2-Aryl-3-hydroxyquinolones, a new class of dyes with solvent dependent dual emission due to excited state intramolecular proton transfer

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Herein, the fluorescence properties of a series of 2-aryl-3-hydroxyquinolones (3HQs) were investigated and compared with the properties of well-studied 3-hydroxyflavone. All these compounds were found to display dual fluorescence with well-separated bands in organic solvents and aqueous solutions. Using steady-state and time-resolved fluorescence spectroscopy, we showed that their dual fluorescence is due to an excited state intramolecular proton transfer reaction. Moreover, the absorption spectra of most 3HQs tested were found to be similar, indicating that they are not sensitive to the substituent at the 2-aryl ring. This was related by quantum chemical calculations to the non-planarity of these molecules which prevents conjugation between the two aromatic moieties. The only exception was the 3HQ derivative with a thiophene ring at position 2 which exhibited a red-shifted spectrum due to its more planar structure. In sharp contrast, the emission spectra and especially the intensity ratio of the two emission bands were highly dependent on the substituents at the 2-aryl ring and at the heterocyclic nitrogen. Moreover, *N*-methyl substituted 3HQs (N–Me 3HQs) demonstrate strong solvatochromic properties, with large changes in their fluorescence band intensity ratio as a function of the solvent polarity. In addition, the logarithm of these intensity ratios varied linearly with the Hammett constant associated with the substituent in the 2-aryl ring, enabling the design of 3HQ dyes with optimized intensity ratios in a given range of solvent polarities. Thus, 3HQs preserve the unique properties of 3-hydroxyflavones, namely dual emission that is highly sensitive to solvent polarity and to chemical substituents. Moreover, in comparison to 3-hydroxyflavones, 3HQ dyes exhibit higher fluorescence quantum yields and 10-fold increased photostability. These properties of the 3HQ derivatives make them prospective candidates for application as polarity-sensitive fluorescent labels for biomolecules.

Introduction

Due to its exquisite sensitivity, fluorescence is one of the most used techniques for investigating molecular events in biological systems. However, this technique relies strongly on the availability of fluorescent probes with optimal properties. In cells and tissues, probes are generally distributed inhomogeneously. As a consequence their fluorescence intensity is dependent on their local concentration. To avoid this limitation, probes with a ratiometric response are strongly desirable. In this respect, dual fluorescence probes exhibiting two well separated emission bands are of particular interest, since they provide a reliable ratiometric signal independent of the probe concentration.[1]

Excited state intramolecular proton transfer (ESIPT) reaction[2] is very effective for the design of probes with dual fluorescence.[3] ESIPT results in the formation of two tautomeric forms in the excited state of the probe: N*—normal and T*—tautomer form. Due to their different photophysical properties, these tautomeric forms exhibit widely separated emission bands on the wavelength scale. The most interesting and characterized representatives of this class of probes are the 3-hydroxyflavone derivatives (3HFs) that have been shown to be highly effective tools for investigating polarity,[3,4] hydration and electronic polarizability,[4] electrostatic effects in different media including lipid membranes[5,6] and proteins.[7,8] Moreover, these dyes were shown to be useful to determine the nature and concentration of cations[9] and anions[10] in solution. However, despite their significant advantages compared to common single-band probes, 3HFs exhibit relatively low photostability and quantum yields that limit their application. As a consequence, the development of new dual fluorescence probes with improved fluorescent parameters is strongly required.

It has been reported[11,12] that 2-aryl-3-hydroxyquinolones (3HQs), which are structural analogs of 3HFs, also undergo ESIPT reaction in organic solvents and thus exhibit two bands in the emission spectrum (Scheme 1). Remarkably, the ratio of the intensities of the two bands changes with the solvent, indicating that these dyes are sensitive to their microenvironment. However, a detailed study of the fluorescent properties

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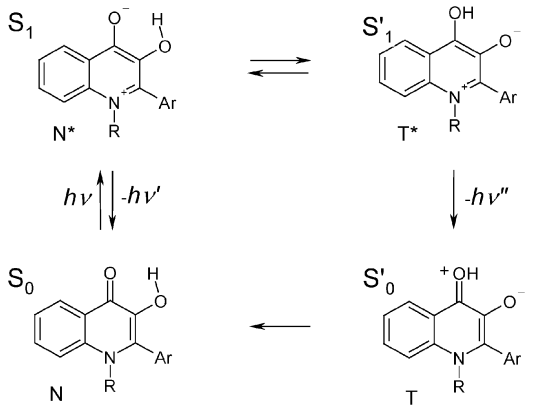


Scheme 1

of this new class of dyes has not yet been done. Moreover, it would be of high interest to compare the properties of 3HQs with the properties of 3HF analogs which have already found a variety of applications. Therefore, the present paper is focused on studies of structure–fluorescence property relationships, solvatochromism and photostability of 3HQs with respect to 3HFs.

