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7-(2-Methoxycarbonylvinyl)-3-hydroxychromones: new dyes with red shifted dual emission

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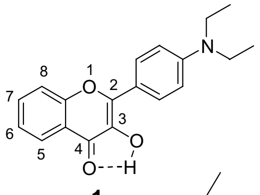
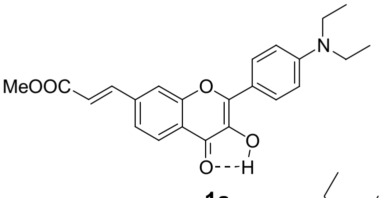
Abstract—7-(2-Methoxycarbonylvinyl)-3-hydroxychromones have been synthesized using Heck coupling reaction from the corresponding 7-bromo-3-hydroxychromones. Introduction of the electron acceptor (2-methoxycarbonylvinyl) group at 7-position of 3-hydroxychromone results in a 30–40 nm red shift in absorption and >50 nm red shifts of both bands in emission. This derivatization allowed us to develop dyes with absorption maxima reaching 480 nm and dual emission in the red region of the spectrum. In comparison to the parent dyes, 7-acryl-3-hydroxychromones demonstrate significantly stronger solvatochromism. This is due to the acceptor group at 7-position, which increases the transfer character of the excited state of the dyes. The new dyes are highly prospective for the development of new fluorescent probes in biological research.

Recently it was demonstrated that 3-hydroxychromones (3HC) are powerful fluorescence probes of intermolecular interactions in condensed media.[1] Due to an excited-state intramolecular proton transfer (ESIPT),[2] these dyes exist in two excited state forms, normal N* and tautomer T*, which emit quanta of different energy resulting in two-band fluorescence.[3] These two bands are highly sensitive to a variety of properties of the microenvironment, which allows the application of these dyes as sensors of polarity,[1,4] and electric fields,[5] and also as probes to study polymers,[6] reverse micelles,[7,8] lipid membranes and proteins.[9–12]

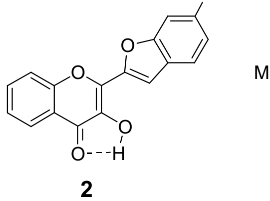
The unique sensing properties of 3HC dyes can be realized in full for studying biological systems only if their absorption and fluorescence properties meet the demands of modern fluorescence microscopy. In this respect, the main disadvantages of the parent 3HC, 3-hydroxyflavone, is its absorption in the ultraviolet, low fluorescent quantum yield and low relative intensity of the N* band with respect to the T* band in most media. These properties were significantly improved with the introduction of a 4′-dialkylmino group (see dye **1**), which shifted the absorption of the dyes up to 400 nm and increased strongly the N* band emission in medium polarity environments.[13,14] We succeeded to improve the fluorescent quantum yield of these dyes by substitution of 2-phenyl with 2-benzofuryl heterocycle.[15,16] Introduction in the latter cycle of electron donor groups (see dye **2**) allowed shifting absorption and emission of the dyes further to the red, to increase the relative intensity of the N* band and to increase the two-band sensitivity of the dyes to solvent polarity.[17,18] In contrast, introduction of an electron donor group (methoxy) from the opposite side of the chromophore resulted in exactly opposite



1



1a



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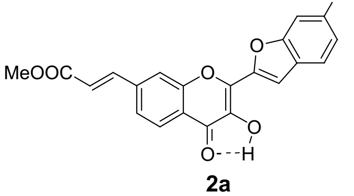

2a

Keywords: 3-Hydroxychromones; Fluorescent dyes; Dual emission; Solvatochromism.

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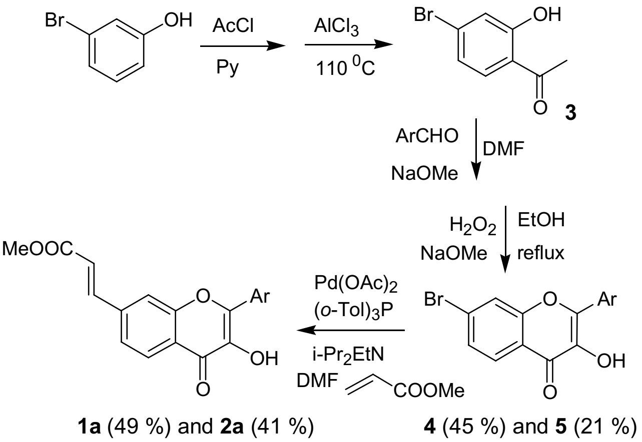
effects.[18] Thus, we have concluded that electron donor groups at the 2-aryl group increases the charge transfer from the 2-aryl to the chromone cycle in the N* excited state of the dyes, while 7-donor group provides the opposite effect, probably by decreasing the electron acceptor properties of the chromone cycle.[18] Therefore, introduction of an electron acceptor group at 7-position could be extremely interesting to provide further enhancement in the charge transfer character of the 3HC dyes.

