

Modulation of the solvent-dependent dual emission in 3-hydroxychromones by substituents

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3-Hydroxychromones (3HCs) are fluorescent dyes, which respond to solvent perturbations by shifts and changes in the relative intensity of the two well-separated bands in the emission spectra. These bands originate from an excited state intramolecular proton transfer (ESIPT) reaction, which can be modulated by different factors, including modifications in the 3HC chromophore. In view of the great importance of 3HCs as prospective basic elements of molecular sensors, we have performed the first systematic study on the correlation between 3HC structure and spectroscopic properties. Two series of known and newly synthesized 2-phenyl-3-hydroxychromones and 2-(2-benzo[*b*]furanyl)-3-hydroxychromones with varied electron-donor substituents, introduced on opposite sides of the chromophore, were compared in solvents of different polarities. The substitution of 2-phenyl for 2-(2-benzo[*b*]furanyl) and introduction of electron donors on the 2-aryl group not only shift the absorption and fluorescence spectra to the red, but also strongly modulate the ESIPT behavior, resulting in a dramatic increase of the intensity ratio of the two emission bands, I_{N^*}/I_{T^*} . In contrast, introduction of a 7-methoxy group results in exactly the opposite spectroscopic effects. All the studied 3HC dyes demonstrate a linear increase in $\ln(I_{N^*}/I_{T^*})$ with the solvent polarity parameter $E_T(30)$. Substitution of 2-phenyl for 2-(2-benzo[*b*]furanyl) or introduction of electron donors on the 2-aryl group in 3HC increases the sensitivity of their I_{N^*}/I_{T^*} ratio to solvent polarity and shifts the optimal range of ratiometric polarity sensing to less polar solvents. The opposite effects are observed for 7-methoxy derivatives. These results allow a new generation of two-band fluorescent sensors based on 3HC that operate by the ESIPT mechanism to be proposed. By proper substituents their photophysical and sensing properties can be tuned over broad ranges.

Introduction

3-Hydroxychromone (3HC) derivatives are very interesting compounds because they exhibit an excited state intramolecular proton transfer (ESIPT) reaction in such a manner that the emission of two excited state species, reactant and reaction product, can be observed simultaneously.¹ This results in two emission bands, well-separated on the wavelength scale. The band in the blue region originates from the normal excited state (N^*) and the other, shifted dramatically to the red, arises from an excited tautomer state (T^*). The spectroscopic behavior of these bands as well as the interplay of their intensities depends strongly on the structure of the 3HC chromophore and the parameters of its microenvironment, which suggests a variety of high technology applications. The most extensively studied representative of this family is 2-phenyl-3-hydroxychromone (**1**; 3-hydroxyflavone, 3HF). It was applied as a laser dye with a large Stokes' shift^{1b} and as a fluorescence probe sensitive to hydrogen bonding perturbations^{1c} in proteins,²

micelles³ and polymers.⁴ 4'-Dialkylamino-3-hydroxyflavones (a typical example is flavone **3**, Chart 1), due to their more advanced fluorescence properties,⁵ recently aroused the interest of researches as two-band fluorescent dyes with a ratiometric response to different perturbations. They were proposed as sensors of ions⁶ and electric fields.⁷ As microenvironment-sensitive probes they were applied in micelles,⁸ model and cell membranes,⁹ and protein molecules.¹⁰

Meantime, a wider application of 3HC derivatives as probes in molecular and cellular biology and in nanoscale sensor technologies has been limited so far due to several important problems. One of them is the need to improve and optimize for particular applications their spectroscopic properties. The absorption maximum, in the near UV region for 3HF, should be shifted to longer wavelengths and the fluorescence quantum yield has to be increased. To resolve this problem the 2-phenyl group of 3HF was substituted with benzo and naphthofuryl groups,¹¹ and, finally, a dialkylamino group was introduced at the 6' position of 2-(2-benzo[*b*]furanyl)-3-hydroxychromone

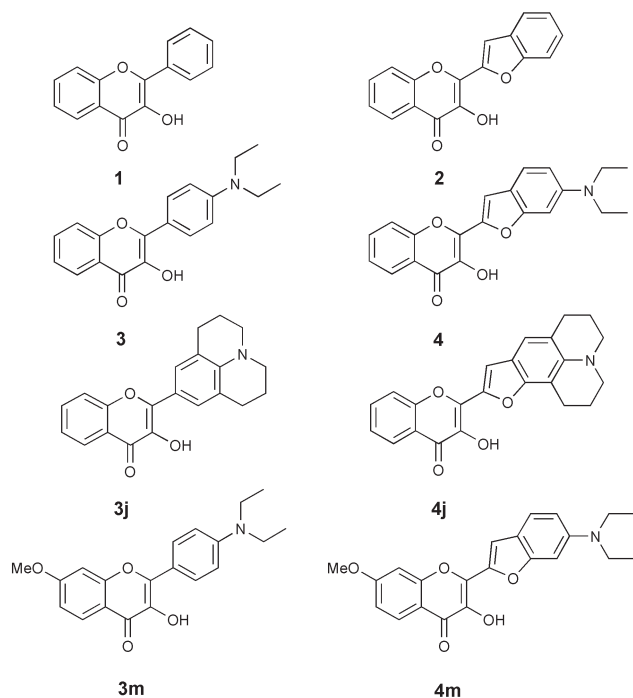


Chart 1 Chemical structures of the studied chromones.

(2), resulting in the development of chromone **4**. The latter shows superior absorption and fluorescence properties to those reported to date for 3HC derivatives.¹² In order to obtain a further improvement of these properties, a more detailed study of an extended range of 3HC derivatives has to be undertaken and some general relationships need to be derived.

The other problem is that the ESIPT behavior, which controls the redistribution of intensities between the two emission bands, I_{N^*}/I_{T^*} , still cannot be predicted and modulated in the desired range. This is the problem of major interest, because its solution can provide a clue for designing new molecular sensors and switches with a dramatic two-band fluorescence ratiometric response allowing fluorescent imaging in real color. The literature data show that the presence of the electron-donating 4'-dialkylamino group in 3HF increases the I_{N^*}/I_{T^*} ratio dramatically.^{5c} Even stronger augmentation of the I_{N^*}/I_{T^*} ratio was recently reported for dialkylamino-substituted chromone **4** with respect to parent compound **2**.¹² However, no systematic data on the effect of electron-donor substitutions at different positions of the 3HC chromophore on the I_{N^*}/I_{T^*} ratio are presently available.