Experimental

Materials and methods

All the solvents and chemicals were purchased from Aldrich. The solvents were of analytical grade. Melting points were determined using a Gallenkamp Melting Point Apparatus. Mass spectra were measured using Mass Spectrometer Mariner System 5155. ^{1}H-NMR spectra were recorded on a Bruker 300 MHz spectrometer. Absorption spectra were recorded on a Cary 4 spectrophotometer (Varian) and fluorescence spectra on a FluoroMax 3.0 (Jobin Yvon, Horiba) spectrofluorimeter. Time-resolved fluorescence measurements were performed with the time-correlated, single-photon counting technique using the frequency doubled output of a Ti-sapphire laser (Tsunami, Spectra Physics) pumped by a Millenia X laser (Tsunami, Spectra Physics).[13] The excitation wavelength was set at 320 nm. The fluorescence decays were collected at the magic angle (54.7°) of the emission polarizer. Data were analyzed by the Maximum Entropy method (MEM) using the Pulse 5.0 software.[14]

The pK_a values of quinolones were calculated by the Fletcher–Pawl algorithm using a nonlinear least squares method, as developed in Doroshenko's program,[15] which minimizes the sum of squared deviations of the experimental and calculated absorbance or fluorescence data (A) assuming a one-step protonation process for each transition. The program utilizes the common expression eqn (1):[16]

$$A = \frac{A_{HA}10^{-pH} + A_A10^{-pK_a}}{10^{-pH} + 10^{-pK_a}}, \qquad (1)$$

where A_{HA} is the absorbance or fluorescence intensity of 3HQ in its neutral form and A_A is the absorbance or fluorescence intensity of the 3HQ anion.

The photostability of the dyes was determined on a FluoroMax 3.0 apparatus. Solutions of 3HQs and 3HF of equal absorbance were irradiated at the excitation wavelength (360 nm) in the same conditions. Emission decays were collected at 520 nm. The intensity of the irradiating light was 2.94 mW cm^{-2} in all cases. Acetonitrile was used because the emission spectra of 3HQs and 3HFs in this solvent are similar in their shape and the ratio of their two bands. This limits errors due to possible differences in the photodegradation rates of the two emission states. Concentrations of the dyes were about 10^{-6} mol L^{-1}. The quantum yields of photodegradation were calculated using eqn (2):[17]

$$\Phi_{pd} = (1 - F_T/F_0)/\{I_0\sigma(\lambda_{exc})\int_0^T [F(t)/F_0]\,dt\}, \qquad (2)$$

where F_0 and F_T are the fluorescence intensities expressed in counts s^{-1} at time 0 and at the end of the measurements, respectively. I_0 is the intensity of the irradiating light expressed in photons cm^{-2} s^{-1}, $\sigma(\lambda_{exc})$ is the one-photon absorption cross-section at λ_{exc} expressed in cm^2 and t is the time in seconds.

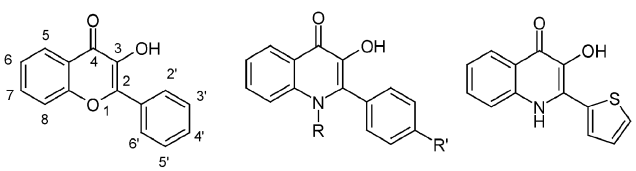
Fluorescence quantum yields φ were determined with quinine sulfate in 0.5 M sulfuric acid (φ = 0.577[18]), taken as a reference.

Quantum-chemical calculations of 3HQ and 3HF torsional enthalpy were performed by the AM1 semi-empirical method using the MOPAC 6.0 program.[19]

Syntheses

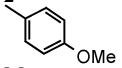
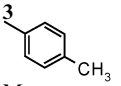
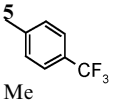
3HQs synthesized in this work are given in Scheme 2 and Table 1. To synthesize them we have used condensation of anthranilic acid esters in polyphosphoric acid as described earlier.[20] The general procedure of synthesis is provided on the example of dye **5**.

1-Methyl-2-(4-(trifluoromethyl)phenyl)-3-hydroxy-4(1*H*)-quinolone (5). A solution of anthranilic acid (3.2 g, 21 mmol) and 4′-(trifluoromethyl)phenacyl bromide (6.38 g, 20 mmol) in 10 ml of DMF was stirred in the presence of dried potassium carbonate (3 g, 21.7 mmol) at 80 °C during 2 h. The solution was poured into water (100 ml), and the 4′-(trifluoromethyl)-phenacylanthranilate formed was filtered off and dried. Then 0.5 g (1.48 mmol) of the latter compound was added to polyphosphoric acid (3.3 g) and stirred at 120° during 2 h. After that, the mixture was poured into 20 g of ice and neutralized by 10% aqueous sodium carbonate. The filtered precipitate after washing, drying and recrystallization from DMF provided the dye **5**. (0.42 g, 89.0%). δ_H(300 MHz; DMSO-d$_6$; Me$_4$Si): 8.34 (d, J 8, 1H, ArH), 7.98–7.73



Scheme 2

Table 1 Spectroscopic properties of the synthesized 3HQs in acetonitrile and phosphate buffer[a]

