

Synthesis and fluorescence properties of 2-aryl-3-hydroxyquinolones, a new class of dyes displaying dual fluorescence

Dmytro A. Yushchenko,^{a,b,*} Mykhailo D. Bilokin,^a Oleksandr V. Pyvovarenko,^a
Guy Duportail,^b Yves Mély^b and Vasyl G. Pivovarenko^{a,b}

^a*Department of Chemistry, Kyiv National Taras Shevchenko University, 01033 Kyiv, Ukraine*

^b*Photophysique des Interactions Biomoléculaires, UMR 7175-LC1 du CNRS, Institut Gilbert Laustriat, Faculté de Pharmacie, Université Louis Pasteur, 67401 Illkirch, France*

Received 25 October 2005; revised 27 November 2005; accepted 30 November 2005

Available online 20 December 2005

Abstract—A series of 2-aryl-3-hydroxyquinolones (3HQs) with different electron donating aryl substituents at the position 2 were synthesized. Their absorption and fluorescence properties were studied in solvents of medium and high polarity. Almost all the synthesized 3HQs display dual fluorescence in the tested solvents, in line with an excited state intramolecular proton transfer reaction. For *N*-methyl substituted compounds, the intensity ratio of the two emission bands was found to be exquisitely sensitive to solvent polarity, with a two orders of magnitude change from toluene to dimethylsulfoxide. Consequently, these compounds appear as prospective polarity fluorescent labels for proteins and nucleic acids.

© 2005 Elsevier Ltd. All rights reserved.

1. Introduction

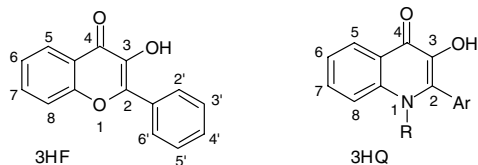
Due to its exquisite sensitivity, fluorescence is one of the most important techniques for investigating molecular events in biological systems. However, this technique strongly relies on the availability of fluorescent probes with optimal properties. Of particular interest are the dual fluorescence probes that exhibit two well separated emission bands.¹ In this case, the ratio of the intensities of the two bands can be used as a signal. This ratiometric response constitutes a strong advantage of the dual fluorescent dyes over the intensimetric response of the common single-band fluorescent probes, since the ratiometric response does not depend on the probe concentration. This advantage is especially of interest in cellular and tissular studies where the local concentrations of the dyes cannot be controlled. Excited state intramolecular proton transfer (ESIPT) reaction^{2,3} is one of the most effective principles used in the design of probes

with dual fluorescence.^{4–7} ESIPT results in the formation of two tautomeric forms in the excited state of the probe. Due to their different photophysical properties, these tautomeric forms exhibit largely separated emission bands on the wavelength scale. Moreover, these two forms also show different sensitivities to their environment, so that the ratio of the intensities of the two emission bands can be used as a sensitive mean to characterize the probe environment.⁸ 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 the polarity,^{4–9} hydration,¹⁰ electronic polarization⁸ and electrostatic effects in different media^{11–14} including microheterogeneous systems and proteins.^{15–18}

However, despite their significant advantages compared to common single-band probes, 3HFs present some drawbacks notably in respect with their limited photostability and quantum yield that limit their applications. As a consequence, the development of new dual fluorescence probes with improved fluorescent properties is strongly required. In this respect, 2-aryl-3-hydroxyquinolones (3HQs), which are structural analogs of 3HFs may constitute potential interesting candidates.

Keywords: 3-Hydroxyquinolones; Absorption; Fluorescence probes; Polarity sensors.

* Corresponding author. Tel.: +33 390 244262; fax: +33 390 244313; e-mail: ydmytro@yahoo.com



Indeed, substitution of the oxygen atom of the 3HF moiety by a nitrogen atom in 3HQs will permit additional modifications, which may further improve the dye properties. However, only very limited spectroscopic data are actually available on 3HQs¹⁹ and no attempts have been done to adjust their spectroscopic properties. In the present work, we describe the synthesis and spectroscopic characterization of a series of 3HQs with different substituents in two key positions of the molecule, namely on the heterocyclic nitrogen atom and the aromatic ring at position 2.

