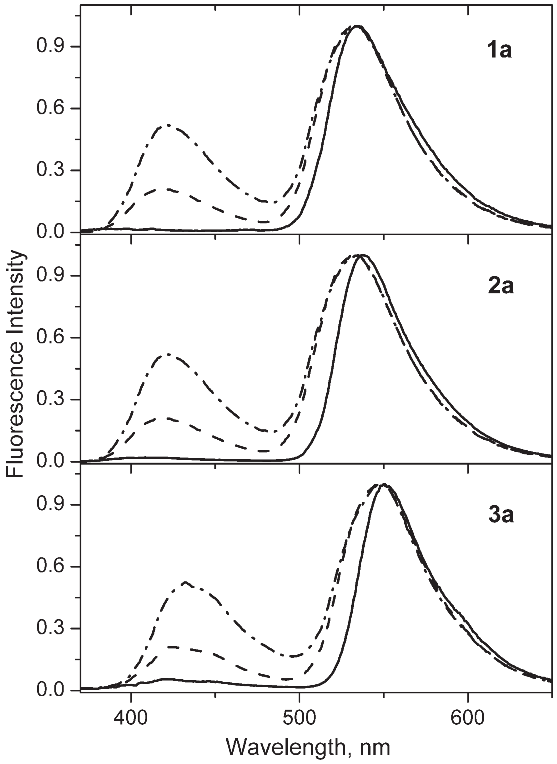

Fig. 1 Fluorescence spectra of chromones **1a**, **2a**, **3a** in different solvents: acetonitrile (solid line), ethanol (dashed line) and methanol (dash-dotted line). The spectra are normalised at the long-wavelength band maximum. Excitation wavelength was 340 (**1a**) or 360 (**2a** and **3a**) nm.

absorption maximum at 418 nm. The obtained pK_a values for 2-furyl- and 2-benzofuryl-3-hydroxychromones **2**, **2a** and **3a** are in the range 8.3–8.4. For 3-hydroxyflavones **1** and **1a** these values are somewhat higher (Table 1).

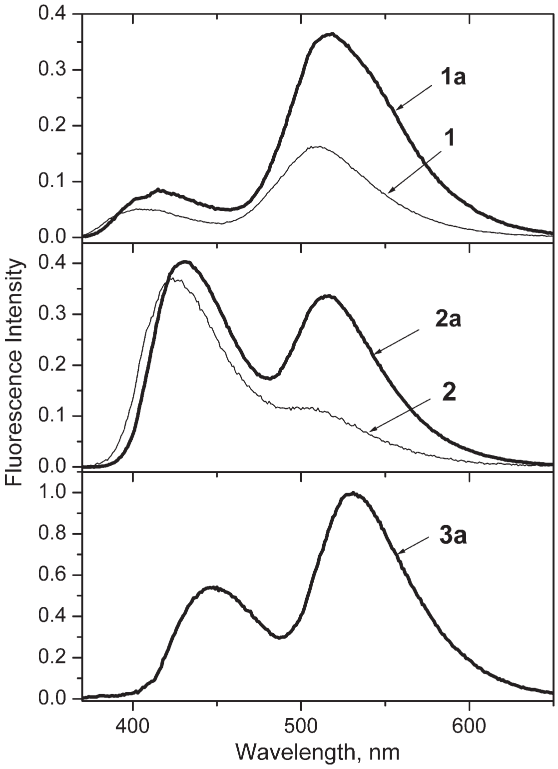

Fig. 2 Fluorescence spectra of the studied dyes in neat water. Within each frame the areas are proportional to the quantum yields. Excitation wavelength was 340 (**1a**) or 360 (**2a** and **3a**) nm.

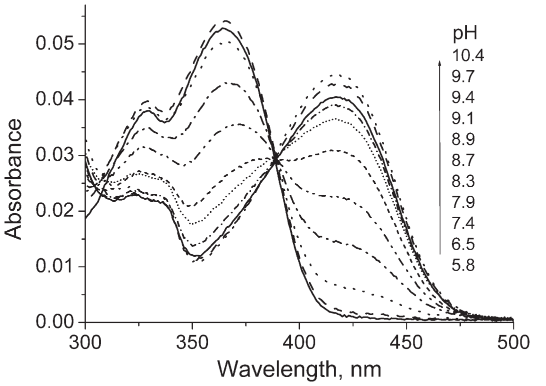

Fig. 3 Absorption spectra of **2a** obtained at different pH (indicated) in 15 mM phosphate–citrate–borate buffer.

