

Synthesis, spectral properties and proton phototransfer reaction in a series of new heterocyclic nitrogen-containing derivatives and analogs of 3-hydroxychromone

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Charge and proton transfer processes are widely spread in nature. Compounds for which these processes are characteristic are actively investigated for over 50 years. 3-Hydroxychromone (3HC) derivatives, which exhibit unique physico-chemical properties as a result of the excited-state intramolecular proton transfer (ESIPT) reaction in their molecules, are subjects of extensive scientific research in this domain. Dual-band emission spectra of these compounds became a basis for development of the novel method of fluorescent analysis – ratiometric detection. The main advantage of this approach is that it doesn't require the use of external standards and reference samples. This particularity can be decisive and crucial in analysis of biological objects. Years of investigations of various possibilities for the practical application of 3HC derivatives showed them to be capable of probing solvent polarity, viscosity, and proton donor or acceptor ability. These fluorescent dyes can also be used as polypeptide markers, as well as indicators and sensors in the studies of physico-chemical properties of biological objects, for example, intracellular pH, surface electric potential of cell membranes and even for early detection of cell apoptosis.

In the past 10 years, the synthesis of 3HC derivatives with additional heterocyclic moieties was extensively developed. Thus, 3HC showing the most long-wavelength fluorescence and the highest quantum yields belong to this series of compounds. 3HC derivatives having heteroaromatic substituents in the position 2 can be considered also as systems with a potential possibility of the alternative Hydrogen bonds formation, and as a result – with a possible alternative excited state proton phototransfer pathway. This particularity opens new and rather intriguing page in the study of the ESIPT process photophysics, and also in the design of new multi-band fluorescent compounds - highly effective ratiometric probes with the increased number of data collecting channels.

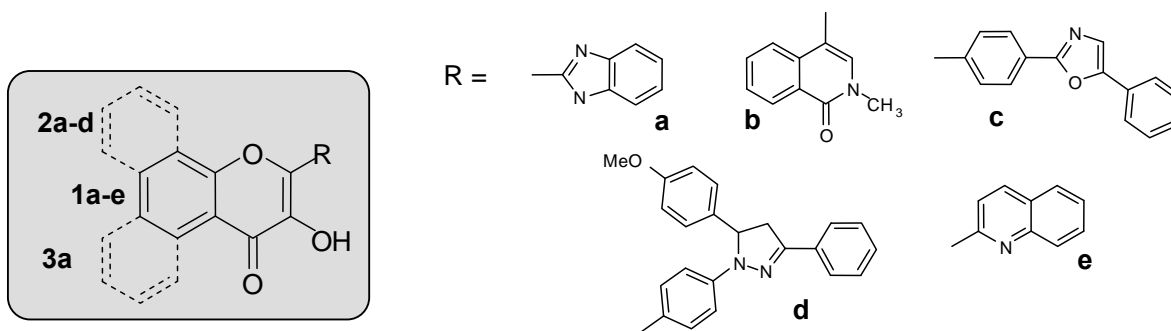


Figure 1. Heterocyclic 3-hydroxychromones under study.

In the present work, a series of the new heterocyclic nitrogen-containing derivatives and analogs of 3HC was synthesized (Fig. 1). In the investigated compounds, benzimidazole, N-methylisoquinolone, diphenyloxazole, triphenylpyrazoline and quinoline fragments were introduced in the position 2 of the parent chromone bicycle. Structures of all compounds were confirmed with ^1H NMR spectroscopy, their purity was controlled with TLC. Spectral and fluorescent properties of the title compounds, as well as their solvatochromism in a number of solvents of different nature (Table 1), and also intramolecular proton transfer reaction in their excited states were investigated (Figure 2). The Reichardt's normalized polarity index E_T^N was selected to investigate polarity influence on spectral behavior. Influence of the substituent nature on the spectral behavior of the 3HC derivatives under study was analyzed.

