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ORIGINAL PAPER

Dual-Fluorescence Probe of Environment Basicity (Hydrogen Bond Accepting Ability) Displaying no Sensitivity to Polarity

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Abstract 3-Hydroxyquinolones (3HQs) are a new class of water soluble dual fluorescence probes that can monitor both polarity and basicity (H-bond accepting ability) parameters. Both parameters play an important role in proteins and lipid membranes. Nevertheless, no method exists actually to measure the basicity parameter separately from the polarity. To achieve this aim, we synthesized 2-benzofuryl-3-hydroxy-4(1H)-quinolone (3HQ-Bf) and characterized its photophysical properties by UV, steady-state and time-resolved fluorescence spectroscopy. Due to its extended conjugation and totally planar conformation, 3HQ-Bf is characterized by a high fluorescence quantum yield. In solution, this dye shows an excited state intramolecular proton transfer (ESIPT) reaction resulting in two tautomer bands in the emission spectra. The ESIPT reaction can be considered as irreversible and is governed by rate constants from 0.6 to 8×10^9 s^{-1}, depending on the solvent. The analysis of the spectral properties of 3HQ-Bf in a series of organic solvents revealed a marginal sensitivity to the solvent polarity, but an exquisite sensitivity to solvent basicity, as shown by the linear dependence of the logarithm of the emission bands intensity ratio, $\log(I_{N*}/I_{T*})$, as well as the absorption or emission maxima wavenumbers as a function of the solvent basicity parameter. This probe may find useful applications through coupling to a protein ligand, for characterizing the H-bond acceptor ability at the ligand binding site as well as for studying the basicity changes of lipid membranes during their chemo- and thermotropic conversions.

Keywords Fluorescence probes · Solvent basicity · Hydrogen-bond acceptor · Ratiometric sensors · Intramolecular proton transfer · 3-hydroxyquinolones

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Introduction

Fluorescence methods are powerful for investigating molecular interactions and especially protein/ligand interactions in complex biological systems [1]. For this reason, there is a strong demand for the development of new fluorescent probes that could sense, for example, in the protein binding site not only the electric field of the surrounding atoms, but also the H-bond donating and H-bond accepting ability. In this respect, probes with a dual emission (two-channel emitters) present the strong advantage over intensiometric probes (single-channel emitters) to give a ratiometric response independent of the probe concentration. Excited state intramolecular proton transfer (ESIPT) [2–4] procures a very effective basis for the design of probes with dual fluorescence. The ESIPT reaction leads to the formation of two isomers (normal N* and tautomer T* forms) in the excited state of the probe. Due to their different photophysical properties, these tautomeric forms exhibit highly separated emission bands. The most interesting and characteristic representatives of ESIPT probes are 3-hydroxyflavones and their derivatives (3HFs) [5, 6]. They

have been shown to be effective tools for investigating the polarity [6–12], H-bond donor ability [12, 13], electronic polarizability [14] and electrostatic effects in different media [15,16] including model lipid membranes [16–19], cell membranes [20] and proteins [21–23]. Moreover, these dyes were shown to be useful to determine the nature and concentration of cations and anions [24–27]. However, despite their advantages over common single-band probes, 3HFs exhibit relatively low photostability and low quantum yields in water that limit their application. As a consequence, the development of new dual-fluorescence probes with improved fluorescent parameters is strongly required.

3-hydroxyquinolones (3HQs) are structural aza-analogs of the 3HFs. They also display a well-expressed dual emission in most tested media [28] due to an ESIPT reaction [28–30]. Spectral properties of 3HQs were up to now less investigated since they are a relatively new class of dyes with more complex synthetic pathways. But importantly, in comparison to 3HFs, most of the synthesized 3HQs are more photostable and have higher fluorescence quantum yields in aqueous solutions [28]. They were shown to be effective in sensing the viscosity of protic solvents [31] as well as both the polarity and basicity of organic media [32]. The last two properties are provided by intra– and intermolecular processes that play a key role in the functions of proteins and lipid membranes. However, in contrast to polarity [33, 34] and H-bond donor ability [12,13], no fluorescence probe reports selectively on the basicity (H-bond acceptor ability) of the surrounding molecules. Thus, it would be important to develop a probe able to sense the basicity independently from the polarity.