2. Results and discussion

2.1. Synthesis

Since some alkaloids are based on the structure of 3HQs, several methods of 3HQs synthesis have been developed.^{19–23} We have selected two of these methods to synthesize our 3HQ derivatives. First, we used the Algar–Flynn–Oyamada reaction, which is largely used in 3HF synthesis. Treatment of 2'-aminochalcones by hydrogen peroxide in basic conditions leads to 3HQs.¹⁹

However, as previously shown,²⁴ we found that the synthesis of 3HQ derivatives by this method requires a more complex procedure than for 3HFs (Scheme 1, pathway 1).

The second used method consists in the synthesis and subsequent conversion of phenacyl anthranilates to 3HQ derivatives in polyphosphoric acid (PPA)^{22,24} or in the presence of a base.²⁴ This method proved to be simple and effective for the synthesis of most of our 3HQ derivatives and was thus preferred (Scheme 1, pathway 2, Table 1).

Table 1. Synthesized 3HQs and their characteristics²⁶

Compd. no.	Ar	R	Total yield (%)	Mp (°C)
1		H	61 ^a /24 ^b	276
2		Me	60 ^a	238
3		Me	71 ^a	257
4		Me	60 ^a /32 ^b	224
5		Me	59 ^a /27 ^b	259
6a		H	55 ^a	293
6b		Me	57 ^a	301
7		Me	49 ^c	316
8		Me	52 ^{c,d}	294

^a Prepared by pathway 2 (Scheme 1).

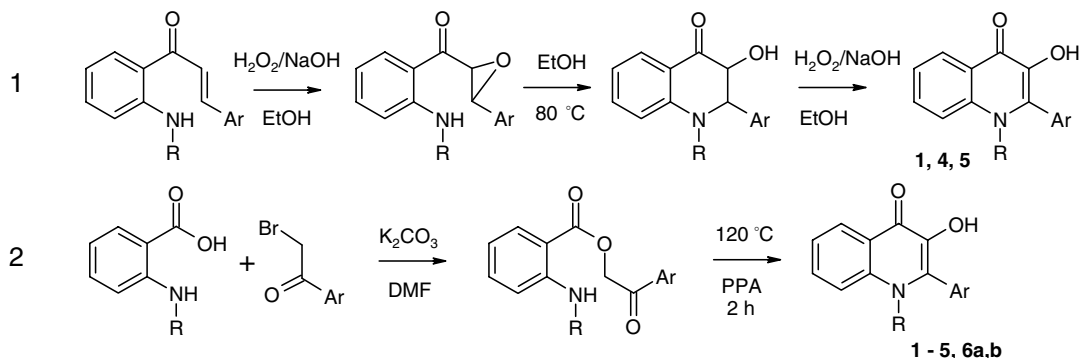
^b Prepared by pathway 1 (Scheme 1).

^c Obtained by nucleophilic substitution of fluorine (Scheme 2).

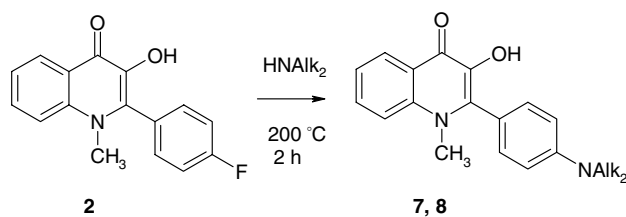
^d DMF as a source of dimethylamine was used instead of the corresponding amine.

Since substitution of the 4' position of the aryl group by the strong electron donor dialkylamino group has been shown to increase the sensitivity of the fluorescence properties of 3HF to its environment,^{5,6,8} 4'-dialkylamino substituted 3HQ compounds (**6a,b**, **7**, **8**) have been synthesized. Since the starting 4'-(*N,N*-dialkylamino)phenyl-2-bromoketones cannot be prepared by the common procedure of bromination, we prepared them through their 2,2-dibromide intermediates as recently described.²⁵ Alternatively, we found that the 4'-dialkylamino substituted 3HQs could also be prepared by substituting the halogen atom in a corresponding fluorine derivative (Scheme 2). We successfully applied this procedure to the synthesis of quinolones **7** and **8**.