For all the studied dyes the shape of the fluorescence spectrum of the dyes in water with excitation at 340/360 nm (neutral form of the dye) does not depend on the pH over a broad range [for dye **2a** see Fig. 4(C)]. For all the dyes excitation spectra recorded at the two emission band maxima at neutral pH superpose completely, demonstrating that these bands originate from the same ground-state precursor. At high pH (>8.2), the emission spectrum with excitation at 420 nm is composed of one band, which can be assigned to emission from the anionic form of the dye [Fig. 4(A,B)]. Importantly, this emission band is not identical to the long-wavelength band observed with 360 nm excitation [Fig. 4(A)]. It differs in its position and shape: its maximum is shifted to the blue by up to 16 nm (600 cm^{-1}) and its bandwidth (full width at half maximum) is up to 1000 cm^{-1} broader (Table 1). The observed essential differences between the anionic emission band (excited at 420 nm at pH > 8.2) and the long-wavelength emission band observed with 340/360 nm excitation at neutral pH show that the latter band cannot be due to emission of the anionic form. The only possible species responsible for the long-wavelength emission is the T* state. So our data suggest that the dual emission of studied 3HC dyes in neutral aqueous media originates from the ESIPT reaction but not from the excited-state anion formation.

To provide an additional test for the possible excited-state deprotonation of the dyes in water, we studied the pH dependence of the fluorescence spectra excited at the isosbestic point (388 nm) for the case of the most representative dye, **2a**. If the excited-state anion is responsible for the long-wavelength band emission, with excitation at the isosbestic point one should

Table 1 Physicochemical properties of the studied dyes in water.[a]

Dye	λ_{abs}/nm	λ^{A}_{abs}/ nm	λ_{T*}/ nm	λ_{A*}/ nm	BW_{T*}/ cm^{-1}	BW_A/ cm^{-1}	pK_a (S_0)
1	342	401	510	498	2570	3590	9.6[b]
1a	343	405	516	511	2490	3580	8.9 ± 0.2
2	358	413	513	501	2530[c]	3050[c]	8.4 ± 0.1
2a	364	418	526	510	2250	3230	8.4 ± 0.1
3a	378	428	533	525	2250	3000	8.3 ± 0.1

[a] λ_{abs} and λ^{A}_{abs} are the absorption maxima of the neutral (pH 7.0) and anionic (pH 8.9) dye forms. λ_{T*} is the emission maximum of the T* band obtained at an excitation wavelength of 340 nm for **1** and **1a** and 360 nm for **2**, **2a** and **3a** (pH independent in the range 6.8–8.9). λ_{A*} is the emission maximum of the anionic form of the dyes excited at 410–420 nm. BW_{T*} and BW_A are the bandwidths (full width at half maximum) of the T* and anionic emission bands. pK_a is the ground state dissociation constant obtained by spectrophotometric titration. [b] Data of ref. 14. [c] The value was obtained from the results of deconvolution (see ref. 13).

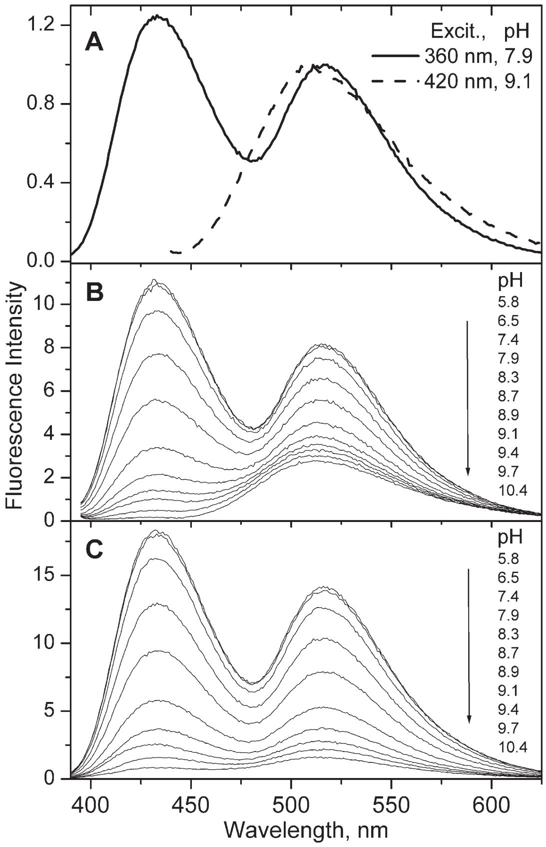

Fig. 4 Results of spectrofluorimetric titrations of dye **2a**. (A) Emission spectra recorded with excitation at 360 nm (pH 7.9) and 420 nm (pH 9.1). Emission spectra as a function of pH recorded with excitation at the isosbestic point of 388 nm (B) and at the band maximum of 360 nm (C).