In this respect, we present herein the design, synthesis and fluorescence properties of a new dye of the 3HQ series, namely 2-benzofuryl–3-hydroxy–4(1H)-quinolone (3HQ-Bf, Scheme 1). This probe has been designed based on more simple analogs–3HQ-Ph and 3HQ–Th - which, being water soluble and possessing good fluorescence quantum yields, exhibit a mixed sensitivity to both solvent polarity and basicity [32]. According to the principles of solvatochromism [33], the sensitivity of a dye to polarity is due to the difference in stabilization of its S_0 and S_1 states by the solvent. Thus, to lower the sensitivity to solvent polarity, this difference should be reduced. In the case of the ESIPT dyes, a decrease of the sensitivity of the ratiometric response to solvent polarity should be achieved by limiting the reorganization of the solvation shell around the proton transfer system. In principle, this requires to minimize the changes in the dipole moment of the dye during its transition from the S_0 to S_1 states. According to quantum chemical calculations [28], this should be achieved by substituting the electron acceptor phenyl or thiophenyl rings by an electron donor benzofuryl ring. In addition, the decrease of the effective size of the five atoms cycle caused by the replacement of the sulfur atom in 3HQ-Th by the oxygen atom in 3HQ-Bf should favor the disruption of the intramolecular H-bond by the solvent [35], thus increasing the sensitivity of the N* emission band to basic molecules. In line with our expectations, all these features brought together resulted in the development of a fluorescence probe that reports about the basicity of the environment, independently from the polarity. As a consequence, we obtained for the first time a pure basicity sensitive fluorescence probe that can find interesting applications in the study of protein binding sites or lipid membranes.

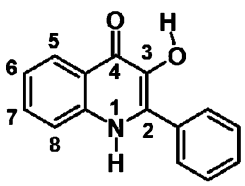
Experimental

Materials and methods

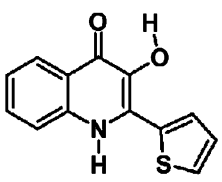
All reagents were purchased from Sigma-Aldrich. Solvents for synthesis were of reagent quality and appropriately dried if necessary. For absorption and fluorescence studies, the solvents were of spectroscopic grade. Melting points were determined on a "VEB Analytik", Dresden hostage microscope melting point apparatus, and were uncorrected. Proton NMR spectra were recorded on a Varian Mercury–400 MHz spectrometer. Tetramethylsilane (TMS) was used as an internal standard in $CDCl_3$ or DMSO−d_6. Mass spectra were measured with a Mass Spectrometer Mariner System 5155.

Absorption spectra were recorded on a Cary 4 spectrophotometer (Varian) and fluorescence spectra on a FluoroMax 3.0 (Jobin Yvon, Horiba) spectrofluorimeter at room temperature. In case of a structured emission spectrum, the middle emission peak which usually was the highest one was chosen to determine the position of the maximum emission wavelength. Fluorescence quantum yields φ were

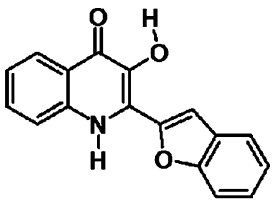
Scheme 1 Chemical structures of the studied 3-hydroxyquinolones



3HQ-Ph



3HQ-Th



3HQ-Bf

determined with quinine sulfate in 0.5 M sulfuric acid (φ= 0.577) as a reference [36]. All measurements were carried out in a temperature-controlled cell at 20±0.1°C. Quantum-chemical calculations were performed by the AM1 semi-empirical method [37] using the MOPAC 2007 program [38]. Configuration interactions in different combinations were taken into account during the calculations of the S_1 state parameters.

Time-resolved fluorescence measurements were performed with the time-correlated, single-photon counting technique using the frequency tripled output of a Ti-Sapphire laser (Tsunami, Spectra Physics), pumped by a Millenia X laser (Tsunami, Spectra Physics) [11]. The excitation wavelength was set at 320 nm. The fluorescence decays were collected at the magic angle (54.7°) of the emission polarizer. The single-photon events were detected with a microchannel plate Hamamatsu R3809U photomultiplier coupled to a Philips 6954 pulse preamplifier and recorded on a multichannel analyzer (Ortec 7100) calibrated at 25.5 ps/channel. The instrumental response function was recorded with a polished aluminum reflector, and its full-width at half-maximum was 50 ps.

The time-resolved decays were analyzed both by the iterative reconvolution method and the Maximum Entropy Method (MEM) [39]. The goodness of the fit was evaluated from the χ^2 values, the plots of the residuals and the autocorrelation function.