Regarding solvent effects, a very strong perturbation of the spectroscopic behavior has been reported for parent 3HF **1** by intermolecular H-bonding with protic solvents^{1e} and for dialkylamino-substituted 3HF **3** by the increase of solvent polarity.⁵ In both cases, the I_{N^*}/I_{T^*} ratio can be increased dramatically, up to complete disappearance of the T^* emission band. As it was recently shown the I_{N^*}/I_{T^*} ratio of **3** on the logarithmic scale increases linearly with solvent polarity as a function of dielectric constant.¹³ A similar effect was reported for chromone **4**^{14a} with respect to the solvent polarity parameter $E_T(30)$.^{14b} In this respect, several other examples can be found in the literature on dyes exhibiting the ESIPT reaction and showing the possibility of solvent dual emission.¹⁵ Meanwhile, the presence of the N^* band in their emission is associated with their conformational isomerization and disruption of their intramolecular hydrogen bonds. Therefore, the connection between the I_{N^*}/I_{T^*} ratio of these dyes and solvent polarity is not straightforward. In 3HCs 3-OH and 4-carbonyl groups are members of the same chromone heterocycle and therefore conformational changes are not involved in their ESIPT reaction coordinate. The N^* band in 3HCs can appear

in aprotic solvents as a result of dielectric stabilization of the N^* state, which due to its charge transfer character may possess a larger dipole moment than the T^* state.^{5,13}

These features suggest that 3HCs may act as unique two-band ratiometric polarity sensors with extremely high sensitivity. In order to design efficient molecular sensors based on 3HC it is primarily needed to resolve the question of how the structure of 3HC derivatives determines their spectroscopic and sensing properties. In this work, we provide comparative solvatochromic studies of known and newly synthesized 3HC derivatives (Chart 1). We demonstrate that, by providing proper substitutions in the chromophore system, there is the possibility of driving the spectroscopic properties in the desired direction (longer wavelengths of light absorption and emission, higher quantum yield) and of optimizing the two-color ratiometric response to the selected range of solvent polarity.

Experimental

Instrumentation

Melting points of synthesized compounds were determined on a Büchi 512 melting point apparatus and are presented as uncorrected values. Microanalyses were performed with a Carlo Erba 1106 Elemental Analyzer. Proton NMR spectra were recorded at 200 MHz on a JEOL PMX 270 MHz spectrometer. Tetramethylsilane (TMS) was used as the internal standard in all NMR spectra run in $CDCl_3$ or $[D_6]DMSO$. Mass spectra were recorded on a Kratos MS-25 mass spectrometer using EI or FAB methods. All column chromatography was performed on silica gel (Merck, Kieselgel 60H, Art 7736). Absorption spectra were recorded on a Cary 3 Bio spectrophotometer (Varian). Fluorescence spectra were recorded on a Quanta Master spectrofluorometer (Photon Technology International).

Reagents and solvents

All the reagents were purchased from Aldrich-Sigma Chemical Company. Solvents for synthesis were of reagent quality and were appropriately dried if necessary. For absorption and fluorescence studies the solvents were of spectroscopic grade. Flavone **1** was purchased from Aldrich Chemical Company. Chromones **2** and **3** were prepared as described elsewhere.^{5b,16}

6-Diethylaminobenzo[b]furan-2-carbaldehyde (5). To 11.5 mmol of 3-diethylaminophenol in 10 ml of dry THF, 13.8 mmol of NaH (55% in paraffin) was slowly added while stirring. The solvent was evaporated and to the resultant mixture dissolved in 8 ml of DMSO, 14.0 mmol of bromoacetaldehyde diethylacetal and 11.5 mmol of KI were added. After heating at 50 °C for 3 h, water was added. The product, *N,N*-diethyl-3-(2,2-diethoxyethoxy)aniline, was extracted with benzene, purified on silica gel column (hexane–ethylacetate, 4:1), and used directly for the next step. Yield was 90%.

To 26.9 mmol of $POCl_3$, cooled in an ice bath, 8.5 mmol of DMF was added, while stirring. Then, 7.1 mmol of *N,N*-diethyl-3-(2,2-diethoxyethoxy)aniline was added and stirring was continued for 14 h at 50 °C. The mixture was poured into ice, neutralized and extracted with ethylacetate. The product **5** was purified on silica gel column (hexane–ethylacetate, 4:1). Yield 21%. IR ν 1655 cm^{-1} (HC=O); 1H NMR (200 MHz, $CDCl_3$) δ 1.21 (6H, t, J 7.1 Hz), 3.33 (3H, q, J 7.1 Hz), 6.70 (1H, d, J 2.3 Hz), 6.76 (1H, dd, J 8.8, 2.3 Hz), 7.40 (1H, s), 7.49 (1H, d, J 8.8 Hz), 9.61 (1H, s); EI-MS m/z 217.1 (M^+), 202.1, 193.1, 174.0, 159.0, 145.0, 118.0, 89.0.

2,3,6,7-Tetrahydro-1*H*,5*H*-furo[2,3-*f*]pyrido[3,2,1-*ij*]quinoline-10-carbaldehyde (6). To 11.5 mmol of 9-formyl-8-hydroxyjulolidine in 10 ml of dry THF, 13.8 mmol of NaH (55% in

paraffin) was slowly added while stirring. The solvent was evaporated and to the resultant mixture dissolved in 8 ml of DMSO, 14.0 mmol of bromoacetaldehyde diethylacetal and 11.5 mmol of KI were added. After heating at 60 °C for 24 h, water was added. The product, 8-(2,2-diethoxyethoxy)-2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinoline-9-carbaldehyde, was extracted with ethyl acetate, purified on silica gel column (dichloromethane–ethylacetate, 10:1) and used directly for the next step. Yield was 60–70%.