In the present work we report on the synthesis of 3HC dyes bearing at 7-position an electron acceptor (2-methoxycarbonylvinyl) group (see dyes **1a** and **2a**). Our results show that this group provides dramatic red shifts in the absorption and emission spectra of the dyes, increases the N* band relative intensity and enhances their solvatochromism.

The target 3HC dyes were synthesized in five steps from 3-bromophenol (Scheme 1). The latter was acylated with acetic anhydride in pyridine and the product was converted into 4′-bromo-2′-hydroxyacetophenone **3** in the presence of aluminium chloride at 110 °C. Applying a recently developed procedure[19] based on Algar–Flynn–Oyamada method,[20,21] acetophenone **3** was converted into the corresponding 3HC **4** or **5** in two steps.[22] At the first step, **3** was condensed with 4-(diethylamino)benzaldehyde or 6-(diethylamino)benzo[*b*]furan-2-carbaldehyde[17] in dry DMF in the presence of 3 mol excess of sodium methoxide. On the next step the reaction mixture was diluted with two volumes of ethanol followed by addition of 12 mol excess of sodium methoxide and 10 mol excess of 35% hydrogen peroxide. After reflux for 3–5 min, the mixture was poured into ice, neutralized and the precipitate of the corresponding product **4** or **5** was filtered. The purified **4** or **5**[22] was converted into corresponding **1a** or **2a** by palladium-catalyzed Heck coupling reaction with methyl acrylate (Scheme 1). Solution of 100 μmol of **4** or **5**, 150 μmol of methyl acrylate, 1 μmol of palladium (II) acetate and 2 μmol tri-*o*-tolylphosphine in 0.4 mL of N,N-diisopropylethylamine and 1 mL of DMF was heated for 4 h at 120 °C. After cooling, the reaction mixture was poured into water and neutralized with 1 M hydrochloric acid. The obtained precipitate of the corresponding product **1a** or **2a** was filtered, dried and crystallized from butanol.[23]



Scheme 1. Synthesis of 3-hydroxychromones **1a** and **2a**.

Absorption and emission spectra of the dyes **1a** and **2a** were studied in five hydrophobic solvents of different polarity and the data were compared with those for the parent compounds **1** and **2**.[24] The results show that in all the studied solvents, the one band absorption spectrum of the new dyes is significantly shifted to the red with respect to the parent dyes (Table 1, Fig. 1). Similar strong red shifts are observed for both emission bands in the fluorescence spectra (Table 1, Fig. 1). Furthermore, these shifts are accompanied by a dramatic increase in the relative intensity of the N* band with respect to the T* band.

Previously we showed that a 7-donor (methoxy) group provides blue shifts in absorption and emission maxima and decrease in the I_{N^*}/I_{T^*} ratio.[18] Thus, the 7-acceptor group (2-methoxycarbonylvinyl) provides the opposite spectroscopic effects. In this respect, the effects of the 7-acceptor group are very similar to those of a donor aryl group at 2-position[18] and, therefore, can be related to the increase in the charge transfer character of the excited state of 3HC dye as a result of the increase in the acceptor properties of its chromone cycle.

Similar to the parent compounds the new dyes **1a** and **2a** demonstrate a dual emission that is strongly sensitive to solvent polarity. Indeed, an increase in solvent polarity from heptane ($E_T(30) = 31.1$)[25] to trichloroethylene ($E_T(30) = 35.9$) results in an increase of the relative intensity of the N* band accompanied by its red shift (Fig. 2). Noticeably, the increase in the I_{N^*}/I_{T^*} ratio with solvent polarity is larger in the case of dyes **1a** and **2a** as compared to their parent compounds (Table 1). Indeed, **1a** and **2a** show a 12-fold growth of the I_{N^*}/I_{T^*} ratio in toluene compared to heptane, while the I_{N^*}/I_{T^*} ratio of **1** and **2** increases only 4.4 and 7 times, respectively. This suggests that the 7-acceptor group enhances the sensitivity of the new dyes to solvent polarity. Previously, we have shown that the sensitivity of the dual emission of 3-hydroxychromones can be improved by increasing the electron donor properties of the 2-aryl, which was connected with the increase in the charge transfer from the 2-aryl to the chromone ring in the N* excited state.[18] However, the T* state was less dependent on the donor properties of 2-aryl, probably because in this state the 1-oxygen heteroatom is involved more in the electron donation than the 2-aryl group.[18] In dyes **1a** and **2a**, 7-acceptor group produces a very similar effect. Thus, both the higher values of the I_{N^*}/I_{T^*} ratio and the stronger solvatochromism of **1a** and **2a**, compared to their parent compounds **1** and **2**, is probably connected with their more pronounced charge transfer in the excited state, due to an increase in the acceptor properties of the chromone heterocycle. The increased charge transfer character for the new compounds is also evidenced from the strong fluorescence quenching in more polar solvents like ethyl acetate ($\varphi = 0.10$ for **1a**) and acetone ($\varphi = 0.008$ for **1a**). A very similar quenching with