Synthesis

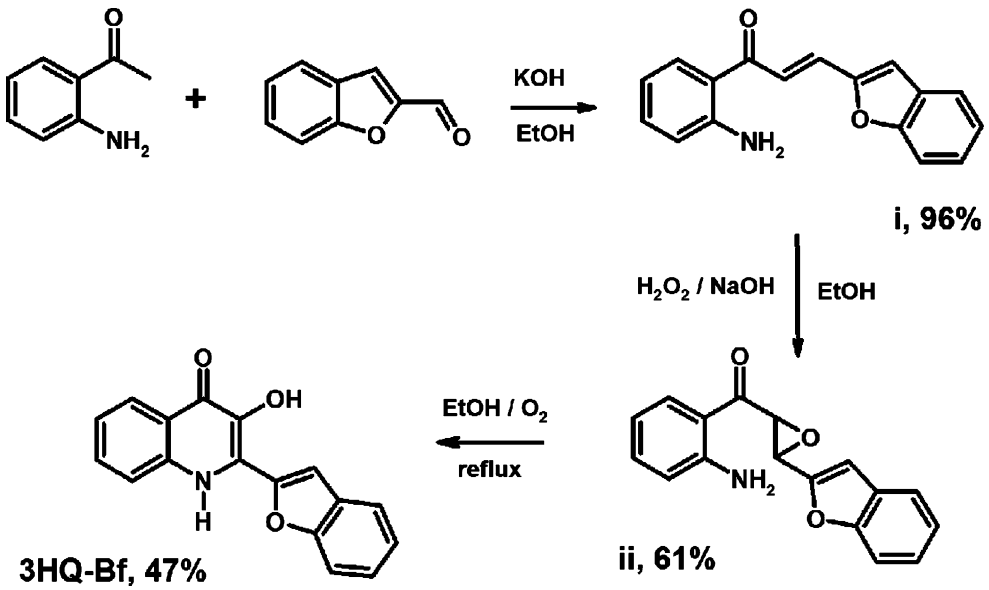
Synthesis (Scheme 2) and purification of 3HQ-Ph and 3HQ-Th were performed as previously described [28]. For the preparation of 2–(2-benzofuryl)-3-hydroxy-4(1H)-quinolone (3HQ-Bf) 0.5 ml of 30% aqueous solution of KOH was slowly added to a solution of 2-aminoacetophenone (0.675 g, 5 mmol) and 2-formylbenzofurane (0.759 g, 5.2 mmol) in 2 ml of ethanol. The resulting solution was stirred at room temperature during 45 min. Then, water (10 ml) was added to the reaction mixture, resulting in a yellow precipitate which was isolated by filtration and recrystallized from ethanol to give i as light yellow needles (96% yield). On the next step, the obtained product was dissolved in a mixture of ethanol and 10% aqueous NaOH (1:1 in v/v). 0.5 ml of 30% hydrogen peroxide was added drop-wise to the resulting mixture at 0°C up to the formation of the corresponding epoxide ii (TLC-controled). The resulting mixture was poured into 10 g of ice and neutralized by diluted aqueous acetic acid. The filtered precipitate was dissolved in 20 ml ethanol and refluxed during 20 h, until the precipitate of 3HQ-Bf was formed (TLC-controled). The crude product after washing and recrystallization from ethanol, gives light yellow needles of 3HQ-Bf. Yield 0.381 g (47%), m.p. 265–266°C (from ethanol), LC/MSD:%, m/z 278 $[M+1]^+$, ^1H -NMR data (TMS, DMSO−d_6) δ, (J, Hz): 7.27t, J=8.1 Hz, 1H(H-5′); 7.35t, J=6.9 Hz, 1H(H-6′); 7.61dd, J_1=8.3 Hz, J_2=3.1 Hz, 2H(H−7′, H-4′); 7.74 m, 2H(H-3′, H-8); 7.95d, J=2.1 Hz, 1H(H-6); 8.05d, J=8.7 Hz 1H(H-5); 11.07c, 1H (NH).

Results and discussion

Absorption properties

The absorption spectra of 3HQ-Bf and 3HQ-Ph exhibit dramatic differences in their profiles as well as in the

Scheme 2 Synthesis of 3HQ-Bf



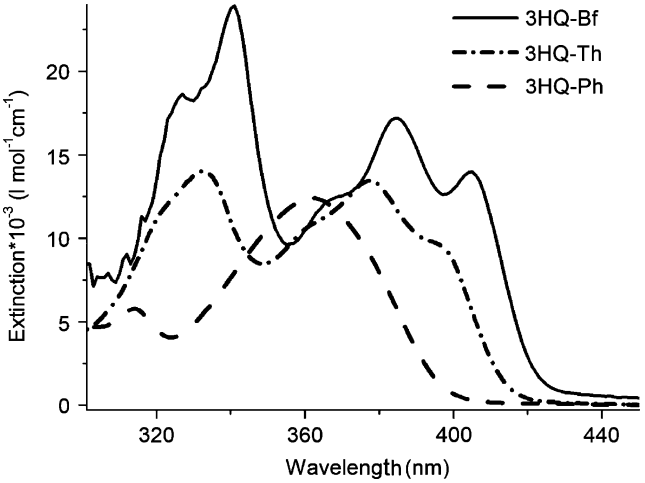


Fig. 1 Absorption spectra of 3HQs in ethanol

positions of their bands (Fig. 1), evidencing strong conformational differences between the two compounds. For 3HQ-Ph, the structureless profile and the short-wavelength position of the bands are in line with a distribution of nonplanar conformations with a low degree of π-conjugation between the rings [28]. On the contrary, the absorption spectrum of 3HQ-Bf is well structured and presents a 30 nm red-shift suggesting a distribution of more planar conformations and a larger degree of π-conjugation. In further line with this conclusion, 3HQ-Bf shows a ~1.5-fold higher molar extinction coefficient in solution as compared to 3HQ−Ph and other dyes of the 3HQ series [28] (Table 1). The absorption spectrum of 3HQ-Th shows an intermediate behavior, being less structured than the 3HQ-Bf one, indicating that 3HQ-Th exhibits less planar conformations than 3HQ-Bf.