Table 1. Spectral properties of some 3-hydroxychromone derivatives

Comp.	Solvent	ν_{abs} , cm^{-1}	ν_{fl}^N , cm^{-1}	$\Delta\nu_{ST}^N$, cm^{-1}	ν_{fl}^T , cm^{-1}	$\Delta\nu_{ST}^T$, cm^{-1}	ϕ
1a	toluene	26820	24190	2630	19000	7820	0.34
	1,2-dichloroethane	26860	23140	3720	18950	7910	0.44
	acetonitrile	27180	22790	4390	18690	8490	0.65
1b	toluene	28700	23740	4960	18020	10680	0.01
	1,2-dichloroethane	30260	25485	4775	18160	12100	0.03
	acetonitrile	30380	25400	4980	18160	12220	0.04
1c	toluene	25460	22420	3040	17940	7520	0.57
	1,2-dichloroethane	25580	21520	4060	18100	7480	0.70
	acetonitrile	26020	20340	5680	18120	7900	0.51
1d	toluene	22960	20180	2780	16990	5970	0.55
	1,2-dichloroethane	22660	19060	3600	16705	5955	0.30
	acetonitrile	23380	18420	4960	16440	6940	0.48
1e	toluene	25660	22540	3120	17850	7810	0.005
	1,2-dichloroethane	25960	20880	5080	16990	8970	0.004
	acetonitrile	26100	19770	6330	16930	9170	0.003

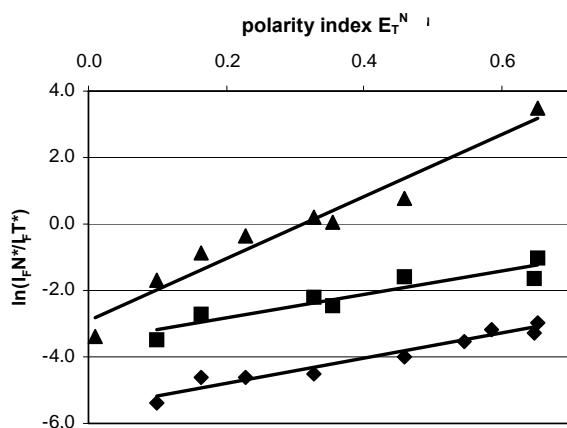


Figure 2. Dependence of the normal-to-
tautomer fluorescence intensity on the
solvent polarity for compounds **1a** (◆),
1c (■) and **1d** (▲).

Combining the chromone bicycle with molecular fragments being known as effective fluorophores is shown to result in the obtaining of the new highly effective multi-banded fluorescent dyes exhibiting excited-state intramolecular proton phototransfer. Contrary to such behavior, strong electron acceptor substituents in the position 2 lead to significant decrease of quantum yields of fluorescence. The same effect was observed upon annealing of the benzene ring to the parent chromone bicycle.

Introduction of a strong electron donor fragment to the position 2 of the 3-hydroxychromone significantly increased the charge-transfer character of the normal form. This makes the normal fluorescence band much more intense and increases its sensitivity to the solvent polarity. Extremely high solvatofluorochromism of the normal emission band of the compound **1d** makes it one of the most effective solvatofluorochromic dyes in the 3-hydroxychromone family. Good response of the normal-to-phototautomer ratio to the solvent polarity makes such compounds prospective for their application as ratiometric fluorescent probes. Sensitivity of their phototautomer form emission to the solvent polarity allows to keep the band separation suitable for dual-band ratiometric measurements in a wider range of solvent polarities.

Our experiments proved that the proton phototransfer reaction is realized not only for the molecules with charge transfer directed from the substituent in the position 2 to the chromone bicycle, which is traditional for this family of compounds (Figure 3, right), but also for the molecules exhibiting charge transfer in the opposite direction upon excitation (Figure 3, left).