Table 1. Spectroscopic properties of the studied chromones[a]

Solvent $E_T(30)$	3HC	λ_{max} abs (nm)	λ_{max} fl N* (nm)	λ_{max} fl T* (nm)	I_{N^*}/I_{T^*}	φ
Heptane 31.1	**1**	403	425	553	0.010	0.14
	1a	425	482	600	0.054	0.36
	2	439	457	583	0.078	0.20
	2a	457	511	629	0.158	0.26
CCl_4 32.4	**1**	411	442	560	0.026	0.21
	1a	434	499	605	0.180	0.41
	2	450	481	596	0.277	0.31
	2a	471	536	649	0.511	0.26
CS_2 32.8	**1**	423	459	578	0.049	0.16
	1a	448	518	625	0.386	0.39
	2	466	502	618	0.487	0.26
	2a	487	556	666	0.958	0.26
Toluene[b] 33.9	**1**	408	456	566	0.044	0.14
	1a	434	531	612	0.652	0.45
	2	446	508	613	0.55	0.26
	2a	470	571	657	1.30/1.9[c]	0.24
Trichloroethylene 35.9	**1**	412	462	563	0.119	0.19
	1a	439	534	610	1.55[c]	0.50
	2	452	515	609	0.943	0.46
	2a	478	577	655	2.9[c]	0.15

[a] $E_T(30)$—empirical polarity index of solvents from Ref. 25. λ_{max} abs—positions of absorption maxima, λ_{max} fl N* and λ_{max} fl T*—positions of the fluorescence maxima of the N* and T* bands. φ—the fluorescence quantum yield determined with **1** as a reference (φ = 0.52 in ethanol, see Ref. 13).

[b] Data from Ref. 18.

[c] Values evaluated from deconvolution of the spectra to two bands approximated by log-normal function using the Siano program kindly provided by the author (A.O. Doroshenko from the Karazin University, Kharkov, Ukraine).

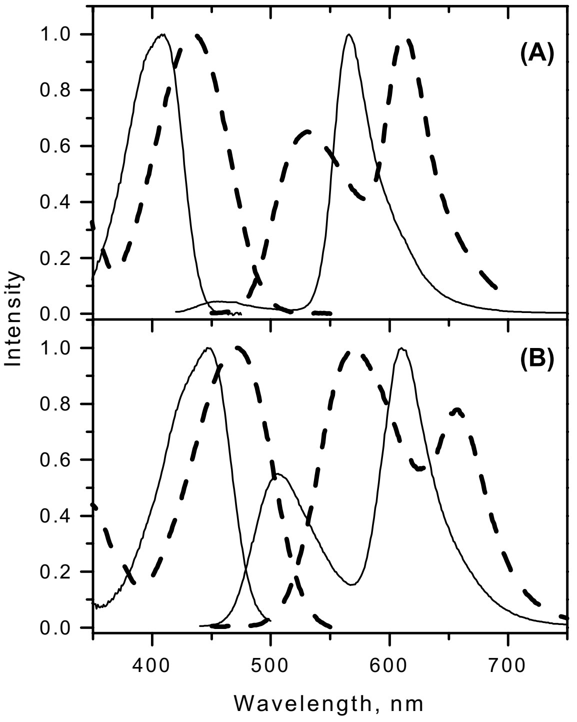


Figure 1. Absorption and fluorescence spectra of dyes **1a** (A) and **2a** (B) (dashed lines) in toluene with respect to their parent compounds **1** and **2** (solid lines).