To further strengthen our conclusions on the conformations of the 3HQ derivatives, quantum-chemical calculations were performed. In good agreement with the above spectral data, the calculated torsional enthalpy values show that the 3HQ derivatives with thiophene and phenyl rings are favoring non-planar conformations ([28], Fig. 2) with optimal angle values between the aromatic rings of 28° for 3HQ-Th and 42° for 3HQ-Ph, respectively. Meantime, the deep energy minimum observed for 3HQ-Bf indicates a narrow distribution of totally planar conformations at room temperature, in full agreement with the well-structured absorption spectrum. Thus, 3HQ-Th and 3HQ-Bf are a good example of molecules with close chemical structures, but with markedly different distribution of conformations. The main reason for this difference is likely the smaller size of the more compact oxygen-containing furyl ring that limits the steric hindrance with the 3HQ moiety.

Fluorescence properties

3HQ-Bf was found to exhibit high quantum yield in all types of solvents (Table 1), in full line with its highly planar structure. Furthermore, 3HQ-Bf displayed dual fluores-

Table 1 Spectroscopic properties of 3HQ-Bf in different solvents

№	Solvent	ε	β	λ_{abs}	$E \cdot 10^{-3}$	λ_{N^*}	λ_{T^*}	I_{N^*}/I_{T^*}	φ
1	Water	78.4	0.35	381	18.2	443	503	0.296	0.102
2	DMSO	46.8	0.88	391	15.3	453	532	1.433	0.408
3	Ethane-1,2-diol	40.2	0.78	389	18.6	447	524	0.500	0.462
4	DMFA	37.2	0.74	387	19.5	449	532	0.633	0.294
5	Acetonitrile	35.7	0.32	381	16.9	436	523	0.086	0.263
6	Methanol	32.6	0.47	384	17.7	444	526	0.213	0.143
7	N-methyl-pyrrolidone	32.6	0.76	389	17.5	450	536	0.523	0.309
8	HMPA	29.0	1.00	392	17.9	453	541	0.892	0.302
9	Ethanol	24.9	0.48	386	17.2	444	527	0.128	0.288
10	1,1,3,3-Tetra-methylurea	24.5	0.78	388	15.0	448	535	0.326	0.315
11	1-Propanol	20.5	0.48	385	17.5	447	524	0.121	0.310
12	Acetone	20.5	0.49	384	14.7	438	528	0.116	0.134
13	1-Butanol	17.3	0.51	385	16.8	446	525	0.130	0.374
14	Dichlormethane	9.02	0.05	383	17.1	437	521	0.050	0.320
15	Tetrahydrofuran	7.43	0.48	386	13.9	444	531	0.135	0.422
16	Ethyl acetate	5.99	0.45	383	16.9	438	527	0.068	0.250
17	Bromobenenzene	5.41	0.09	379	14.2	437	530	0.038	0.425
18	1,2-Dibromoethane	4.93	0.17	380	18.9	441	527	0.040	0.453
19	Diisopropyl ether	3.38	0.41	383	17.3	435	530	0.060	0.306
20	Dioxan	2.27	0.64	386	17.8	449	531	0.235	0.274

[a] ε – dielectric constant at 298 K. β – Abraham's hydrogen bond basicity [42], λ_{abs}: position of the absorption maximum (nm), E – molar extinction coefficient $l\ mol^{-1}\ cm^{-1}$ at λ_{abs}, λ_{N^*}, λ_{T^*} and I_{N^*}/I_{T^*}–positions of the fluorescence maxima and fluorescence intensity ratio of the N* and T* forms, respectively, φ is the fluorescence quantum yield.

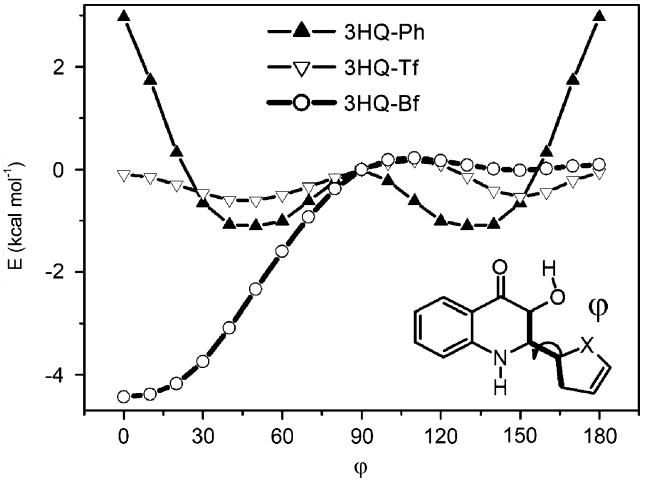


Fig. 2 Torsion enthalpy profiles of 3HQ derivatives

cence in all tested media (Fig. 3). Both emission bands are well resolved, with a separation between their maxima of 60-100 nm (2700−4200 cm^{-1}). Appearing in the blue and yellow-green regions of the visible spectrum, both emission bands showed only limited solvatochromic shifts, suggesting that the dipole moment values in the S_1 and S_0 electronic states are close. In spite of its poor solubility in low polar solvents, we succeeded to obtain solutions of monomeric dye starting from bromobenzene and dioxane. The excitation spectra of 3HQ-Bf recorded at the maximum emission wavelengths of the N* and T* bands were found to superimpose with the absorption spectrum, proving that the two emission bands belong to excited tautomeric forms. In addition, the ESIPT reaction for 3HQ-Bf was further confirmed by the identical fluorescence lifetimes values τ_1 for both N* and T* bands as well as by the appearance of a negative amplitude associated with the τ_1 value in the time-resolved fluorescence decay of the T* band (see below).