observe an increase in the absolute intensity of the long-wavelength band and a decrease in that of the short-wavelength band as a result of an increase in pH. A significantly different picture is observed in our results [Fig. 4(B)]. The increase in pH results in a significant decrease of the fluorescence intensity of both of the bands, which is especially large near the pK_a range of values of pH. In addition, in this pH range we observe a dramatic transformation of the emission spectrum: a disappearance of the short-wavelength band and a blue shift of the long-wavelength band accompanied by its broadening [Fig. 4(B)]. The fact that transformation of the fluorescence spectrum is observed in the pK_a range of pH demonstrates that it occurs due to ground-state deprotonation. Even more important is the strong decrease in fluorescence intensity of both emission bands with the increase in pH. This shows that the fluorescence quantum yield of the anionic form is much lower (more than 2 times) than that of the form responsible for the long-wavelength emission band at neutral pH. The observed difference in the fluorescence quantum yields is direct evidence that the latter species is not an anionic form of the dye, thus excluding excited-state deprotonation as a reason for the long-wavelength emission at neutral pH.

Additional strong support for the ESIPT origin of the dual emission in water is provided by the fact that dual emission (excited at 340/360 nm) is independent of pH in the range 6.8–7.9 for all the dyes. For the case of **2a**, which was studied additionally over the extended pH range (5.8–10.4), the results show invariant dual emission profile in the range 5.8–7.9 [Fig. 4(C)]. At pH > pK_a in the fluorescence spectrum excited at 360 nm we observe a slight increase of relative intensity of the long-wavelength band, which can be completely explained by partial excitation of the ground-state anion that also emits in this long-wavelength region. If it were the excited-state anion form present in equilibrium with the N* form at neutral pH, then in a pH titration experiment the change of pH by 1 unit should result in a change of the intensity ratio of the two emission bands by one order of magnitude. The absence of a pH dependence for the shape of the fluorescence spectrum of the studied 3HCs shows that the second (long-wavelength) band is a result of a unimolecular reaction, which can be conclusively assigned to ESIPT.

Since we observe the results of only the ground-state but not of the excited-state deprotonation, we can conclude that at moderately basic pH the studied 3HCs dissociate more readily in the ground state than in the excited state. A similar conclusion was made by Wolfbeis *et al.* for the parent 3HF **1**.[14] Moreover, this conclusion is also in-line with previous studies of 3-hydroxyflavone **1** in the highly polar solvent formamide, showing that in this solvent **1** exists partially in the ground-state anionic form, while excited-state dissociation under these conditions is not detectable.[19] A similar observation was recently reported for **1** in alcohols.[20] This is a very unusual behaviour for common phenolic dyes, which are known to undergo intermolecular proton transfer in the excited state much more readily that in the ground state.[21]

One of the possible explanations of this interesting feature is that in the 3HC excited state the 3-OH group cannot play the role of a strong electron donor, because no keto form can be attributed to the 3-OH group in the excited state. In the meanwhile, the OH group of common phenolic dyes, like 2-naphthol,[22] 2-(2′-hydroxyphenyl)benzimidazole,[23] 7-hydroxycoumarins,[24] *etc.* is a strong excited-state electron donor and thus in the excited state it is much more acidic than in the ground state.[21] In 3HCs the function of the electron donor is played by the benzopyran ring (including the 1-oxygen heteroatom) and the 2-aryl group.[13,25] Therefore, the increase in acidity of the 3-OH group of 3HC in the N* excited state may not be as strong as in the other phenolic dyes. A second effect is the strong increase in basicity of the 4-carbonyl group in the excited state due to an increase of its effective negative charge.[2,25] As a result, the intramolecular H-bond in the excited state becomes stronger than the intermolecular interactions with water and the deprotonation becomes energetically less favourable than the intramolecular proton transfer. Therefore, the emission of the anionic form can be detected only in cases when the anion is already formed in the ground state and is excited directly. The suggestion that strengthening of the intramolecular H-bonding prevails over deprotonation is supported by the observations of excited-state behaviour of the typical ESIPT dye 2-(2′-hydroxyphenyl)benzimidazole.[23] It was shown that excited-state deprotonation of this molecule occurs only when the ground-state conformer that does not have intramolecular H-bonding is excited. In contrast, the conformer with an intramolecular H-bond shows only the tautomer (ESIPT product) emission without any contribution from the anionic form.[23]