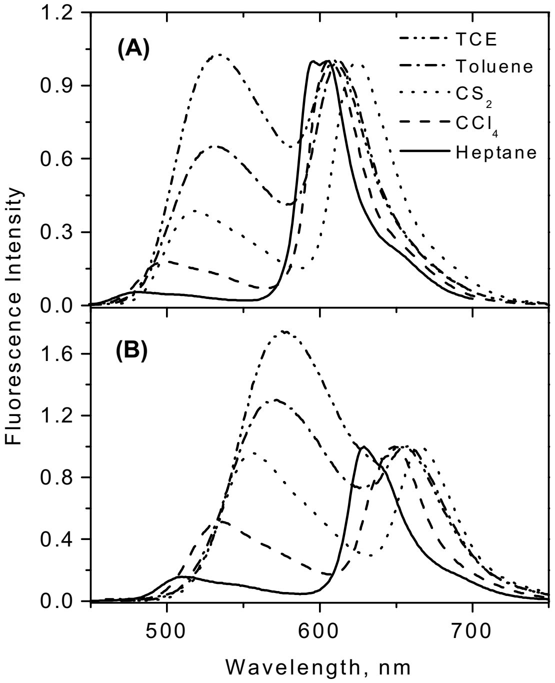


Figure 2. Fluorescence spectra of dyes **1a** (A) and **2a** (B) in solvents of different polarity. TCE refers to trichloroethylene.

increase in solvent polarity was observed for the julolidine analog of dye **2** (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), bearing the strongest 2-aryl electron donor group studied so far on 3-hydroxychromones.[18]

In conclusion, we have synthesized new 3-hydroxychromone dyes characterized by significantly red shifted absorption and emission and enhanced solvatochromism. This was achieved by the introduction of a 7-acceptor group in the 3-hydroxychromone ring. The latter methodology provides a facile route to the development of new two-color fluorescence dyes with red shifted absorption and emission, which are highly demanded as fluorescence probes in biological research.[26,27]

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22. *7-Bromo-4′-(diethylamino)-3-hydroxyflavone* (**4**): Crystallized from butanol; yield 45%; mp 156 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 1.23 (6H, t, *J* 7.0 Hz), 3.45 (4H, q, *J* 7.0 Hz), 6.77 (2H, *J* 9.4 Hz), 6.85 (1H, s), 7.49 (1H, dd, *J* 8.5, 1.9 Hz), 7.76 (1H, d, *J* 1.9 Hz), 8.08 (1H, d, *J* 8.5), 8.13 (2H, d, *J* 9.4 Hz); ESI *m*/*z* ($M^+ + 1$) 388.1. *7-Bromo-2-(6-(diethylamino)benzo[b]furan-2-yl)-3-hydroxychromone* (**5**): Crystallized from DMF; yield 21%; mp 257 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 1.24 (6H, t, *J* 7.0 Hz), 3.45 (4H, q, *J* 7.0 Hz), 6.755 (1H, d, *J* 8.9 Hz), 6.84 (1H, s), 6.89 (1H, s), 7.475 (1H, d, *J* 8.3 Hz), 7.53 (1H, d, *J* 8.9), 7.62 (1H, s), 7.88 (1H, s), 8.10 (1H, d, *J* 8.3 Hz); ESI *m*/*z* ($M^+ + 1$) 428.1.
23. *Methyl 3-{7-[4′-(diethylamino)-3-hydroxyflavonyl]}acrylate* (**1a**): Yield 49%; mp 193 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 1.25 (6H, t, *J* 7.1 Hz), 3.47 (4H, q, *J* 7.1 Hz), 3.86 (3H, s), 6.61 (1H, d, *J* 16.1 Hz), 6.79 (2H, d, *J* 9.1 Hz), 6.90 (1H, s), 7.54 (1H, d, *J* 8.3 Hz), 7.66 (1H, s), 7.77 (1H, d, *J* 16.1 Hz), 8.18 (2H, d, *J* 9.1 Hz), 8.23 (1H, d, *J* 8.3 Hz); ESI *m*/*z* ($M^+ + 1$) 394.2. *Methyl 3-{7-[2-(6-(diethylamino)benzo[b]furan-2-yl)-3-hydroxychromonyl]}acrylate* (**2a**): Yield 41%; mp 236 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 1.26 (6H, t, *J* 7.1 Hz), 3.51 (4H, q, *J* 7.1 Hz), 3.86 (3H, s), 6.62 (1H, d, *J* 16.1 Hz), 6.77 (1H, d, 8.8 J Hz), 6.87 (1H, s), 7.49 (1H, d, *J* 8.8 Hz), 7.56 (1H, d, *J* 8.4 Hz), 7.65 (1H, s), 7.77 (1H, d, *J* 16.1 Hz), 7.79 (1H, s), 8.26 (1H, d, *J* 8.4 Hz); ESI *m*/*z* ($M^+ + 1$) 434.2.
24. All the solvents were of spectroscopic grade. Absorption and fluorescence spectra were recorded on a Cary 400 spectrophotometer (Varian) and FluoroMax 3.0 (Jobin Yvon, Horiba), respectively. Excitation wavelength for the fluorescence measurements was 410 nm for **1** and **1a** and 430 nm for **2** and **2a**. Concentration of the dyes in the solutions corresponded to absorbance close to 0.1.
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