The latter product was kept in an excess of 50% sulfuric acid at 100 °C for 4 h affording 2,3,6,7-tetrahydro-1*H*,5*H*-furo[2,3-*f*]pyrido[3,2,1-*ij*]quinoline-10-carbaldehyde (**6**), pure enough for the next step. Yield 20%. ¹H NMR (200 MHz, CDCl₃) δ 2.00 (4H, m), 2.83 (2H, t, *J* 6.3 Hz), 2.98 (2H, t, *J* 6.5 Hz), 3.26 (4H, m), 7.08 (1H, s), 7.33 (1H, s), 9.57 (1H, s); EI-MS *m/z* 241.1 (M⁺), 212.0, 184.1

General strategy and modified general procedure for the synthesis of new 3-hydroxychromones

The common two-step procedure for the preparation of 3-hydroxychromones¹⁷ was found to be inefficient for the synthesis of new 3HC derivatives having electron-donor groups, **3m**, **3j**, **4m** and **4j**. Its first step, condensation of the corresponding aldehydes with 2-hydroxyacetophenones, which requires sodium hydroxide in aqueous ethanol, takes place for several weeks without complete transformation. Then, oxidative heterocyclization of the obtained 1-(2'-hydroxyphenyl)-2-propene-1-ones (Algar–Flynn–Oyamada reaction^{17a}) with hydrogen peroxide and sodium hydroxide in aqueous ethanol provides only traces of the target chromones. Therefore, for both of the steps we applied a recently developed modified procedure.¹⁸

2-Hydroxyacetophenones were condensed with the appropriate aldehydes in the presence of sodium methoxide in DMF for 1–6 h. The mixture was diluted with ethanol, then, subsequently, 20 mol excess of sodium methoxide and 15 mol excess of 30% hydrogen peroxide were added. The mixture was refluxed for less than 1 min, cooled to room temperature and poured into water. After neutralization with diluted HCl, the resultant precipitate was filtered. The target chromones **3m**, **3j**, **4m**, **4** and **4j** were purified by recrystallization or column chromatography. The obtained 3HCs were pure according to thin-layer chromatography and ¹H NMR spectra.

4'-Diethylamino-3-hydroxy-7-methoxyflavone (3m). Prepared from 2-hydroxy-4-methoxyacetophenone and 4-diethylaminobenzaldehyde. Crystallized from ethanol; yield 36%; mp 172 °C. UV (ethanol) ε(406 nm) 42 000 l mol⁻¹ cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 1.22 (6H, t, *J* 7.0 Hz), 3.44 (4H, q, *J* 7.0 Hz), 3.92 (3H, s), 6.76 (2H, d, *J* 9.1 Hz), 6.87 (1H, s), 6.94 (1H, s), 6.96 (1H, d, *J* 6.6 Hz), 8.10 (1H, d, *J* 6.6 Hz), 8.12 (2H, d, *J* 9.1 Hz); EI-MS *m/z* 339.1 (M⁺), 324, 295.

3-Hydroxy-2-(2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinolin-9-yl)chromone (3j). Prepared from 2-hydroxyacetophenone and 9-formyljulolidine. Yield 35%; purified on silica gel column chromatography (dichloromethane); mp 207 °C. UV (ethanol) ε(429 nm) 41 000 l mol⁻¹ cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 2.00 (4H, m), 2.84 (4H, t, *J* 6.3 Hz), 3.28 (4H, t, *J* 5.7 Hz), 6.88 (1H, s), 7.33–7.41 (1H, m), 7.54–7.69 (2H, m), 7.75 (2H, s), 8.21 (1H, dd, *J* 8.1, 1.2 Hz); EI-MS *m/z* 333 (M⁺), 304.

2-(6-Diethylaminobenzo[*b*]furan-2-yl)-3-hydroxychromone (4). Prepared from 2-hydroxyacetophenone and aldehyde **5**. Crystallized from acetonitrile; yield 25% mp 226 °C. UV (ethanol) ε(444 nm) 36 000 l mol⁻¹ cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 1.23 (6H, t, *J* 7.1 Hz), 3.45 (4H, q, *J* 7.1 Hz), 6.76 (1H, dd, *J* 8.8, 2.2 Hz), 6.87 (1H, d, *J* 2.2 Hz), 7.38–7.46

(1H, m), 7.48 (1H, d, *J* 8.8 Hz), 7.64 (1H, s), 7.64–7.75 (2H, m), 8.25 (1H, dd, *J* 8.2); EI-MS *m/z* 349.2 (M⁺), 334.1, 305.1, 276.1, 248.1, 167.0.

2-(6-Diethylaminobenzo[*b*]furan-2-yl)-3-hydroxy-7-methoxychromone (4m). Prepared from 2-hydroxy-4-methoxyacetophenone and aldehyde **5**. Crystallized from toluene; yield 15%, mp 214 °C. UV (ethanol) ε(438 nm) 41 000 l mol⁻¹ cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 1.23 (6H, t, *J* 7.0 Hz), 3.45 (4H, q, *J* 7.0 Hz), 3.93 (3H, s), 6.75 (1H, dd, *J* 8.7, 2.2 Hz), 6.93 (1H, d, *J* 2.2 Hz), 6.99 (1H, dd, *J* 8.8, 2.3 Hz), 7.05 (1H, d, *J* 2.3 Hz), 7.47 (1H, d, *J* 8.7 Hz), 7.57 (1H, s), 8.12 (1H, d, *J* 8.8 Hz); EI-MS *m/z* 379.1 (M⁺), 364.1, 355.1, 324.1.

3-Hydroxy-2-(2,3,6,7-tetrahydro-1*H*,5*H*-furo[2,3-*f*]pyrido[3,2,1-*ij*]quinolin-10-yl)chromone (4j). Prepared from 2-hydroxyacetophenone and aldehyde **6**. Crystallized from butanol; yield 26%; mp 295 °C (dec.). UV (ethanol) ε(458 nm) 44 000 l mol⁻¹ cm⁻¹; ¹H NMR (200 MHz, [D₆]DMSO) δ 1.93 (4H, m), 2.79 (2H, t, *J* 7.3 Hz), 2.95 (2H, t, *J* 6.4 Hz), 3.20 (4H, m), 7.11 (1H, s), 4.41–7.49 (1H, m), 7.52 (1H, s), 7.60–7.82 (2H, m), 8.10 (1H, dd, *J* 8.0, 1.1 Hz); EI-MS *m/z* 373.1 (M⁺), 344.1, 316.1

Absorption and fluorescence studies

The solutions of 3HC derivatives for absorption and fluorescence spectroscopy were used in concentrations corresponding to an absorbance close to 0.1. Quantum yields ϕ for **3**, **3m**, **3j**, **4**, **4m** and **4j** were determined with respect to a solution of **3** in ethanol as the reference ($\phi = 0.52$).^{5b} Excitation wavelength for the fluorescence studies was 350 nm for **1** and **2** and 420 nm with all other dyes. All the spectroscopic data for compounds **3** and **4** reported previously¹² were reproduced and corrected. Deconvolution of fluorescence spectra in the cases when the two bands were overlapped was made using the program Siano, kindly provided by the author (Dr. A.O. Doroshenko from Karazin University, Kharkov, Ukraine). The program uses an iterative non-linear least-squares method based on the Fletcher–Powell algorithm. The shapes of individual emission bands were approximated by a log-normal function,¹⁹ which accounts for the asymmetry of the spectral bands.