	No	**1a**	**1b**	**2**	**3**	**4**	**5**	**6**
	Ar							
	R	H	Me	Me	Me	Me	Me	H
Phosphate buffer	λ_{abs}/nm	352	351	356	353	353	353	364
	λ_{N^*}/nm	413	422	423	423	422	429	419
	λ_{T^*}/nm	495	499	492	498	496	518	501
	I_{N^*}/I_{T^*}	0.12	0.72	1.60	1.12	0.76	0.46	0.14
	$\varepsilon \times 10^{-3}$/l mol^{-1} cm^{-1}	13.0	13.3	13.1	12.3	15.1	9.9	11.5
	φ	0.45	0.13	0.096	0.13	0.12	0.13	0.31
Acetonitrile	λ_{abs}/nm	362	366	366	366	366	366	375
	λ_{N^*}/nm	424	419	419	422	418	433	433
	λ_{T^*}/nm	510	525	521	523	523	541	518
	I_{N^*}/I_{T^*}	0.02	0.10	0.17	0.11	0.11	0.12	0.03
	$\varepsilon \times 10^{-3}$/l mol^{-1} cm^{-1}	11.5	11.5	10.6	12.0	12.0	9.6	10.5
	φ	0.32	0.14	0.078	0.12	0.14	0.17	0.31
	Φ_{pd} (%)[b]	0.28	0.12	0.11	0.11	0.11	0.14	0.23

[a] Ar and R are the 3HQ substituents at position 2 and the heterocyclic nitrogen, respectively. λ_{abs} is the position of the absorption maximum (nm), λ_N* and λ_T* are the positions of the fluorescence maxima of the N* and T* forms, respectively (nm), ε is the molar extinction coefficient (l mol^{-1} cm^{-1}), φ is the fluorescence quantum yield, Φ_{pd} is the quantum yield of photodegradation. [b] For 3HF, Φ_{pd} = 1.2%.

(m, 6H, ArH, Ar′H), 7.42 (t, *J* 7, 1H, ArH), 3.54 (s, 3H, NCH_3); *m*/*z* 342.21 ($M^+ + H$); mp 234 °C.

2-Phenyl-3-hydroxy-4(1*H*)-quinolone (1a). Prepared by using the experimental procedure described above for **5**. δ_H(300 MHz; DMSO-d_6): 11.59 (br s, 1H, NH), 8.15 (d, *J* 8.5, 2H, ArH), 7.81 (d, *J* 7, 2H, Ar′H), 7.72 (d, *J* 8, 1H, ArH), 7.57 (m, 4H, ArH, Ar′H), 7.27 (t, *J* 7, 1H, Ar′H); *m*/*z* 238.24 ($M^+ + H$); mp 276 °C.

1-Methyl-2-phenyl-3-hydroxy-4(1*H*)-quinolone (1b). Prepared by using the experimental procedure described above for **5**. δ_H(300 MHz; DMSO-d_6): 8.31 (d, *J* 8, 1H, ArH), 7.74 (m, 2H, ArH), 7.57 (m, 3H, Ar′H), 7.45 (d, *J* 7, 2H, Ar′H), 7.38 (dd, *J* 7, 1H, ArH), 3.52 (s, 3H, NCH_3); *m*/*z* 252.22 ($M^+ + H$); mp 262 °C.

1-Methyl-2-(4-methoxyphenyl)-3-hydroxy-4(1*H*)-quinolone (2). Prepared by using the experimental procedure described above for **5**. δ_H(300 MHz; DMSO-d_6): 8.33 (d, *J* 8, 1H, ArH), 7.79 (m, 2H, ArH), 7.35 (m, 3H, ArH, Ar′H), 7.09 (d, *J* 7, 2H, Ar′H), 3.87 (s, 3H, OCH_3), 3.58 (s, 3H, NCH_3); *m*/*z* 282.31 ($M^+ + H$); mp 259 °C.

1-Methyl-2-(4-methylphenyl)-3-hydroxy-4(1*H*)-quinolone (3). Prepared by using the experimental procedure described above for **5**. δ_H(300 MHz; DMSO-d_6): 8.31 (d, *J* 8, 1H, ArH), 7.69 (m, 2H, ArH), 7.33 (m, 5H, ArH, Ar′H), 3.55 (s, 3H, NCH_3), 2.44 (s, 3H, 4′-CH_3); *m*/*z* 266.30 ($M^+ + H$); mp 224 °C.

1-Methyl-2-(4-fluorophenyl)-3-hydroxy-4(1*H*)-quinolone (4). Prepared by using the experimental procedure described above for **5**. δ_H(300 MHz; DMSO-d_6): 8.30 (d, *J* 6.9, 1H, ArH), 7.70 (m, 2H, ArH), 7.50 (m, 2H, ArH), 7.40 (m, 2H, ArH), 3.53 (s, 3H, NCH_3); *m*/*z* 270.11 ($M^+ + H$); mp 238 °C.

2-(2-Thienyl)-3-hydroxy-4(1*H*)-quinolone (6). Prepared by using the experimental procedure described above for **5**. δ_H(300 MHz; DMSO-d_6): 11.35 (br s, 1H, NH) 8.14 (d, *J* 8.5, 1H, ArH), 8.06 (d, *J* 4, 1H, Het), 7.86 (m, 2H, ArH, HetH), 7.63 (t, *J* 7.5, 1H, ArH), 7.32 (m, 2H, ArH, HetH); *m*/*z* 244.16 ($M^+ + H$); mp 296 °C.