The prominent feature of the 3HC dyes of the present study is that their fluorescent quantum yields in water are significantly higher than those reported so far for 3HC derivatives. Thus, substitution of the 2-phenyl ring by 2-furyl or 2-benzofuryl results in a 2–3 fold increase of the fluorescence quantum yield in water and other polar solvents. This effect is in-line with the previous studies of parent compounds **1**–**3** in polar solvents and is attributed to the higher planarity of the 2-furyl- and 2-benzofuryl-3-hydroxychromones in comparison to their 2-phenyl analogues.[11] Moreover, introduction of the 7-acetamido group results in a nearly 2-fold increase of the fluorescence quantum yield in water for **1a** and **2a** (Table 2), while in organic solvents this effect is observed only with **2a**. The effects of the 7-donor group on the quantum yield of the 3-hydroxychromones in polar solvents can be connected with a decrease in the asymmetry of the electron distribution in their excited states. Thus, the parent 3-hydroxyflavone **1** shows an

Table 2 Spectroscopic characteristics of 3HC derivatives.[a]

Solvent	Dye	λ_{abs}/nm	λ_{N^*}/nm	λ_{T^*}/nm	I_{N^*}/I_{T^*}	ϕ/%
CH_3CN	**1**	339	394	525	0.028	5
	1a	340	389	535	0.017	5
	2	348	409	533	0.098	6
	2a	351	411	537	0.019	12
	3	358	418	542	0.040	17
	3a	377	423	550	0.050	15
EtOH	**1**	343	402	532	0.270	3
	1a	346	419	534	0.209	3
	2	357	416	531	0.644	8
	2a	360	419	534	0.210	11
	3	366	425	543	0.220	15
	3a	375	427	548	0.209	14
MeOH	**1**	343	403	528	0.442	3
	1a	345	422	534	0.517	3
	2	356	416	527	1.71	8
	2a	360	422	534	0.521	10
	3	370	428	540	0.570	18
	3a	375	434	548	0.525	14
Water[b]	**1**	342	408	510	0.312	2
	1a	343	414	516	0.230	4
	2	358	427	513[c]	3.19[c]	5
	2a	364	432	516	1.20	7
	3[d]	375	442	526	1.43	–
	3a	380	448	533	0.538	13

[a] λ_{abs} is the position of absorption maximum, λ_{N^*} and λ_{T^*} are the positions of fluorescence maxima of the N* and T* forms. ϕ is the fluorescence quantum yield. Data on ϕ for **1**, **2** and **3** in organic solvents are from ref. 13 [b] Data in neat water and in HEPES buffer at pH 7.0 are the same. [c] Values obtained from the results of deconvolution (see ref. 13). [d] Data obtained for a water–methanol mixture (10:1 v/v).

almost one order of magnitude higher quantum yield in water as compared with 4′-dialkylamino-3-hydroxyflavones (2% *vs.* 0.3%[9c]), dyes characterised by a significant chromophore asymmetry due to the excited-state charge transfer.[4,12,13] Introduction of a 7-methoxy group into the structure of 4′-dialkylamino-3-hydroxyflavones, which evidently decreases this charge transfer, results in a significant increase of the quantum yields in organic solvents.[13] In the case of the present compounds the introduction of a 7-donor group may result in a further restoration of the chromophore symmetry, so that on electronic excitation the charge transfer from the 2-aryl ring is counter-balanced with that from the 7-acetamido group. This effect is especially important in the case of **2a**, a dye containing the strongest 2-aryl electron donor (Table 2).