Results

Absorption and fluorescence properties of the new 3-hydroxychromones were studied in six solvents of different polarity: hexane, toluene, ethyl acetate, chloroform, acetonitrile and ethanol. We found that all the new compounds exhibit two solvent-dependent bands in the fluorescence spectra, which is a characteristic feature of 3-hydroxychromones.^{1,5} Comparative studies of the 3HC derivatives reveal the key relationships between the chromophore structure and its spectral properties.

Effects of chromophore extension

2-(Benzo[*d*]furan-2-yl)-3-hydroxychromones (benzofurylchromones, BF-3HCs) **2**, **4**, **4j** and **4m** exhibit absorption spectra shifted to longer wavelengths in comparison with their 2-phenyl analogs (flavones, 3HFs) **1**, **3**, **3j** and **3m**, respectively (Fig. 1).

In the fluorescence spectra the extension of conjugation by one furan heterocycle also results in red shifts of the N* and T* bands. These shifts are much stronger for dialkylamino-substituted chromones in comparison to their non-substituted analogs (Table 1). Thus, in chloroform, compound **4** exhibits N* and T* bands shifted to the red, with respect to **3**, by 2440 and 1750 cm⁻¹, respectively, while for **2**, with respect to **1**, the corresponding shifts are only 660 and 710 cm⁻¹.

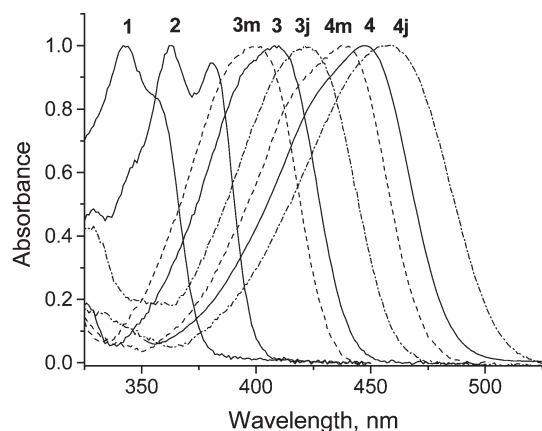


Fig. 1 Normalized absorption spectra of the studied 3-hydroxychromones in toluene.

The intensity ratio of these bands, I_{N^*}/I_{T^*} , which is an important characteristic of the ESIPT reaction, is also affected dramatically by the nature of the 2-aryl substituent. In all the studied solvents, BF-3HCs **2**, **4**, **4j** and **4m** exhibit much higher I_{N^*}/I_{T^*} ratios than the corresponding flavones **1**, **3**, **3j** and **3m** (Table 1), so that the ESIPT reaction is depressed for the former. This effect is especially strong for dialkylamino-substituted chromones. Whereas for **2**, I_{N^*}/I_{T^*} is less than 2 times higher than that of **1**, it becomes 8–15 times higher for **4**, **4j** and **4m** in comparison with corresponding dyes **3**, **3j** and **3m**.

For BF-3HCs we observe also a significantly increased fluorescence quantum yield in most of the studied solvents. Usually it is 2–5 times higher than that of the corresponding flavones (Table 1).

Effects of different electron donors

The newly synthesized dyes allow comparison of spectroscopic effects produced by introduction of electron-donor groups on

opposite sides of the 3HC molecule. Chromones **3** and **4** with an introduced diethylamino group, show absorption spectra strongly shifted to longer wavelengths with respect to their non-substituted analogs **1** and **2** (Fig. 1). Introduction of a diethylamino group at the 6' position of chromone **2** results in a red shift of the absorption maximum by 78–88 nm (4800–5320 cm^{-1}). When this group is introduced at the 4' position of 3-hydroxyflavone **1**, this shift is somewhat smaller, 61–69 nm (4470–4860 cm^{-1}). Moreover, chromones **3j** and **4j** with stronger π -electron donation from the nitrogen fixed in the julolidine cycle show an additional red shift of the absorption maxima with respect to the corresponding diethylamino derivatives **3** and **4** (Table 1). In contrast, chromones **3m** and **4m** with the introduced electron-donor methoxy group at the 7 position show absorption spectra shifted to the blue (Fig. 1).

Similarly to the shifts in absorption spectra, the shifts of both the N^* and T^* bands in emission depend strongly on the nature and position of the electron-donor substituents in 3-hydroxychromones. For both chromone series, **1-3j** and **2-4j**, an increase of electron donation on the 2-aryl side results in sequential red shifts of the emission bands (Table 1). It is stronger in the case of the N^* band compared to the T^* band. Introduction of the electron-donor methoxy group on the opposite side of the 3-hydroxychromone moiety at the 7 position results for flavone **3m** in a very uncommon observation: shifts of the N^* and T^* bands in opposite directions (Fig. 2). This increases the separation between these bands by 12–15 nm (450–630 cm^{-1}). In the case of **4m**, the introduced methoxy group affects mainly the N^* band, shifting it to the blue by 8–16 nm (300–490 cm^{-1}), while the T^* band does not undergo any considerable and systematic shifts in the different studied solvents (Table 1).

