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.

Excited state intramolecular proton transfer (ESIPT) reaction is very effective for the design of probes with dual fluorescence. 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, hydration and electronic polarizability, electrostatic effects in different media including lipid membranes and proteins. Moreover, these dyes were shown to be useful to determine the nature and concentration of cations and anions 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 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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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 Mar-
iner System 5155. 1H-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). 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.

The pKₐ values of quinolones were calculated by the
Fletcher–Pawl algorithm using a nonlinear least squares meth-
method, as developed in Doroshenko’s program, 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):\(^{16}\)

\[
A = \frac{A_{HA}10^{-pH} + A_{A}10^{-pK_a}}{10^{-pH} + 10^{-pK_a}},
\]

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 Fluoro-
Max 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):\(^{17}\)

\[
\phi_{\text{pr}} = \frac{1 - F_T/F_0}{\left[I_0\sigma(\lambda_{\text{exc}})\right] \int_0^T \left[F(t)/F_0\right] dt},
\]

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. \(\sigma(\lambda_{\text{exc}})\) is the one-photon absorption
cross-section at \(\lambda_{\text{exc}}\) expressed in cm² and \(t\) is the time in
seconds.

Fluorescence quantum yields \(\phi\) were determined with qui-
nine sulfate in 0.5 M sulfuric acid (\(\phi = 0.577\))\(^{18}\), 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(1H)-
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 °C 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%). \(\delta_H(300 \text{ MHz;}
\text{DMSO-d}_6; \text{Me}_4\text{Si}): 8.34 (d, 1H, ArH), 7.98–7.73
Table 1  Spectroscopic properties of the synthesized 3HQs in acetonitrile and phosphate buffer

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<th></th>
<th>1a</th>
<th>1b</th>
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<td></td>
<td>λνt/nm</td>
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<tr>
<td></td>
<td>ε × 10⁻³/l mol⁻¹ cm⁻¹</td>
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<td>11.5</td>
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<td>φ</td>
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<td>0.14</td>
<td>0.078</td>
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<td>0.14</td>
<td>0.17</td>
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<td>Φpd (%)*</td>
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<td>0.12</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
<td>0.14</td>
</tr>
</tbody>
</table>

* Ar and R are the 3HQ substituents at position 2 and the heterocyclic nitrogen, respectively. λabs is the position of the absorption maximum (nm), λνt and λτt 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, Φpd is the quantum yield of photodegradation. For 3HF, Φpd = 1.2%.

2-Phenyl-3-hydroxy-4(1H)-quinolone (1a). Prepared by using the experimental procedure described above for 5. δH(300 MHz; DMSO-d6): 11.59 (br s, 1H, NH), 8.15 (d, 8, 1H, ArH), 7.81 (d, J 7, 2H, Ar'H), 7.72 (d, J 8, 1H, Ar'H), 7.57 (m, 4H, ArH, Ar'H), 7.27 (t, J 7, 1H, Ar'H); m/z 324.21 (M⁺ + H); mp 234 °C.

1-Methyl-2-phenyl-3-hydroxy-4(1H)-quinolone (1b). Prepared by using the experimental procedure described above for 5. δH(300 MHz; DMSO-d6): 8.31 (d, J 8, 1H, Ar'H), 7.74 (m, 2H, Ar'H), 7.57 (m, 3H, Ar'H), 7.45 (d, J 7, 2H, Ar'H), 7.38 (dd, J 7, 1H, Ar'H), 3.52 (s, 3H, NCH₃); m/z 252.22 (M⁺ + H); mp 262 °C.

1-Methyl-2-(4-methoxyphenyl)-3-hydroxy-4(1H)-quinolone (2). Prepared by using the experimental procedure described above for 5. δH(300 MHz; DMSO-d6): 8.33 (d, J 8, 1H, Ar'H), 7.79 (m, 2H, Ar'H), 7.35 (m, 3H, ArH, Ar'H), 7.09 (d, J 7, 2H, Ar'H), 3.87 (s, 3H, OCH₃), 3.58 (s, 3H, NCH₃); m/z 282.31 (M⁺ + H); mp 259 °C.