Taken together, our data indicate that pathway 2 through phenacylanthranilates is the most appropriate one for 3HQ synthesis. Even dialkylamino derivatives can be easily obtained with substitution of the fluorine



Scheme 1.



Scheme 2.

atom by an amino group on the last step of this pathway.

2.2. Fluorescence properties

As expected, all the 3HQ compounds exhibit two emission bands in almost all tested solvents, evidencing the occurrence of ESIPT for all these compounds. Though most 3HQ derivatives exhibit similar absorption spectra, their emission spectra were found to depend on the aromatic group in position 2. Indeed, the electron donating properties of this aromatic group substantially influence the N^*/T^* ratio (Fig. 1). A detailed study of this phenomenon will be published separately. Here, we will discuss only how the solvent polarity influences the fluorescence properties of 3HQs.

All the studied 3HQs exhibit similar fluorescence quantum yields in all tested solvents (Table 2). These quan-

tum yields are generally higher than those reported for corresponding 3HFs.⁵

According to the intensity of the short wavelength band (N^* -band) in the fluorescence spectra, the 3HQ derivatives can be divided into three groups that differ considerably by their fluorescence properties. In the first group composed of compounds **1** and **6a** (with $R = H$), the short wavelength band exhibits low intensity and limited sensitivity to solvent polarity, as could be seen from the changes of the maxima emission wavelengths and two band intensity ratios (Fig. 2). The second group comprises the *N*-methyl substituted quinolones **2–5**. In this group, considerable changes of the two band intensity ratio were observed upon changes in the solvent polarity (Fig. 3, Table 2). Such peculiar emission of these 3HQs is very similar to fluorescence that was observed for some 3HFs and can be explained in the same way.¹⁰ As an example, the intensity ratio changes by two orders of magnitude from the less polar toluene (0.04) to DMSO (2.04) for 3HQ **2**. As a consequence, the compounds of this group may constitute suitable polarity sensors in a wide range of solvents. Finally, the third group is composed with the dialkylamino substituted compounds **6b**, **7** and **8**. The two emission bands of these compounds were observed to collapse in protic solvents (spectra not shown). This undesired effect may be related to the formation of hydrogen bonded complexes between the protic solvent molecules and both tautomeric forms of the dialkylamino 3HQ derivatives. These complexes markedly shift the N^* band to the red region

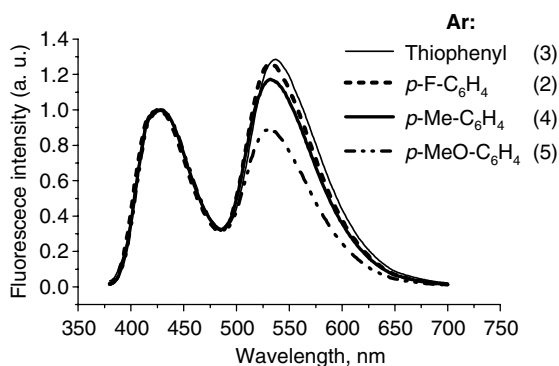


Figure 1. Normalized fluorescence spectra of *N*-methyl-3HQs **2–5** in DMF. The two bands correspond to the emission of the normal (N^*) and tautomeric (T^*) forms resulting from ESIPT.

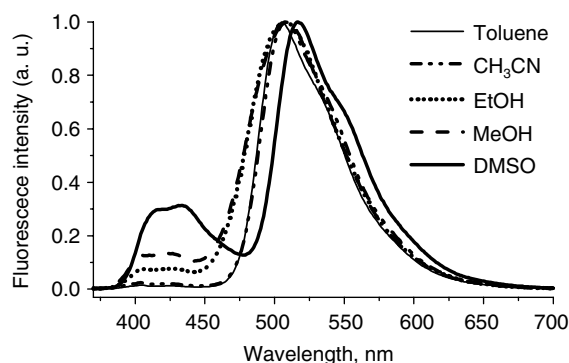


Figure 2. Normalized fluorescence spectra of 3HQ **1** ($R = H$) in organic solvents.