A particularly strong effect of the electron-donor groups is observed on the fluorescence intensity ratio, I_{N^*}/I_{T^*} . Chromones **3** and **4**, compared to the corresponding parent compounds **1** and **2**, show dramatically increased I_{N^*}/I_{T^*} ratios, by 84 and 210-fold, respectively (Table 1). It can be noted that in the case of BF-3HC **4**, the effect of introduction of the

Table 1 Spectroscopic properties of the studied 3-hydroxychromones^a

Solvent	$E_T(30)$	3HF	$\lambda_{\text{max}}^{\text{abs}}/\text{nm}$	$\lambda_{\text{max}}^{N^*}/\text{nm}$	$\lambda_{\text{max}}^{T^*}/\text{nm}$	I_{N^*}/I_{T^*}	% ϕ	BF-3HC	$\lambda_{\text{max}}^{\text{abs}}/\text{nm}$	$\lambda_{\text{max}}^{N^*}/\text{nm}$	$\lambda_{\text{max}}^{T^*}/\text{nm}$	I_{N^*}/I_{T^*}	% ϕ
Hexane	31.0	3m	394	—	562	—	28	4m	431	450	588	0.053	29
		3	403	425	553	0.010	14	4	439	457	583	0.078	20
		3j	414	440	564	0.021	13	4j	451	480	602	0.247	23
		1	343	—	527	—	29 ^b	2	364	406	547	0.008	41
Toluene	33.1	3m	400	447	572	0.018	30	4m	439	498	610	0.242	30
		3	408	456	566	0.044	14	4	446	508	613	0.550	26
		3j	422	480	582	0.174	17	4j	457	532	634	1.75	44
		1	340	—	529	—	3.6	2	358	410	545	0.029	17
EtOAc	38.1	3m	394	464	573	0.062	11	4m	429	524	618	0.914	30
		3	401	475	570	0.253	5.0	4	436	537	623 ^c	2.67 ^c	26
		3j	415	507	586	1.01	11	4j	446	567	655 ^c	10.8 ^c	35
		1	344	404	521	0.008	19	2	365	415	541	0.020	36
CHCl_3	39.1	3m	407	475	566	0.252	24	4m	445	535	616	1.58	46
		3	413	481	560	0.669	19	4	453	545	621 ^c	4.2 ^c	46
		3j	430	511	579	1.71	50	4j	468	578	—	—	38
		1	339	394	525	0.028	5.1	2	358	418	542	0.040	17
CH_3CN	45.6	3m	397	498	577	0.350	9.0	4m	431	566	643 ^c	8.7 ^c	34
		3	404	509	571	1.30	9.0	4	437	582	—	—	25
		3j	420	541	598 ^c	—	32	4j	450	618	—	—	5.0
		1	343	402	532	0.270	2.9	2	366	425	543	0.220	15
EtOH	53.7	3m	406	515	568 ^c	4.56 ^c	50	4m	438	590	—	—	22
		3	412	523	—	—	52 ^d	4	444	600	—	—	9.0
		3j	429	552	—	—	12	4j	458	626	—	—	2.0

^a $\lambda_{\text{max}}^{\text{abs}}$ is the position of the absorption maximum, $\lambda_{\text{max}}^{N^*}$ and $\lambda_{\text{max}}^{T^*}$ are the positions of the fluorescence maxima of the N^* and T^* forms, respectively, ϕ is the fluorescence quantum yield. Data on ϕ for **1** and **2** are from Ref. 20. ^b Ref. 5c. ^c The value was evaluated from the results of the deconvolution. ^d Ref. 5b.

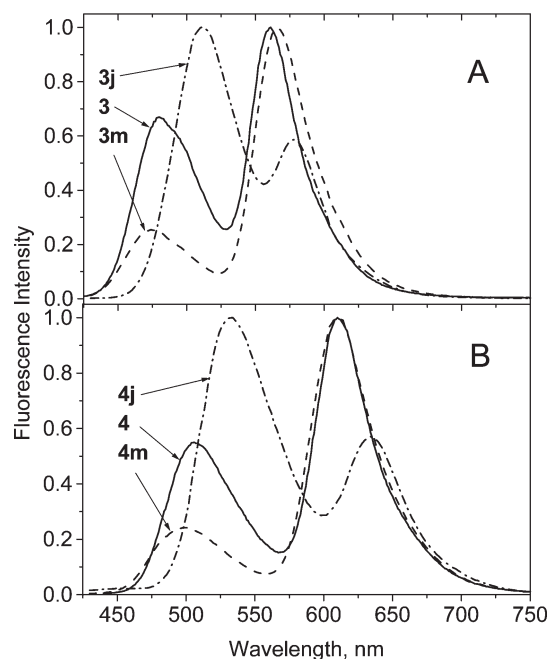


Fig. 2 Fluorescence spectra of the studied flavones **3**, **3j** and **3m** in chloroform (A) and benzofurylchromones **4**, **4j** and **4m** in toluene (B). Excitation wavelength of 420 nm.

diethylamino group is stronger than that for flavone **3**. Fixing the amino group in chromones **3j** and **4j** results in additional growth of I_{N^*}/I_{T^*} , up to 4-fold (Table 1), which can be due to a further increase of the electron donation. The opposite effect is observed on introduction of a methoxy group on the opposite side of the chromophore, at the 7 position in chromones **3m** and **4m**; the I_{N^*}/I_{T^*} ratio decreases by 4 and 3 times, respectively (Fig. 2).

The presence of an electron-donor group exerts a significant influence on fluorescence quantum yield. However, the magnitude and direction of the effect depend strongly on the nature of the solvent. The general trend observed is higher quantum yields of **3m** and **4m** in most of the studied solvents (Table 1).

Solvent effects

The correlation of spectroscopic effects with the solvent polarity was established based on the most popular empirical polarity scale, $E_T(30)$, which is based on the solvatochromic shifts of betaine dye.^{14b} According to our data, the absorption spectra of all studied chromones are almost independent of solvent polarity, and only in the case of the BF-3HCs **4**, **4j** and **4m** was a small positive solvatochromy detected (Table 1). This is in line with the recent detailed solvatochromic data on **3**, showing that its absorption maximum is almost polarity insensitive.¹³

In contrast, fluorescence spectra show a clear dependence on solvent polarity and this property is strongly dependent on the nature of the 2-aryl group. For the parent 3-hydroxyflavone **1**, both emission bands do not reveal any solvent-dependent shifts (Fig. 3, Table 1). For non-substituted BF-3HC **2**, the N^* band is already solvent polarity dependent, while its T^* band is not. The introduction of a diethylamino group results in a dramatic increase of the solvent dependence of the N^* band in the compounds of both the flavone and BF-3HC series. This effect is much stronger for the N^* band, which demonstrates a gradual shift to the red with an increase in polarity (Fig. 3). The T^* band positive solvatofluorochromy is considerable only for the BF-3HCs (Table 1).