Results and discussion

Light absorption properties

The absorption spectra for all tested 3HQs with an aromatic ring at position 2 were found to be marginally dependent on the nature of the substituents at this ring and on the quinolone nitrogen atom (Fig. 1). The absorption maximum in acetonitrile of all these derivatives is 365 ± 2 nm. This constitutes a major difference with 3HFs, where the absorption properties are very sensitive to the donor substituents at position 2.[3]

The only exception is compound **6** with a five-membered aromatic ring substituent that differs significantly from the other compounds by its absorption maximum being red-shifted by 9 nm and by the appearance of a shoulder at 394 nm. These differences may be attributed to the lower steric hindrance provided by the smaller ring in compound **6**. To further assess this hypothesis, quantum chemical calculations were performed for compounds **1a**, **1b** and **6** in comparison with 3HF (Fig. 2). Like 3HF, the 3HQ derivatives are

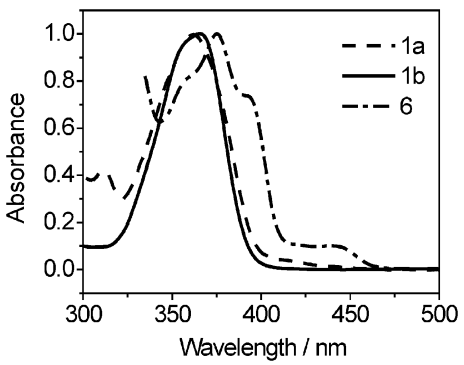


Fig. 1 Absorption spectra of 3HQs in acetonitrile. Spectra of compounds **2–5** are superimposed on that of compound **1b**.

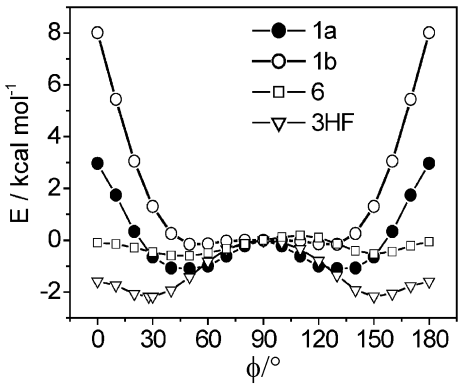


Fig. 2 Torsional enthalpy profiles (superimposed at $\phi = 90°$) of 3HF and 3HQ derivatives.

characterized by two planar moieties that can adopt non-planar conformations with dihedral angles between the two moieties governed by steric factors. Depending on these angles, substantial differences on the conjugation of the rings and thus in the absorption properties are expected. Crystallographic data show that in flavones the dihedral angle ϕ between the aryl and heterocycle moieties is in the range 5–30° depending whether a hydrogen atom or a more bulky hydroxyl group is at position 3 of the molecule.[21] According to our quantum chemical calculations, this angle is close to 30° and the energy of the planar conformation ($\phi = 0°$) is much lower than that of the twisted ($\phi = 90°$) conformation (Fig. 2). This relatively small angle still allows good conjugation between the two moieties and as a consequence, the possibility of controlling the absorption and fluorescence properties of 3HF by changing the substituents on the aryl ring. In contrast, the torsion energy profiles for 3HQ derivatives **1a**, **1b** (Fig. 2) suggest that the favorable dihedral angles are close to 45°, in line with recent X-ray data.[22] Moreover, in contrast to the energy profiles of 3HF, the planar conformation of 3HQ derivatives is of much higher energy than the twisted one (Fig. 2). These large angles and the high energy of the planar conformation likely prevent conjugation between the two aromatic moieties and explain the limited influence of the aromatic ring at position 2 on the light absorption properties.

In sharp contrast, conformations with smaller dihedral angles can be populated in the derivative **6** with a thiophene ring at position 2 (Fig. 2). Moreover, the energy differences between the various conformations of compound **6** are much lower than for the other 3HQ compounds (for instance, compare **6** with **1a** in Fig. 2). Consequently, better conjugation between the moieties is possible. This should enable us to adjust the absorption and fluorescence properties by varying the substituents on the five-membered ring at position 2.

Fluorescence properties: evidence of ESIPT in 3HQs

Due to their structural similarities with 3HFs, the 3HQ derivatives are also expected to exhibit an ESIPT reaction. In line with our previous results,[11] all the synthesized compounds exhibit two emission bands in phosphate buffer (Fig. 3a) and organic solvents (Fig. 4, Tables 1 and 2). Moreover, the excitation spectra of 3HQs recorded at the two emission band maxima are the same and match well with the corresponding absorption spectra. These observations on excitation and emission spectra are strongly indicative of an excited state process. Since in addition, the positions of the two maxima are close to those observed for 3HF, it is strongly suggested that 3HQ derivatives also undergo ESIPT. Additional experiments were performed to exclude other photophysical reactions such as excimer formation or photodissociation. Experiments performed with dye **4** demonstrated a stable ratio of the two band intensities I_{N^*}/I_{T^*} in the 10^{-7}–10^{-5} M concentration range and a linear growth of the fluorescence intensities with the dye concentration in this range. Both observations exclude excimer formation as the cause of the observed dual fluorescence.