Table 2. Spectroscopic properties of 3HQ **2** in organic solvents²⁷

Solvent	Absorption λ_{abs} (nm)	Extinction coefficient	Fluorescence		Intensity ratio I_{N^*}/I_{T^*}	Fluorescence quantum yield (%) ^a
			λ_{N^*} (nm)	λ_{T^*} (nm)		
Toluene	372	11,600	419	524	0.040	26.2
Chloroform	368	12,500	418	515	0.066	24.0
Acetonitrile	365	11,600	418	521	0.11	16.1
Ethanol	365	12,300	428	519	0.48	13.4
Methanol	365	10,600	420	515	0.93	21.7
DMF	369	12,000	425	531	0.79	13.5
DMSO	370	11,800	430	529	2.03	31.9

^a As a reference was used quinine sulfate in 1.0 N H_2SO_4 .

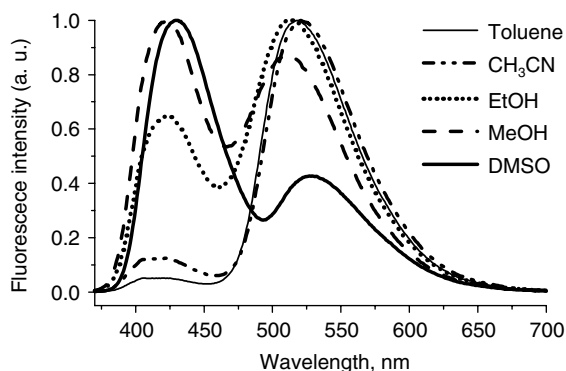


Figure 3. Normalized fluorescence spectra of 3HQ 4 (R = Me) in organic solvents.

and the T* band to the blue region of the spectrum, thus inducing their collapse. The same trends but on a smaller scale were already observed for dialkylamino 3HF derivatives.

Thus, in contrast to the compounds of the second group, the dialkylamino derivatives will not be useful as polarity sensors.

3. Conclusions

In this study the substituted 2-aryl-3-hydroxyquinolones have been shown to exhibit dual fluorescence. All the synthesized dyes show similar absorption properties that weakly depend on the nature of the substituent on the quinolone nitrogen atom and the aromatic ring at position 2. Based on their fluorescence properties, these compounds can be divided into three groups among which the *N*-methyl substituted group is the most promising one. Indeed, the two band intensity ratio of the compounds of this group was found to be exquisitely sensitive on solvent polarity. As a consequence, these compounds may be used as ratiometric probes for sensing the polarity in a large range, from toluene to dimethylsulfoxide. Moreover, they were having an increased quantum yield compared to 3HFs in a series of protic and aprotic solvents. They may be also used as polarity-sensitive fluorescent labels for nucleic acids and proteins.

Acknowledgements

This work was supported by Association Française contre les Myopathies (Contract #LSHB-CT 2003-50311480) and Eco-Net Program from French Ministère des Affaires Etrangères. D. Yushchenko is a member of Collège Doctoral Européen and was supported by the TRIOH European Program and CNRS. The laboratory of Drs André Mann and Jean Suffert is acknowledged for providing support for organic synthesis. A special acknowledgment is addressed to Dr. Andrey S. Klymchenko for discussions and advices. We are also thankful to Professor A. P. Demchenko for the comments.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.tetlet.2005.11.160](https://doi.org/10.1016/j.tetlet.2005.11.160).