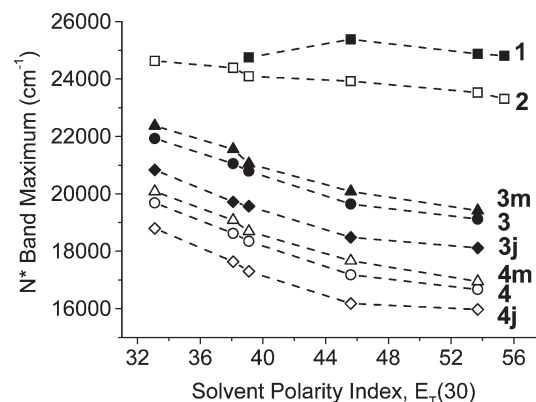


Fig. 3 Positions of N^* band maxima of the studied chromones as a function of the solvent polarity index $E_T(30)$. The solvents are toluene, ethyl acetate, chloroform, acetonitrile, ethanol and methanol.

The common feature of all studied 3-hydroxychromones is the high sensitivity of the intensity ratio of the emission bands, I_{N^*}/I_{T^*} , to solvent polarity (Table 1). The logarithm of this ratio shows an almost linear increase with solvent polarity for all studied 3HCs with relatively good correlation coefficients of the linear fits (Table 2). For dialkylamino-substituted 3HCs this is demonstrated in Fig. 4. A considerable and systematic deviation from this linearity is observed only for the dialkylamino-substituted 3HF dyes in chloroform, the solvent with a tendency for specific H-bond interactions, resulting in an increase of its apparent polarity. Interestingly, this deviation is the strongest for **3m** and negligible for **3j** (Fig. 4).

It is remarkable that the level of sensitivity to the polarity of the I_{N^*}/I_{T^*} ratio is also determined by the chromone structure. The slopes of the linear fits for BF-3HCs **4**, **4j** and **4m** are steeper than those for the corresponding flavones **3**, **3j** and **3m**, representing a higher sensitivity to solvent polarity (Table 2). Furthermore, the electron-donor substituents can modulate this sensitivity. For both chromone series, **1-3-3j** and **2-4-4j**, an increase of electron donation at the 2-aryl side increases the sensitivity of I_{N^*}/I_{T^*} to solvent polarity (Table 2). Meanwhile, for methoxy-substituted **3m** and **4m**, it is lower in comparison to the corresponding parents **3** and **4**.

In both series of compounds the fluorescence quantum yield ϕ exhibits broad variations. For the 3HF derivatives **3** and **3m**, the ϕ values follow a similar trend, being low in acetonitrile and high in ethanol (Table 1). BF-3HCs **2** and **4** exhibit relatively high ϕ values (18–44%) in all studied solvents. Meanwhile, **4j**, with the strongest electron-donor substituent, demonstrates in highly polar solvents a dramatic ϕ decrease, from 44% in toluene down to 2% in ethanol (Table 1). A similar, though less dramatic ϕ drop is observed for compound **4**. It is interesting to note that the introduction of the methoxy group always increases the ϕ values and makes them substantially less solvent-dependent for both flavone **3m** and BF-3HC **4m** (Table 1).

Table 2 Data obtained from linear fits of $\ln(I_{N^*}/I_{T^*})$ vs. $E_T(30)^a$

3HF	Slope	$\Omega_{E(30)}$	r	BF-3HC	Slope	$\Omega_{E(30)}$	r
1	0.260	59.0	0.996	2	0.170	61.4	0.972
3m	0.250	48.4	0.885	4m	0.400	38.0	0.994
3	0.341	43.0	0.949	4	0.475	35.9	0.991
3j	0.524	37.9	0.992	4j	0.525	33.0	0.992

^a The slope is that of the corresponding linear fit. $\Omega_{E(30)}$ is the $E_T(30)$ index of a virtual solvent, in which the two emission bands are equal in intensity. r is the correlation factor of the linear fit.

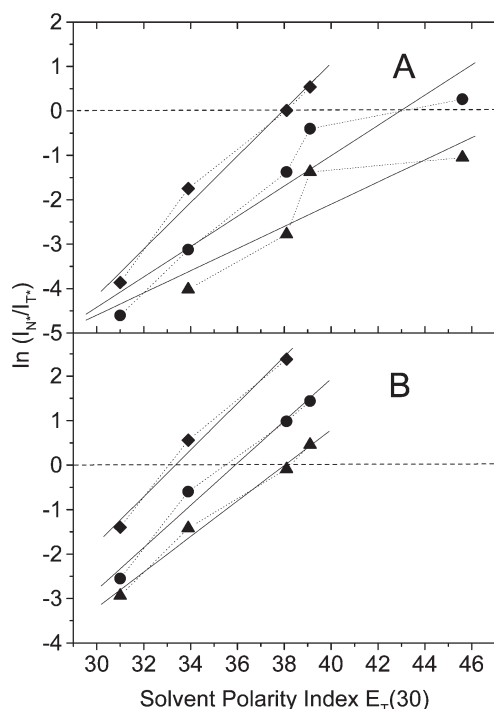


Fig. 4 Dependence of the spectral parameter $\ln(I_{N^*}/I_{T^*})$ of the studied 3-hydroxychromones on the solvent polarity index $E_T(30)$. (A) flavones **3** (●), **3j** (◆) and **3m** (▲); (B) benzofurylchromones **4** (●), **4j** (◆) and **4m** (▲). The solvents are hexane, toluene, ethyl acetate, chloroform and acetonitrile. The straight lines correspond to linear fits of the data. Their crossing with the horizontal dashed line that corresponds to $\ln(I_{N^*}/I_{T^*}) = 0$ determines $\Omega_{E(30)}$ as the point of equality in the intensity of the N^* and T^* bands on the polarity scale.