Moreover, the intensity ratio of the two bands of dye **4** displayed only limited changes in the pH range 5–8. Formation of dye **4** anion was observed only at pH > 8 as monitored

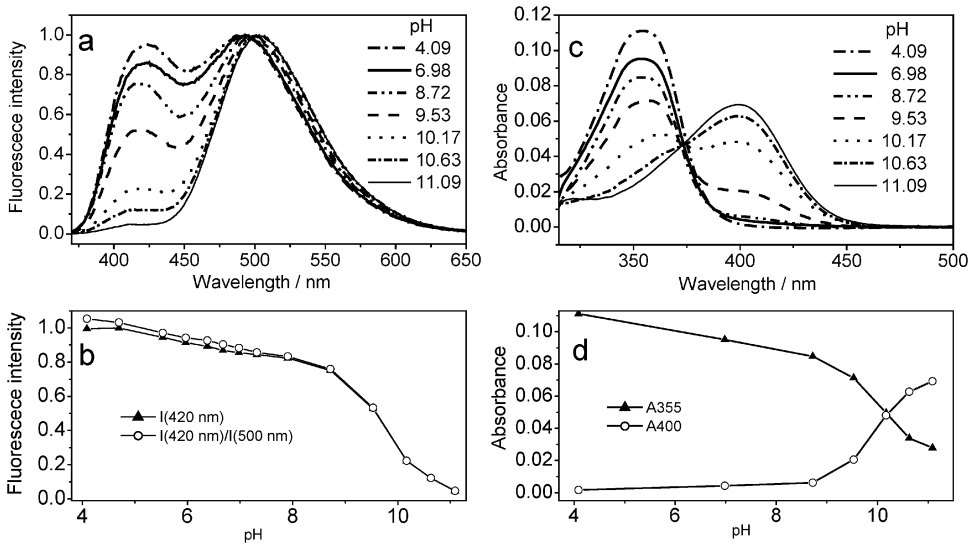


Fig. 3 pH dependence of the fluorescence and absorption spectra of dye **4**. **a** Emission spectra. **b** Fluorescence intensity at 420 nm and I_{420}/I_{500} intensity ratio. **c** Absorption spectra. **d** Absorbances at 355 and 400 nm.

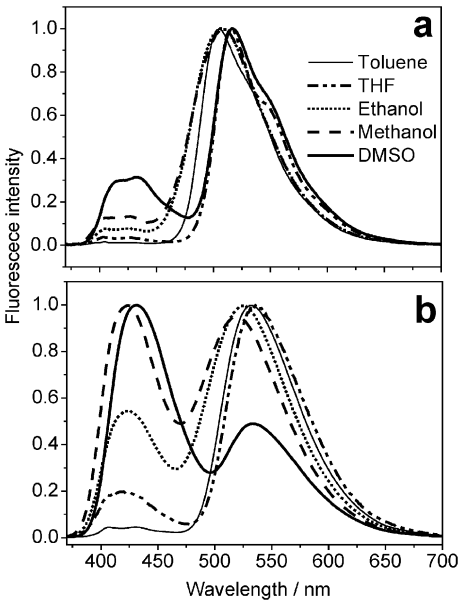


Fig. 4 Normalized emission spectra of 3HQ **1a** (R = H, panel a) and **1b** (R = Me, panel b) in organic solvents.

from the disappearance of the short wavelength band (Fig. 3a). Evaluation of the excited state pK_a by the dependence of the fluorescence intensity at 420 nm or the intensity ratio I_{420}/I_{500} on pH gives a value of 9.58 ± 0.04 (Fig. 3b). This excludes the possibility that photodissociation of dye **4** occurs at neutral pH in aqueous solutions and thus provides additional support for the ESIPT mechanism of the dual emission in 3HQs. Moreover, both the absence of excited state photodissociation in aqueous solutions and the preference of 3HQ molecules to undergo ESIPT are fully in line with previous observations on 3HF derivatives.[23]

Acid–base properties in the ground state are an additional important characteristic of fluorescence probes. In aqueous solutions, the anionic form of **4** appears only at pH ≥ 9 and exhibits an absorption maximum at 400 nm (Fig. 3c). A pK_a value of 10 ± 0.1 was calculated using the dependences of the absorbances at 355 and 400 nm *versus* pH (Fig. 3d). The fluorescence spectrum of the pure anionic form of dye **4** was obtained at pH > 11 using an excitation wavelength of 400 nm and exhibited a single band with a maximum at 504 nm (not shown).