Due to the weak solvatochromism exhibited by 3HQ-Bf in its absorption as well as in its emission spectra, the positions of the band maxima are clearly non-effective parameters for characterizing the solvent polarity. In contrast, the ratio of the emission intensities of the two tautomers I_{N*}/I_{T*} displays dramatic changes in protic as well as in aprotic solvents (Fig. 3, Table 1). In contrast to 3-hydroxyflavones [12,13] and N-methyl-3HQs [30,32], where the I_{N*}/I_{T*} intensity ratio was largely governed by the solvent polarity, the I_{N*}/I_{T*} ratio of 3HQ-Bf showed no regular dependency on either the empirical $E_T(30)$ [33] or SPP [34] polarity parameters or the theoretical Lippert [40,41] polarity-polarisability function (Fig. 4a). Meantime, a linear relationship was found when $\log(I_{N*}/I_{T*})$ was plotted as a function of the H-bond acceptor ability expressed by the Abraham's basicity parameter β [42,43] (Fig. 4b, $log(I_{N*}/I_{T*})=-1.59+1.62\beta$, $r^2=0.9$). In addition, in spite of their limited variations, the positions of the maxima of both absorption and emission spectra were also found to linearly depend on the β value (Fig. 5). Thus, in line with our expectations, the spectroscopic properties of 3HQ-Bf appear to be strongly sensitive to the basicity parameter β, but not to the polarity.

An additional demonstration of the sensitivity of 3HQ-Bf emission to solvent basicity was obtained from the study of its spectral properties in mixtures of two solvents with close dielectric constant, but different basicity. For this purpose, acetonitrile ($\varepsilon=35.7$, $\beta=0.32$) and dimethylformamide ($\varepsilon=37.2$, $\beta=0.74$) were chosen as an appropriate pair of solvents. A linear relationship was found when the logarithm of the intensity ratio changes $\log(\Delta I_{N*}/I_{T*})$ was plotted as a function of the logarithm of the concentration of dimethylformamide in the mixture (Fig. 4c, $r^2=0.990$) or when the intensity ratio I_{N*}/I_{T*} was plotted as a function of the proportion of dimethylformamide in the mixture (Fig. 4 d,

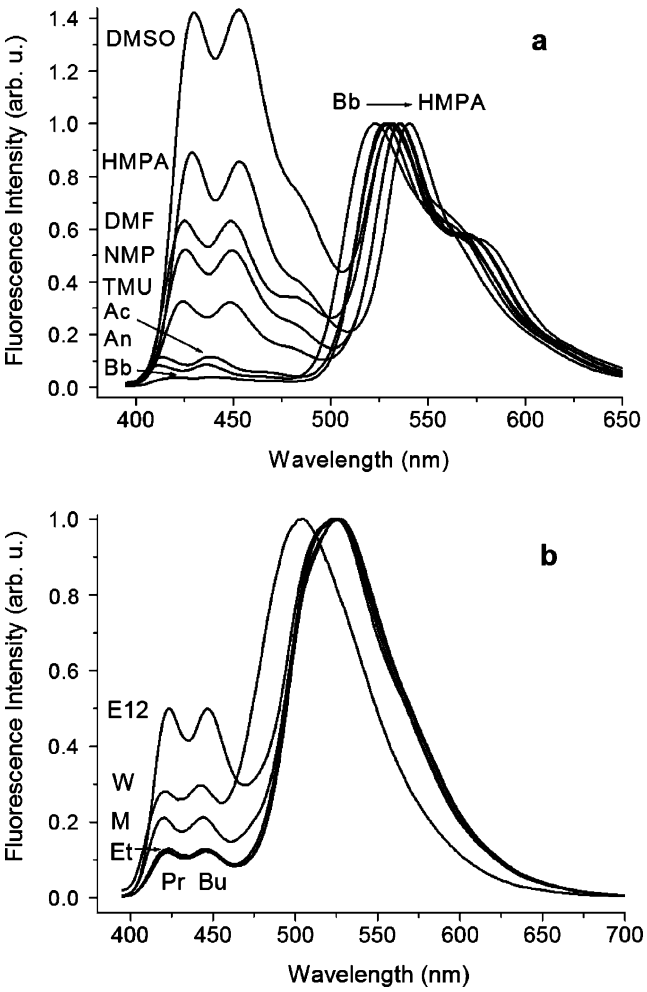


Fig. 3 Normalized fluorescence emission spectra of 3HQ-Bf in aprotic **a** and protic **b** solvents. Abbreviations: NMP - N-methylpyrrolidone, TMU - tetramethylurea, Ac - acetone, An - acetonitrile, Bb - bromobenzene, E12 - Ethane-1,2-diol, W - water, M - methanol, Et - ethanol, Pr - 1-propanol, Bu - 1-butanol