Discussion

The spectral shifts

We observe that the positions of the absorption bands depend strongly on the length of the chromophore and on the excited state electron-donor and -acceptor ability of the groups. In 3HC derivatives the electron-acceptor group is 4-carbonyl, while the role of the electron donor can be played by the 1-oxygen heteroatom or 2-aryl group. Participation of the 2-aryl group in electronic excitation can be observed by a comparison of the two studied series of compounds, 3-hydroxyflavones (3HFs) and 2-benzofuryl-3-hydroxychromones (BF-3HCs). Since benzofuryl in comparison to phenyl has a higher π -electron density and a stronger electron-donor ability, the absorption and emission spectra of BF-3HCs are shifted to the red. The other factor that may contribute to the observed red shift is the smaller size of the five-membered furan ring in comparison to the six-membered benzene ring, which can allow a more planar orientation with respect to the 3HC system resulting in a better conjugation.²⁰ The introduction of an electron-donor diethylamino group in the 2-aryl group of 3HC should increase the charge transfer character of the excited state, which explains the strong red shift in absorption and emission spectra of **3** and **4** with respect to **1** and **2**. Fixing the amino group into the julolidine cycle results in an additional red shift in absorption and emission spectra. This effect is opposite to that expected from twisted intramolecular charge transfer (TICT), which involves rotation of the dialkylamino group. The latter was proposed previously for 4'-dimethylamino-3-hydroxyflavone, an analog of **3**.^{5c} Our results show that TICT probably does not take place in chromones **3** and **4** and the most important factor determining the spectroscopic properties of **3j** and **4j** is the increase of electron donation.

The blue shift of absorption and N^* emission bands observed with the introduction of the 7-methoxy group can

be explained by the decrease in π -electron-acceptor ability of the chromone part. Thus, the effect of electron donation from the 7 position on the absorption and emission spectra in 3HC chromophore is the opposite of that at the 2 position.

Importantly, the electron-donor substituents at both sides of the chromophore affect more strongly the position of the N^* emission band as compared to that of the T^* band. This means that increase or decrease of the charge transfer character of the excited state due to substituents stabilizes or destabilizes, respectively, the N^* state with respect to the T^* state, which should substantially affect the ESIPT reaction.

We can analyze the factors that make 3HCs strongly solvatochromic. The small solvent dependence of the emission maxima observed for non-substituted 3HCs **1** and **2** shows that their dipole moments do not increase significantly on electronic excitation.²¹ The N^* band becomes strongly solvent dependent with introduction of dialkylamino substituents, which increases the charge-transfer character and, correspondingly, the dipole moment of the N^* state. The latter is supported by the recent data on direct measurements of dipole moments of 4'-dialkylamino-substituted 3HF.²² Our results show also that the charge separation of the T^* state is small and it is not influenced significantly by the provided substitutions.

The ESIPT modulation

The ESIPT rates in both parent 3HF^{1,23} and its 4'-diethylamino-substituted analog **3j** are much higher than the rates of emission. This allows us to assume that in 3HC dyes the major part of the emission proceeds from both N^* and T^* states when they reach dynamic equilibrium.^{5a} Based on this consideration, the intensity ratio of the two emission bands can be correlated with the relative populations of the states at equilibrium, which is a function of the relative energies of the corresponding states. This is in accordance with the recent spectroscopic data on dyes **3** and **4**.^{13,24} As it follows from the data on spectral shifts, the increase in the size and electron-donor ability of the 2-aryl π -electron system in the 3HC chromophore results in selective stabilization of the N^* state. This increases the population of this state with respect to the T^* state and, therefore, increases the relative intensity of the N^* band in emission. Indeed, the red shifts of the N^* band observed as a result of these modifications are always accompanied by an increase in the fluorescence intensity ratio, I_{N^*}/I_{T^*} (Table 1). Meanwhile, the electron-donor methoxy group at the 7 position, which destabilizes the N^* state, shifting its band to the blue for both **3m** and **4m**, produces the opposite effect—a decrease of the I_{N^*}/I_{T^*} ratio (Fig. 3). Thus, the ESIPT reaction in 3HC system can be modulated in opposite directions by π -electron donation from the two opposite sides of the chromophore.

With the increase of solvent polarity, the same correlation is observed: the increase in the I_{N^*}/I_{T^*} ratio is always accompanied by a red shift of the N^* band (Table 1). Thus, the increase of solvent polarity results in selective dielectric stabilization of the N^* state with respect to the T^* state, which increases its relative population and, therefore, the relative intensity of the corresponding band.

The observed effects of chemical substituents and solvent polarity reveal the essential common features, which suggests that ESIPT in both of these cases is controlled by the same mechanism—a change in the relative energies of the N^* and T^* states.

The quantum yields

The fluorescence quantum yield ϕ of 3-hydroxychromones is known to vary significantly from solvent to solvent.^{1,5c,20} This is especially characteristic for 3-hydroxyflavones, the ϕ values of which do not follow a simple dependence on solvent

polarity but are mostly influenced by specific solute-solvent interactions.^{13,20} In contrast, BF-3HCs show relatively higher ϕ values in most of the tested solvents and their solvent-dependent variations are smaller.^{11,14a} For the BF-3HCs presented in this work, the ϕ values are also high in most of the studied solvents (Table 1). The dramatic decrease in quantum yield of the 3HF **1** in hydrogen-bond-acceptor solvents has recently been proposed to be a result of hydrogen bonding of the 3-hydroxy group with solvent molecules, which can produce additional distortion of the planarity of 2-phenyl with respect to the 3HC moiety.²⁰ Similar ϕ variations are observed for flavones **3** and **3m**, with the exception of **3j**, which possesses the strongest electron-donor group in the 4' position. In the case of BF-3HCs, these variations are much less pronounced, which is probably due to the smaller size of the furan heterocycle, which does not allow a significant distortion of the planarity by the solvent.

The pronounced ϕ decrease for **3j**, **4** and **4j** in the highly polar solvent ethanol may be connected with the strong electronic asymmetry in their excited states and their strong solvation with the formation of electron traps.²⁵ This effect is most clearly observed in the case of **4j**, which possesses the strongest electron donor and the most extended conjugation. Therefore, the substituent that restores the π -electronic symmetry can provide an increase in the ϕ value, which is exactly what is observed with the introduction of the 7-methoxy group.