1-Methyl-2-(4-phenylphenyl)-3-hydroxy-4(1H)-quinolone (3). Prepared by using the experimental procedure described above for 5. δH(300 MHz; DMSO-d6): 8.31 (d, J 8, 1H, Ar'H), 7.69 (m, 2H, Ar'H), 7.33 (m, 5H, ArH, Ar'H), 3.55 (s, 3H, NCH₃), 2.44 (s, 3H, 4-Ch₂); m/z 266.30 (M⁺ + H); mp 224 °C.

1-Methyl-2-(4-fluorophenyl)-3-hydroxy-4(1H)-quinolone (4). Prepared by using the experimental procedure described above for 5. δH(300 MHz; DMSO-d6): 8.30 (d, J 6.9, 1H, Ar'H), 7.70 (m, 2H, Ar'H), 7.50 (m, 2H, ArH), 7.40 (m, 2H, ArH), 3.53 (s, 3H, NCH₃); m/z 270.11 (M⁺ + H); mp 238 °C.

2-(2-Thienyl)-3-hydroxy-4(1H)-quinolone (6). Prepared by using the experimental procedure described above for 5. δH(300 MHz; DMSO-d6): 11.35 (br s, 1H, NH) 8.14 (d, J 8.5, 1H, Ar'H), 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 marginal 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 3HF, where the absorption properties are very sensitive to the donor substituents at position 2.6

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...
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 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. 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 suggest that the favorable dihedral angles are close to 45°, in line with recent X-ray data. 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, 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 \(10^{-7}\)–\(10^{-3}\) 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...
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 mono-exponential 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

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<tr>
<th>Solvent</th>
<th>PPSa</th>
<th>3HQ</th>
<th>1a</th>
<th>1b</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>Toluene</td>
<td>0.655</td>
<td>$I_{N*/T*}$</td>
<td>0.01</td>
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<td>0.03</td>
<td>0.02</td>
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<td></td>
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<td>$\phi$</td>
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<td>0.22</td>
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<td>0.22</td>
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<td></td>
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<td></td>
<td>$\phi$</td>
<td>0.51</td>
<td>0.32</td>
<td>0.25</td>
<td>0.31</td>
<td>0.37</td>
<td>0.28</td>
<td>0.39</td>
</tr>
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</table>

a PPS and $\phi$ designate the solvent polarity–polarizability scale and the fluorescence quantum yield, respectively.

**Fig. 4** Normalized emission spectra of 3HQ 1a (R = H, panel a) and 1b (R = Me, panel b) in organic solvents.
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*/T*} intensity ratio. For example, in phosphate buffer at pH 7.0 the I_{N*/T*} ratio varies from 0.46 for dye 5 containing an electron-withdrawing CF3 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 k0 and k are the rate constants of a given reaction with a non-substituted (R' = H) and a substituted compound, respectively. The constant \(\rho\) characterizes the type of reaction and \(\sigma^+\) characterizes the electron-withdrawing properties of the substituent, taking into account a high level of \(\pi\)-conjugation.

Interestingly, the dependence of the logarithm of the I_{N*/T*} intensity ratio versus the \(\sigma^+\) 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 N–Me 3HQs and that its rate increases with the electron-withdrawing property \(\sigma^+\) 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*/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*/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*/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*/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*/T*} ratio on the environment.3–5 To check whether this dependence also exists for N–Me 3HQs, we have determined their I_{N*/T*} ratio and quantum yield in different organic solvents (Table 2).

The correlations between the I_{N*/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*/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*/T*} ratio linearly increases with the PPS index in aprotic solvents. In contrast, the I_{N*/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,8 the hydroxyl groups of protic solvents probably form

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

![Emission spectra of 3HQs 1b–5 in phosphate buffer (pH 7.0) normalized at the long-wavelength band maximum: 1b (- - - - -), 2 (- - -), 3 (--), 4 (- - -), 5 (- - - -).](attachment:image.png)
hydrogen bonds with 3HQs, decreasing the ESIPT rates and therefore increasing the $I_{N^*/I^*}$ 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 $I_{N^*/I^*}$ ratio, only limited differences in 3HQ quantum yields were observed as a function of the solvent polarity. Moreover, as in the case of 3HF's, 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 3HQ 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 generally higher than those reported for 3HF's. This constitutes an additional advantage of 3HQs over 3HF.

**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 3HF's. 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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References