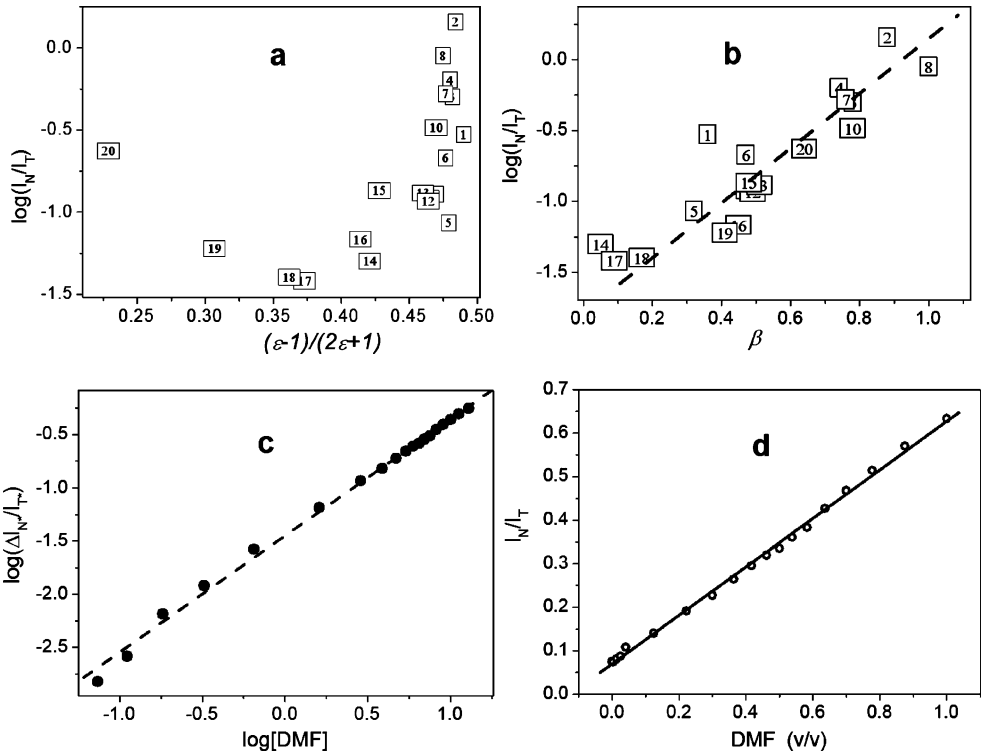


Fig. 4 Dependence of $\log(I_{N*}/I_{T*})$ on the polarity function **a** and the Abraham's hydrogen bond acceptor basicity parameter **b**. Numbering of solvents is as in Table 1. Influence of the solvent basicity in MeCN-DMF mixtures on the I_{N*}/I_{T*} ratio **c**, **d**. $\Delta I_{N*}= I_{N*}(\text{mixture})-I_{N*}(\text{pure acetonitrile})$

r^2=0.999). This shows the absence of preferential solvation of 3HQ-Bf by DMF in acetonitrile and points on the close values of the solvation energies of 3HQ-Bf in acetonitrile and DMF. The two linear relationships unambiguously confirmed the direct dependence of the 3HQ-Bf fluorescence properties on the solvent basicity.

Noticeably, several solvents show some deviations from the linear plot of $\log(I_{N*}/I_{T*})$ as a function of the solvent basicity β (Fig. 4b). For instance, the $\log(I_{N*}/I_{T*})$ values where found to be below the linear fit in 8–HMPA, 10–TMU, and 19–diisopropyl ether, while in contrast, $\log(I_{N*}/I_{T*})$ values significantly above the fit were observed in 1–water, 2–DMSO, 4–DMF, and 6 – methanol. In the former solvents (8, 10 and 19), the low $\log(I_{N*}/I_{T*})$ values could result from the poor accessibility of the spatially hindered basic (H-bond acceptor) center of the solvent molecules to the proton-transfer system. The relatively low concentrations of these basic centers (from 5.6 to 9.8 mole·l^{-1}) resulting from the high molar volume of these solvents should also contribute to this behaviour. In contrast, in the latter solvents (1, 2, 4 and 6), the high $\log(I_{N*}/I_{T*})$ values may be related to their comparatively higher molar concentrations (from 13 to 55 mole·l^{-1}). The deviation observed with dichloromethane (14) is less clear and may result from the cooperative effect of the two basic chlorine atoms localized in close proximity.