To further characterize the ESIPT reaction in aqueous medium, time-resolved measurements of compound **4** were performed with a 320 nm excitation wavelength. The fluorescence decay of the N* form recorded at 400 nm was monoexponential with a 0.38 ns lifetime. The fluorescence decay of the T* form recorded at 530 nm was bi-exponential with two lifetime values, 0.38 and 1.16 ns. These two lifetimes were associated with negative and positive pre-exponential coefficients, respectively. The negative pre-exponential coefficient for the long-wavelength band is a clear indication of an excited state reaction.[24] Moreover, the identity of the short lifetime components of the two emission bands suggests that the long-wavelength emissive species is generated from the short-wavelength emissive species. These results provide additional evidence for the ESIPT reaction being the mechanism for the dual emission of compound **4**. However, the observed ESIPT related lifetime component is much longer than that observed for 3HFs in organic solvents, which indicates that in this particular case the ESIPT reaction is relatively slow. The latter could be connected both with an intrinsic property of the 3HQ fluorophore and with the solvation effect of water. The absence of long lifetime component in the case of the N* band suggests that in the present case the ESIPT reaction is probably an irreversible process,[24] which is in contrast with reversible ESIPT in 3HF derivatives observed in organic solvents.[13]

Taken together, our data indicate that the long-wavelength band of 3HQs in aqueous solution at neutral pH results from an emission of the ESIPT product. This behavior being similar to that of 3HFs, 3HQs appear as prospective two-color fluorescent dyes for biological applications.

Dependence of the emission spectra on the substituent at the heterocyclic nitrogen

The ratio of the two band intensities and the sensitivity of this ratio to the solvent were found to depend on the substituent at the heterocyclic nitrogen. For instance, compounds **1a** and **6** with R = H are characterized by a low intensity of the short

Table 2 Spectroscopic properties of 3HQs in organic solvents

Solvent	PPS[a]	3HQ	**1a**	**1b**	**2**	**3**	**4**	**5**	**6**
Toluene	0.655	I_{N*}/I_{T*}	0.01	0.03	0.03	0.02	0.02	0.11	0.02
		φ	0.38	0.22	0.16	0.22	0.23	0.22	0.34
Chloroform	0.786	I_{N*}/I_{T*}	0.02	0.07	0.10	0.07	0.06	0.09	0.03
		φ	0.44	0.23	0.14	0.21	0.23	0.27	0.40
THF	0.838	I_{N*}/I_{T*}	0.04	0.2	0.25	0.19	0.20	0.45	0.08
		φ	0.28	0.13	0.07	0.11	0.12	0.13	0.22
Ethanol	0.853	I_{N*}/I_{T*}	0.09	0.51	1.02	0.58	0.59	0.42	0.11
		φ	0.36	0.13	0.08	0.12	0.12	0.18	0.33
Methanol	0.857	I_{N*}/I_{T*}	0.34	1.01	2.10	1.12	1.18	0.60	0.16
		φ	0.35	0.13	0.07	0.11	0.11	0.17	0.30
DMF	0.954	I_{N*}/I_{T*}	0.16	0.77	1.14	0.78	0.84	0.87	0.23
		φ	0.39	0.19	0.12	0.17	0.37	0.2	0.34
DMSO	1	I_{N*}/I_{T*}	0.34	2.00	3.66	2.13	2.14	1.12	0.48
		φ	0.51	0.32	0.25	0.31	0.37	0.28	0.39

[a] PPS and φ designate the solvent polarity–polarizability scale and the fluorescence quantum yield, respectively.

wavelength band and thus a limited sensitivity of its intensity ratio to the nature of the solvent (Fig. 4a, Table 2). In contrast, compounds **1b–5** with R = Me display a pronounced variation of their intensity ratio in different solvents (Fig. 4b, Table 2).

One possible explanation for the increase of the N* form intensity in N–Me 3HQs in comparison with N–H derivatives is the decrease of the acidity of their 3-OH group in the excited state. This acidity can decrease due to the higher electron-donating properties of Me as compared to H. Alternatively, the higher relative intensity of the N* band in the case of N–Me substituted 3HQs can also be assigned to the larger size of the methyl group which disrupts the planarity of the molecule in the excited state due to steric hindrance (see above). This conclusion is in line with previous reports on 3-hydroxychromones, showing that an *ortho*-methyl group on the 2-aryl ring produced similar steric effects and increased the N* form emission.[25]

Dependence of the emission spectra of *N*-methyl substituted 3HQs on the substituent at position 2

Though N–Me 3HQ derivatives exhibit similar absorption spectra, their emission spectra were found to depend on the nature of the aromatic group at position 2. Indeed, the electron-donor properties of this aromatic group substantially influence the I_{N^*}/I_{T^*} intensity ratio. For example, in phosphate buffer at pH 7.0 the I_{N^*}/I_{T^*} ratio varies from 0.46 for dye **5** containing an electron-withdrawing CF_3 group to 1.6 for dye **2** with an electron-donating OMe group in the *para* position of the aromatic ring (Fig. 5).

To evaluate the electronic influence of substituents on the chemical reaction rates of aromatic compounds, the Hammett equation can be used:[26]

$$\log k/k_0 = \sigma^+\rho$$

where k_0 and k are the rate constants of a given reaction with a non-substituted (R′ = H) and a substituted compound, respectively. The constant ρ characterizes the type of reaction and σ^+ characterizes the electron-withdrawing properties of the substituent, taking into account a high level of π-conjugation.