References and notes

- Zbigniew, R. G.; Krystyna, R.; Wolfgang, R. *Chem. Rev.* **2003**, *103*, 3899–4031.
- Formosinho, J. S.; Arnaut, G. L. *J. Photochem. Photobiol. A: Chem.* **1993**, *75*, 21–48.
- Le Gourrier, D.; Ormson, S. M.; Brown, R. G. *Progr. React. Kinet.* **1994**, *19*, 211–275.
- Klymchenko, A. S.; Ozturk, T.; Pivovarenko, V. G.; Demchenko, A. P. *Can. J. Chem.* **2001**, *79*, 358–363.
- Klymchenko, A. S.; Ozturk, T.; Pivovarenko, V. G.; Demchenko, A. P. *Tetrahedron Lett.* **2001**, *42*, 7967–7970.
- Klymchenko, A. S.; Ozturk, T.; Demchenko, A. P. *Tetrahedron Lett.* **2002**, *43*, 7079–7082.
- Klymchenko, A. S.; Ozturk, T.; Pivovarenko, V. G.; Demchenko, A. P. *New J. Chem.* **2003**, *27*, 1336–1343.
- Ercelen, S.; Klymchenko, A. S.; Demchenko, A. P. *Anal. Chim. Acta* **2002**, *464*, 273–287.
- Klymchenko, A. S.; Pivovarenko, V. G.; Demchenko, A. P. *J. Phys. Chem. A* **2003**, *107*, 4211–4216.
- Klymchenko, A. S.; Demchenko, A. P. *Phys. Chem. Chem. Phys.* **2003**, *5*, 461–468.
- Roshal, A. D.; Grigorovich, A. V.; Doroshenko, A. O.; Pivovarenko, V. G.; Demchenko, A. P. *J. Phys. Chem.* **1998**, *102*, 5907–5914.
- Klymchenko, A. S.; Demchenko, A. P. *Langmuir* **2002**, *18*, 5637–5639.
- Klymchenko, A. S.; Duportail, G.; Ozturk, T.; Pivovarenko, V. G.; Mély, Y.; Demchenko, A. P. *Chem. Biol.* **2002**, *9*, 1199–1208.
- Klymchenko, A. S.; Duportail, G.; Ozturk, T.; Mély, Y.; Demchenko, A. P. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 11219–11224.
- Bondar, O. P.; Pivovarenko, V. G.; Rowe, E. S. *Biochim. Biophys. Acta* **1998**, *1369*, 119–130.
- Duportail, G.; Klymchenko, A. S.; Mély, Y.; Demchenko, A. P. *J. Fluorescence* **2002**, *12*, 181–185.
- Ercelen, S.; Klymchenko, A. S.; Demchenko, A. P. *FEBS Lett.* **2003**, *538*, 25–28.
- Klymchenko, A. S.; Avilov, S. V.; Demchenko, A. P. *Anal. Biochem.* **2004**, *329*, 43–57.
- Gao, F.; Johnson, K. F.; Schlenoff, J. B. *J. Chem. Soc., Perkin Trans. 2* **1996**, *2*, 269–273.
- Beney, C.; Hadjeri, M.; Mariotte, A.-M.; Boumendjel, A. *Tetrahedron Lett.* **2000**, *41*, 7037–7039.
- Coppola, G. M. *J. Heterocycl. Chem.* **1982**, *19*, 727–731.
- Hradil, P.; Jirman, J. *Collect. Czech. Chem. Commun.* **1995**, *60*, 1357–1366.
- Smith, M. A.; Neumann, R. M.; Webb, R. A. *J. Heterocycl. Chem.* **1968**, *5*, 425–426.
- Hradil, P.; Hlavac, J.; Lemr, K. *J. Heterocycl. Chem.* **1999**, *36*, 141–144.
- Diwu, Z.; Beachdel, C.; Klaubert, D. H. *Tetrahedron Lett.* **1998**, *39*, 4987–4990.
- Synthetic procedures are described in [Supplementary data](#).
- Absorption and fluorescence spectra were recorded at room temperature on a Cary 4 spectrophotometer (Varian) and a FluoroMax 3.0 (Jobin Yvon, Horiba) spectrofluorometer, respectively. Excitation wavelength for emission spectra was 360 nm in all cases.