Solvent polarity sensing

Polarity is a simple integrating characteristic of weak non-covalent interactions in the condensed phase, which may be an important characteristic of unknown media on the molecular dimension scale. Before addressing the problem of polarity sensing in these media,^{14b} prospective fluorescent sensors are often tested for their sensitivity to solvent polarity. The linear dependence of $\ln(I_{N^*}/I_{T^*})$ on the solvent polarity index $E_T(30)$, previously reported for BF-3HC **4**,^{14a} and demonstrated presently for all the studied 3HCs, is a very important property of these dyes, making them prospective polarity sensors with the possibility to perform quantitative measurements. In the analysis presented below we use the solvent polarity scaling as the tool for evaluating the sensitivity of our new dyes to external perturbations and for deriving the general rules of their response.

The slope of the linear fit of $\ln(I_{N^*}/I_{T^*})$ vs. $E_T(30)$ may serve as an important parameter to characterize the sensitivity of the two-band ratiometric response to solvent polarity. The results presented in Fig. 4 show that both the substitution of 2-phenyl with 2-benzofuryl and the introduction of electron donors at 2-aryl makes the 3HC chromophore more sensitive to polarity. This probably reflects a stronger increase in dipole moment of the N^* state compared to the T^* state. In this respect, it is remarkable that the 7-methoxy group produces the opposite effect. Thus, the variation of the 2-aryl group and the introduction of electron-donor substituents on different sides of the 3HC chromophore can provide a fine modulation of dye sensitivity to solvent polarity.

In order to measure the intensity ratio of the two bands most easily and with high precision, these bands have to be of comparable intensity. It is important therefore to specify experimental conditions in which the two N^* and T^* bands are observed in equal intensities. Regarding polarity sensing, we introduce a new parameter, $\Omega_{E(30)}$, the $E_T(30)$ polarity index of such a virtual solvent, in which the two emission bands are equal in intensity. Its values can be obtained by a linear interpolation of the functions presented in Fig. 4 at $\ln(I_{N^*}/I_{T^*}) = 0$. The data of Table 2 show that this parameter depends strongly on the chromone structure. The BF-3HC dyes **4**, **4j** and **4m** exhibit much lower values of $\Omega_{E(30)}$ than the corresponding flavones **3**, **3j** and **3m**. So, their sensing

range is dramatically shifted to lower polarities. Introduction of the diethylamino group and its fixation results in a strong sequential decrease of $\Omega_{E(30)}$. In contrast, the introduction of the 7-methoxy group provides a change in the opposite direction—the point of band equality on the polarity scale is shifted in the direction of higher polarities.

Thus, we demonstrate that the ratiometric sensing effect of chromones presented in this work, due to the variation of substituents, may already cover the whole polarity range of known solvents (Fig. 5). For non-substituted **1** and **2** the range of optimal sensitivity is between ethanol and water. For flavone **3** it is in the range of medium polarity, between ethyl acetate and acetonitrile. For chromone **4**, it is already in the range of low polar solvents, between toluene and ethyl acetate. An additional shift towards lower polarity can be achieved by fixation of the amino group, while the opposite can be achieved by introduction of the 7-methoxy group (Table 2). Since the polarity scale reflects a number of universal and specific solute-solvent interactions,^{14b} these results point to the possibility of adapting the ratiometric response to a particular specific range of interactions that may be required for the design of highly specified sensors.

Conclusions

On the basis of 3-hydroxychromone we suggest a new generation of two-band fluorescence dyes that operate by the ESIPT mechanism, providing high two-band sensitivity to microenvironment polarity. The photophysical and sensing properties of the dyes can be finely tuned by introduction of the appropriate groups at the 2 and 7 positions of 3-hydroxychromone. (a) The absorption and fluorescence spectra can be shifted to longer wavelengths and the I_{N^*}/I_{T^*} ratio can be increased by substitution of 2-phenyl for 2-(2-benzo[*b*]furanyl) and by introduction of an electron donor in the 2-aryl group. The change of spectroscopic properties in the opposite direction can be provided by introduction of an electron-donor methoxy group at the

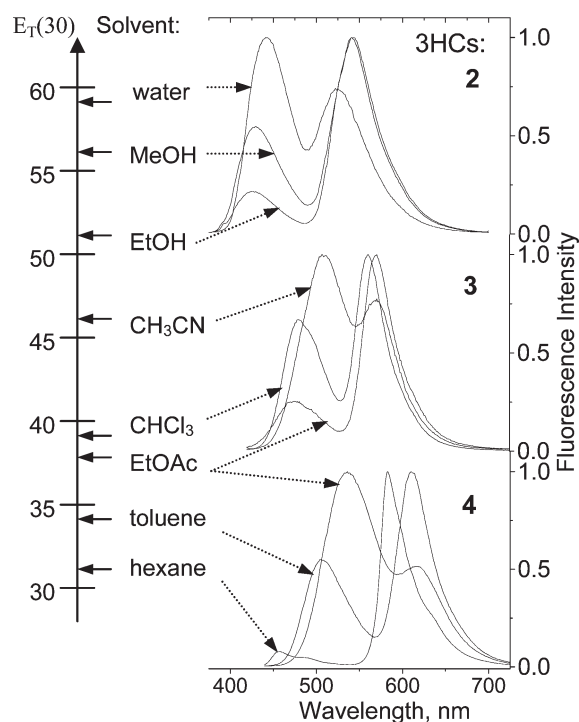


Fig. 5 The two-band fluorescence ratiometric responses of chromones **2**, **3** and **4** adapted for three different ranges of solvent polarity: polar, medium-polar and low-polar, respectively. The spectrum that refers to water was recorded in the presence of 10% methanol. Excitation wavelength of 350 nm for **2** and 420 nm for **3** and **4**.

7 position. The fluorescence quantum yield in 3-hydroxychromones can be increased by substitution of 2-phenyl for 2-(2-benzo[*b*]furanyl) and by introduction of a 7-methoxy group. (b) The two-band sensitivity of 3-hydroxychromones to solvent polarity can be increased and its optimal polarity range can be shifted to less polar solvents by substitution of 2-phenyl for 2-(2-benzo[*b*]furanyl) and by introduction of an electron donor in the 2-aryl group. The change of sensing properties in the opposite direction can be provided by the introduction of the π -electron-donor methoxy group at the 7 position. Thus, we demonstrate that for any particular range of intermolecular interactions (the polarity range from hexane to water) a 3HC derivative can be chosen with optimal color-change sensitivity. These new design possibilities make the studied family of 3-hydroxychromone dyes very attractive as molecular sensors for a wide range of applications in analytical chemistry, colloid chemistry and in material and biological sciences.

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