A connection between the spectroscopic properties in water and in organic solvents, where the parent compounds **1**–**3** and some of their analogues were studied in detail,[2,3,11,13] could provide additional information on the photophysical behaviour of the studied dyes and the spectroscopic effects of the 7-acetamido group. A comparison of the parameters from the absorption and fluorescence emission spectra of **1a**–**3a** with their parent analogues **1**–**3** in organic solvents and in water is presented in Table 2. We observe that the introduction of the 7-acetamido group does not change significantly the spectral positions of the absorption and emission bands. As a general trend, a small red shift (3–7 nm) is observed for the absorption and both emission bands. A remarkable decrease in the intensity ratio I_{N^*}/I_{T^*} is observed on introduction of this group in the case of **2a** (and for **3a** in water only). Previously we showed that in the case of 4′-dialkylamino-substituted 3HCs the electron donor (methoxy group) at the 7-position decreases the I_{N^*}/I_{T^*} ratio.[13] In this respect the strong effect of the 7-donor (acetamido) group on I_{N^*}/I_{T^*} in the case of **2a** can be because the 2-furanyl group is a stronger excited-state electron donor as compared to phenyl or 2-benzo[*b*]furanyl groups.

We observe that the new dyes, like their non-substituted analogues, show high two-band sensitivity to the type and polarity of solvent (Fig. 1, Table 2). Thus, in all protic solvents their I_{N^*}/I_{T^*} ratio is much higher than that in the aprotic solvent acetonitrile (Table 2). An increase in the solvent polarity from ethanol to methanol results also in a strong increase of the I_{N^*}/I_{T^*} values. Since the absorption spectra of the new chromones, similarly to their parent compounds **1**–**3**, do not change significantly with solvent (Table 2), the observed changes of the I_{N^*}/I_{T^*} ratio for the new dyes are connected with the influence of solvent on their excited-state behaviour, including the ESIPT reaction. In this respect, for dyes **1**, **1a** and **3a** the similar or lower values of the I_{N^*}/I_{T^*} ratio observed in water as compared to methanol is an anomaly that requires additional studies. However, we observe that the regularity with regards to solvent polarity, including water, is present for the other dyes (Table 2), and it is most clearly manifested for dyes **2** and **2a**, which contain the strongest 2-aryl electron donor (Table 2). In response to solvent variation the latter demonstrates the most significant two-band fluorescence response and the nearly 6-fold increase in the I_{N^*}/I_{T^*} ratio upon going from ethanol to water is particularly impressive.

The strong separation between the N* and T* bands is a favourable property for different molecular sensor applications. We observe that in the present 3HCs this separation is significant even in highly polar solvents, including water. In contrast, in dialkylamino-substituted 3HCs,[4,13] which are commonly used as fluorescence probes,[4–6,8*b*,9] this separation becomes small with increasing polarity, and in view of the strong increase of relative intensity of the N* band, the two bands may become unresolved. In the latter compounds the N* state shows a strong positive solvatochromism, resulting in substantial shifts to longer wavelengths, which causes this overlap. In contrast, the present compounds do not possess the strong electron-donor dialkylamino group on the 2-aryl substituent, so that the charge transfer character of their N* state is much less pronounced. Therefore, even in highly polar solvents the N* band of the present dyes does not undergo significant red shifts and remains well separated from the T* band (Table 2, Figs. 1 and 2).

Conclusions

In the present work we report on 3-hydroxychromone dyes that exhibit dual emission in water with the highest fluorescence quantum yield reported so far for this class of compounds. We provide evidence that this dual emission for all the dyes, including the parent 3-hydroxyflavone **1**, is connected with an excited-state intramolecular proton transfer (ESIPT) reaction. In this respect, the uncommon feature of 3-hydroxychromones is that in the excited state they undergo deprotonation less readily than in the ground state, and that ESIPT prevails over the intermolecular deprotonation process. In comparison to the parent compound **1**, the other dyes, which were studied in water for the first time, show an increased fluorescence quantum yield in highly polar media and a larger variation of their dual emission in these media. More importantly, the separation between the two emission bands remains very large. Since the ESIPT reaction in 3HCs is extremely sensitive to different perturbations of the microenvironment,[3] the development of 3-hydroxychromone dyes with two well-separated emission bands in water is an important step towards the their application in new molecular sensors, which can be used in aqueous solutions in a two-wavelength ratiometric manner. In this respect, the 7-acetamido derivatives are very promising because they are ready precursors for the preparation of a variety of fluorescent labels[26] and probes[27] for microenvironment sensing in nanostructures and biological systems.

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