Interestingly, the dependence of the logarithm of the I_{N^*}/I_{T^*} intensity ratio *versus* the σ^+ value of the substituent R′ (Scheme 2) was found to be linear (Fig. 6), indicating that the same reaction, namely ESIPT, takes place for all studied

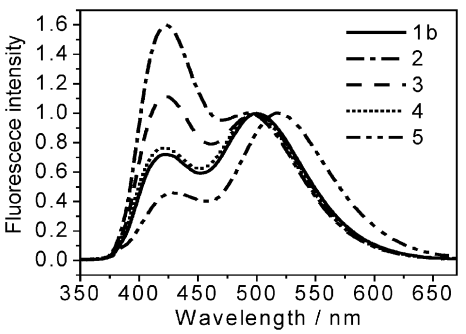


Fig. 5 Emission spectra of 3HQs **1b–5** in phosphate buffer (pH 7.0) normalized at the long-wavelength band maximum: **1b** (–), **2** (--), **3** (···), **4** (-·-) and **5** (– · ·).

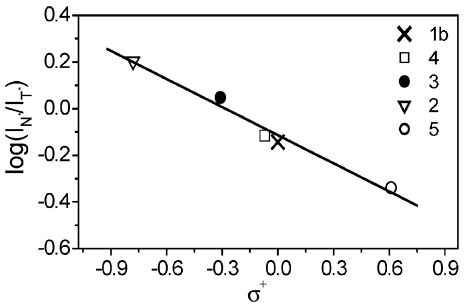


Fig. 6 Dependence of $\log(I_{N^*}/I_{T^*})$ of N–Me 3HQs on the Hammett constant in phosphate buffer. The data are fitted to: $\log(I_{N^*}/I_{T^*}) = -0.114\sigma^+ - 0.402$ ($r^2 = 0.98$).

N–Me 3HQs and that its rate increases with the electron-withdrawing property σ^+ of the substituent. The gradual increase in the ESIPT rate can be explained by the increase in the excited state acidity of the 3-OH group with the electron-withdrawing ability of the substituent. Moreover, since the I_{N^*}/I_{T^*} value is controlled by the relative rates of the various photophysical processes taking place in the excited state,[13] this linear dependence indicates that the other processes (radiative and non-radiative) were not significantly affected by these substituents. Moreover, the effect of the substituent on the emission of 3HQs together with its poor influence on the absorption spectra indicate that the conjugation between the 2-aryl group and the 3HQ moiety is much more pronounced in the excited state than in the ground state.

Thus, the I_{N^*}/I_{T^*} ratio can be modulated by the substituents on the aromatic ring of N–Me 3HQs. Moreover, using the Hammett equation, one can predict the I_{N^*}/I_{T^*} ratio of N–Me 3HQ for various groups in the *para* position. This feature is of high interest for developing 3HQs with programmed fluorescence properties.

Dependence of the I_{N^*}/I_{T^*} ratio and quantum yield of N–Me 3HQs on the solvent polarity

One unique property of 3HF dyes with dual fluorescence is the dependence of their I_{N^*}/I_{T^*} ratio on the environment.[3–5] To check whether this dependence also exists for N–Me 3HQs, we have determined their I_{N^*}/I_{T^*} ratio and quantum yield in different organic solvents (Table 2).

The correlations between the I_{N^*}/I_{T^*} ratios and the solvent polarity were established on the basis of the empirical solvent polarity–polarizability scale (PPS).[27] This scale is based on the solvatochromic shifts undergone by the long-wavelength absorption maximum of two indicators—DMANF and FNF. This scale excludes specific solvent–solute interactions and thus reflects 'pure' polarity.[27]

The I_{N^*}/I_{T^*} ratios of all N–Me 3HQs are highly sensitive to solvent polarity (Table 2). In Fig. 7, it can be seen that for the representative compound **1b**, the logarithm of the I_{N^*}/I_{T^*} ratio linearly increases with the PPS index in aprotic solvents. In contrast, the I_{N^*}/I_{T^*} ratio in the two protic solvents (methyl and ethyl alcohol) was much higher than expected from the PPS scale. This indicates that, as in the case of 3HF derivatives,[5] the hydroxyl groups of protic solvents probably form

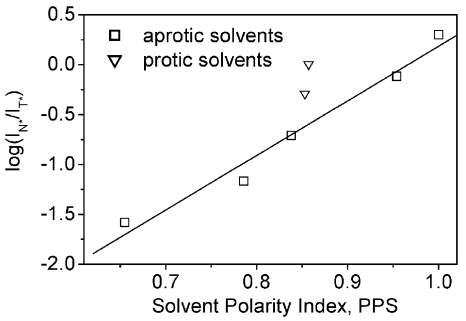


Fig. 7 Dependence of $\log(I_{N*}/I_{T*})$ of the dye **1b** on the solvent polarity index PPS. The aprotic solvents are toluene, chloroform, THF, DMF and DMSO. The protic solvents are ethyl alcohol and methyl alcohol. For aprotic solvents, data are fitted to $\log(I_{N*}/I_{T*}) = 5474 \times \text{PPS} - 5289$ ($r^2 = 0.97$).

hydrogen bonds with 3HQs, decreasing the ESIPT rates and therefore increasing the I_{N*}/I_{T*} ratio.