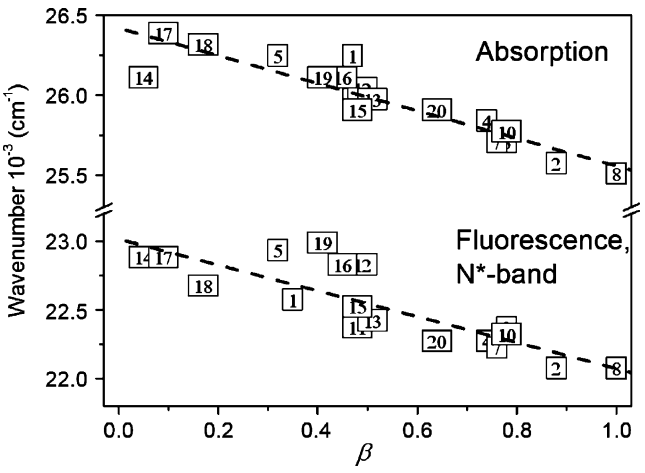


Fig. 5 Dependence of the band maxima positions (cm^{-1}) of the absorption and fluorescence emission spectra of 3HQ-Bf as a function of the Abraham's hydrogen bonding basicity parameter of the solvent. Solvent numbering is as in Table 1

Time-resolved fluorescence measurements

To further characterize the molecular basis of the spectral properties of 3HQ-Bf in comparison with 3HQ-Th, their ESIPT mechanism was investigated by time-resolved fluorescence measurements in methanol, taken as a protic solvent and in aprotic solvents of various basicity. In all cases, the fluorescence decays were biexponential and, both N* and T* forms exhibited the same fluorescence lifetimes (τ_1 and τ_2) but different pre-exponential coefficients (α_1 and α_2). The negative α_1 values for the T* form confirmed that it is produced from the N* state through an ESIPT

Table 2 Time-resolved fluorescence parameters of N* and T* emission bands in organic solvents for the two considered dyes

Dye	Solvent	Band	τ_1[a,b]	α_1[a,c]	τ_2[a,b]	α_2[a,b]	φ_{N^*}[a]	φ_{T^*}[a]	k_+[a]	I/I_{T^*}
3HQ-Bf	DMSO	N^*	1.34	0.91	7.51	0.09	0.240	0.168	0.6	1.43
		T^*	1.27	−0.44	7.57	0.56				
	DMF	N^*	0.82	0.99	6.49	0.01	0.114	0.180	1.0	0.63
		T^*	0.75	−0.66	6.42	0.33				
	MeOH	N^*	0.28	0.99	5.62	0.01	0.025	0.118	3.0	0.21
		T^*	0.27	−0.77	5.64	0.23				
	MeCN	N^*	0.11	0.98	6.01	0.02	0.019	0.244	8.1	0.08
		T^*	0.12	−0.66	5.95	0.33				
3HQ-Th	DMSO	N^*	0.98	0.97	4.79	0.03	0.126	0.264	0.9	0.48
		T^*	0.55	−0.33	7.65	0.66				
	DMF	N^*	0.56	0.99	4.02	0.01	0.064	0.276	1.5	0.23
		T^*	0.32	−0.51	7.76	0.49				
	MeOH	N^*	0.23	0.98	6.07	0.02	0.041	0.259	3.8	0.16
		T^*	0.21	−0.78	6.61	0.22				
	MeCN	N^*	0.06	0.98	5.59	0.02	0.009	0.301	15	0.03
		T^*	0.08	-0.51	5.76	0.49				

[a] τ_1 and τ_2 (ns) are the short-lived and long-lived fluorescence lifetimes respectively; α_1 and α_2 are the corresponding relative amplitudes; φ_{N^*}, φ_{T^*} are the fluorescence quantum yields of the N* and T* forms, respectively. $k_R^{N^*}$, $k_{NR}^{N^*}$ ($\times 10^{-9}$ s^{-1}) are respectively the radiative and non-radiative rate constants of the N^* form; k_+ ($\times 10^{-9}$ s^{-1}) is the forward rate constant of the ESIPT reaction. [b] The error for all lifetime values is ± 10%. The excitation wavelength was 320 nm. The time-resolved data for the N* and T* emission bands were recorded at 425 and 540 nm, respectively. α_1 and α_2 values were normalized according to $|\alpha_1| + |\alpha_2| = 1$.

reaction (Table 2). Furthermore, the very small contribution (< 5%) of the long-lived lifetime τ_2 for the N* form provides a strong argument to consider the ESIPT reaction as an irreversible process. Thus, the ESIPT rate constant k_+ could be calculated as recently described [32, 35].