Comparison of the polarity dependences of all studied N–Me 3HQs in aprotic solvents is given in Fig. 8. Interestingly, the dyes exhibit different sensitivities to polarity, as can be seen from the different slopes. These different sensitivities are probably dependent on the electronic properties of the 2-aryl substituents. For instance, compound **5** with the electron-withdrawing *p*-(trifluoromethyl)phenyl group is less sensitive than **1b** with an unsubstituted phenyl group.

In contrast to the I_{N*}/I_{T*} ratio, only limited differences in 3HQ quantum yields were observed as a function of the solvent polarity. Moreover, as in the case of 3HFs, [3,4] no clear correlation could be established between the quantum yields of 3HQs and the solvent polarity (Table 2). For instance, similar quantum yields were found for all 3HQs in toluene and DMSO, though these solvents differ largely in polarity. However, the quantum yield was found to depend upon the structure of the 3HQs. For instance, N–H 3HQs **1a** and **6** exhibit higher quantum yields than the *N*-methyl derivatives in all solvents studied. This could be explained by an increase in the non-radiative rate constants due to a steric effect of the *N*-methyl group on the 2-aryl substituent that decreases the planarity of the *N*-methyl derivatives. Most interestingly, the quantum yields of the studied 3HQs are

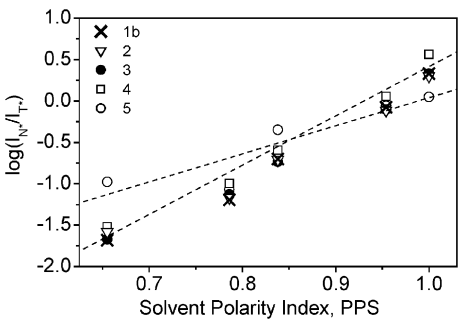


Fig. 8 Dependence of $\log(I_{N*}/I_{T*})$ on the solvent polarity index PPS for the N–Me 3HQs. Linear fits are presented for the most and the least sensitive dyes, **2** and **5**, respectively. The solvents are toluene, chloroform, THF, DMF and DMSO.

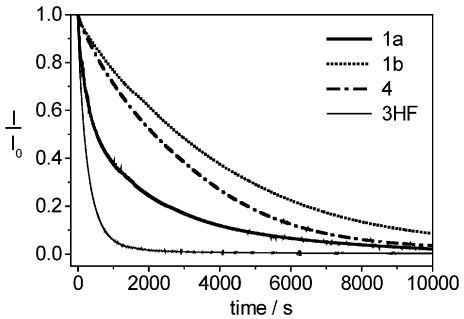


Fig. 9 Dynamics of photodestruction of 3HQs **1a**, **1b**, **4** and 3HF.

generally higher than those reported for 3HFs.[3] This constitutes an additional advantage of 3HQs over 3HFs.

Photostability

Photostability is an important property of fluorescent probes designed for biological applications. This property is notably important for investigating the dynamic properties of biomolecules such as proteins or nucleic acids, when the samples are irradiated for a long time. As could be seen from the quantum yields of photodegradation (Table 1) or the kinetic curves of photodestruction (Fig. 9), all studied 3HQs are one order of magnitude more photostable than 3HF. Moreover, comparison of compounds **1a**, **6** and **1b–5** indicates that N–Me 3HQs are about three times more photostable than the N–H derivatives (Table 1 and Fig. 9).

Conclusions

In this study, a new class of dyes, the substituted 2-aryl-3-hydroxyquinolones, has been shown to exhibit dual fluorescence in organic solvents as well as in aqueous solutions due to an excited state intramolecular proton transfer reaction. We observe that the substituent at the 2-aryl ring does not modify the absorption spectra of 3HQs, probably due to its twisted orientation with respect to the 3HQ moiety. Only the compound with a five-membered aryl substituent exhibits an increased planarity and thus differs considerably in its absorption properties. In contrast, the fluorescence properties of the dyes show a systematic dependence on the substituent. Indeed, a linear correlation of the logarithm of the intensity ratio of the two emission bands *versus* the Hammett constant (electron-withdrawing ability) associated with the substituent in the 2-aryl ring is observed for N–Me 3HQs. Moreover, the fluorescence properties of 3HQs are modulated by the nature of the substituent at the nitrogen heteroatom. While the dual fluorescence of N–H derivatives exhibits limited sensitivity to the polarity of their surroundings, N–Me 3HQs demonstrate strong solvatochromic properties, with large and systematic changes in the relative intensities of the two emission bands as a function of the solvent polarity. Finally, the 3HQ derivatives were found to exhibit higher fluorescence quantum yield and photostability than 3HFs. Consequently, 3HQ derivatives appear as prospective polarity-sensitive fluorescent labels for biomolecules, with fluorescence properties and sensitivity to solvent that can be tuned by the substituents at the 2-aryl ring and the nitrogen heteroatom.

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