For the two dyes, the slowest ESIPT reaction within the series of aprotic solvents is observed for the most basic solvent DMSO, while the ESIPT reaction rate is one order of magnitude faster in the non-basic acetonitrile. The k_+ values were inversely correlated with the I_{N^*}/I_{T^*} ratio, confirming the direct control of this ratio by the forward ESIPT reaction rate in 3HQs [32, 35]. In this respect, the slow reaction rates in basic solvents like DMSO or DMF may be explained by the rate-limiting disruption of the intermolecular H-bond of the N* form [32] between the basic atoms of DMSO or DMF and the proton of the 3HQ hydroxyl group (Fig. 6). This rate-limiting disruption step is required to allow the formation of the intramolecular H bond between the carbonyl oxygen O-4 and the hydroxyl group, which is needed for the ESIPT reaction. The

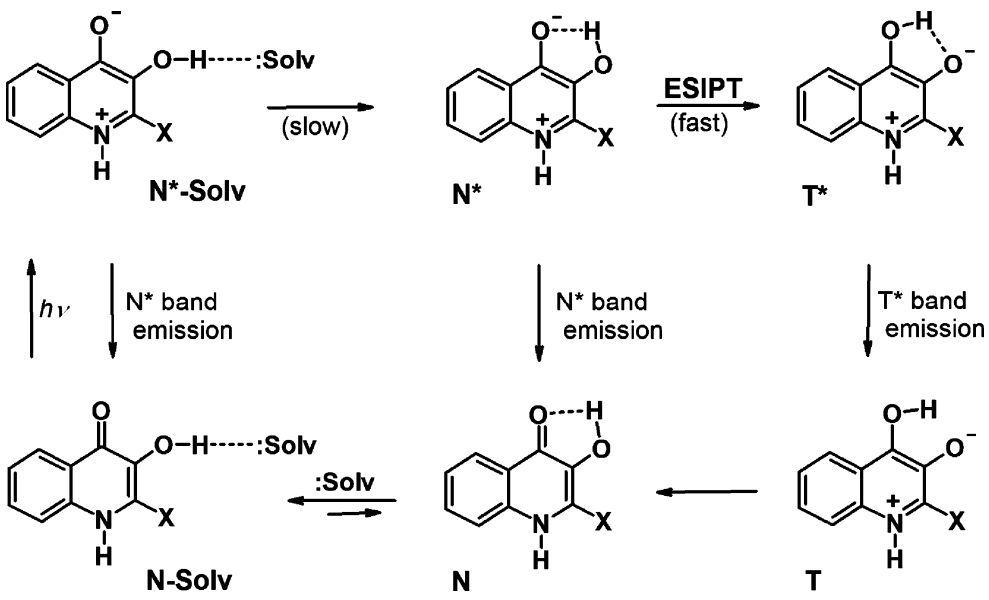


Fig. 6 Proposed excited-state reactions for 3HQ-Bf in basic solvents. In the ground state, 3HQ-Bf is mainly in a solvated form (N-solv) with an intermolecular H-bond between the solvent and the hydroxyl group of 3HQ-Bf. After excitation, the increase of basicity of the O-4 oxygen due to charge displacement in the excited state allows it to compete with the solvent for H-bonding with the hydroxyl group. This results in the rate-limiting formation of the N* form and, its subsequent and irreversible transformation into the T* form by ESIPT. After emission, the T form is thought to rapidly transform into N and finally, N-Solv forms

disruption of the H-bond with the solvent is likely more favored in the excited state than in the ground state, due to the increased basicity of the carbonyl oxygen O-4 in the excited state that allows this oxygen to compete with the solvent for H-bonding with the hydroxyl group. Thus, the excited state reaction rates and the ratio of the emission intensities I_{N*}/I_{T*} of the 3HQ-Bf dye are mainly controlled by the solvent basicity.

Interestingly, 3HQ-Bf exhibits lower ESIPT rate constants than 3HQ-Th in all aprotic solvents. This may result from the smaller steric hindrance provided by the less bulky furyl ring as compared with the thiophene ring on the formation of the intermolecular H-bond between the 3-OH group and the solvent (Fig. 6). As it was shown recently [35], the contact of bulky rings with the 3-OH group of 3HQs increases the ESIPT reaction rate by preserving the intramolecular H-bond for a longer time.

Finally, while the ESIPT reaction rates are different for the two quinolones in aprotic solvents, they appear similar in methanol, indicating that the side ring of the 3HQ derivatives has no influence on the ESIPT reaction in methanol and that the H-bonded complexes are likely different in protic and aprotic solvents.

Conclusions

A novel fluorescent dye, 2-benzofuryl-3-hydroxy-4(1H)-quinolone (3HQ-Bf), was synthesized and studied by absorption, steady-state and time-resolved fluorescence spectroscopy. Due to its totally planar conformation, 3HQ-Bf is characterized by a high fluorescence quantum yield. In solution, this dye demonstrates an excited state intramolecular proton transfer (ESIPT) reaction giving a dual emission resulting from the existence of the two tautomers, N* and T*. The ESIPT reaction in 3HQ-Bf is an irreversible process characterized by rate constants of $0.6–8\times10^{9}$ s^{-1}, depending on the solvent nature. The spectral properties of 3HQ-Bf were found to be exquisitely sensitive to basicity, while being almost non sensitive to polarity. The strong sensitivity of 3HQ-Bf to basicity results from the disruption of its intramolecular H-bond by basic molecules that prevent the ESIPT reaction. As a consequence, since the environment basicity (H-bond acceptor ability) is thought to play an important role in proteins and lipid membranes, 3HQ-Bf appears as a promising fluorophore for the design of probes for studying intermolecular interactions in lipid membranes and proteins.

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