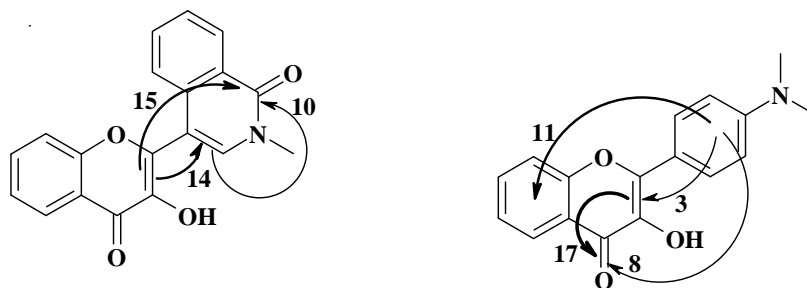
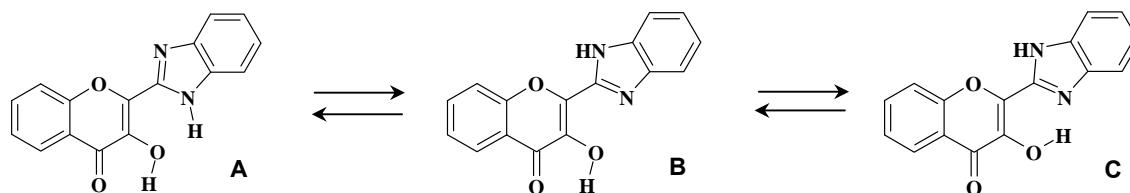


Figure 3. Electron density redistribution upon excitation in the molecules of **1b** (left) and 2-(4'-dimethylaminophenyl)-3-hydroxychromone (**DMAF**, right).

The nature of the intramolecular hydrogen bonds in the molecules of benzimidazole- and quinoline-substituted 3HC was investigated taking into account all three possible rotamers as follows:



Modeling of the possibility of an alternative intramolecular hydrogen bond formation, as well as realization of an alternative pathway for the excited-state intramolecular proton transfer reaction was effectuated. Presence of the alternative intramolecular hydrogen bonds to the nitrogen atom of the substituent's heterocycle was shown by X-ray structure analysis for both compounds in the crystalline state.

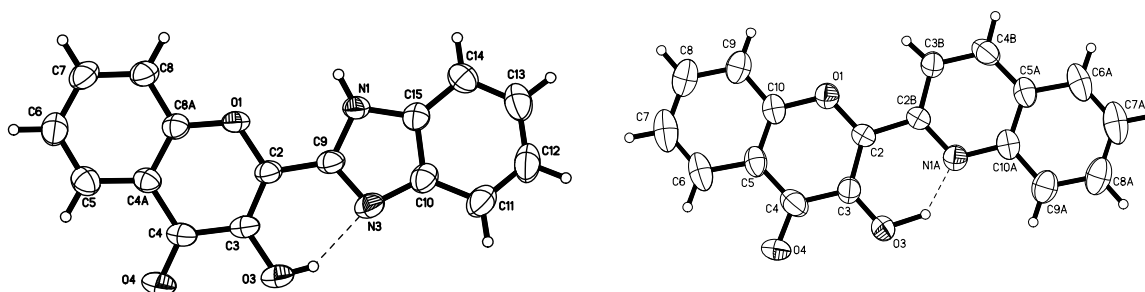


Figure 4. Structures of **1a** and **1e** in the crystalline state (XRD data).

The change of the proton transfer pathway from the “alternative” to the classical “flavonol-like” on going from crystalline state to solution was predicted for the benzimidazole-substituted derivative. At the same time, alternative H-bond appears to be stronger in the case of quinoline-substituted 3HC, thus keeping the proton transfer direction in solutions the same as in the crystalline state.

Spectral characteristics of the investigated compounds are sensitive to their environments polarity changes. This particularity make prospective to develop on their basis ratiometric fluorescent probes and sensors for various physico-chemical and medicobiological investigations. Thus, triphenylpyrazoline-substituted derivatives were shown to be useful for analysis of water or other polar admixtures in organic solvents (Figure 5), while benzimidazole-substituted 3HC could be used for water-organic solvent systems analysis in the wide range of concentrations.

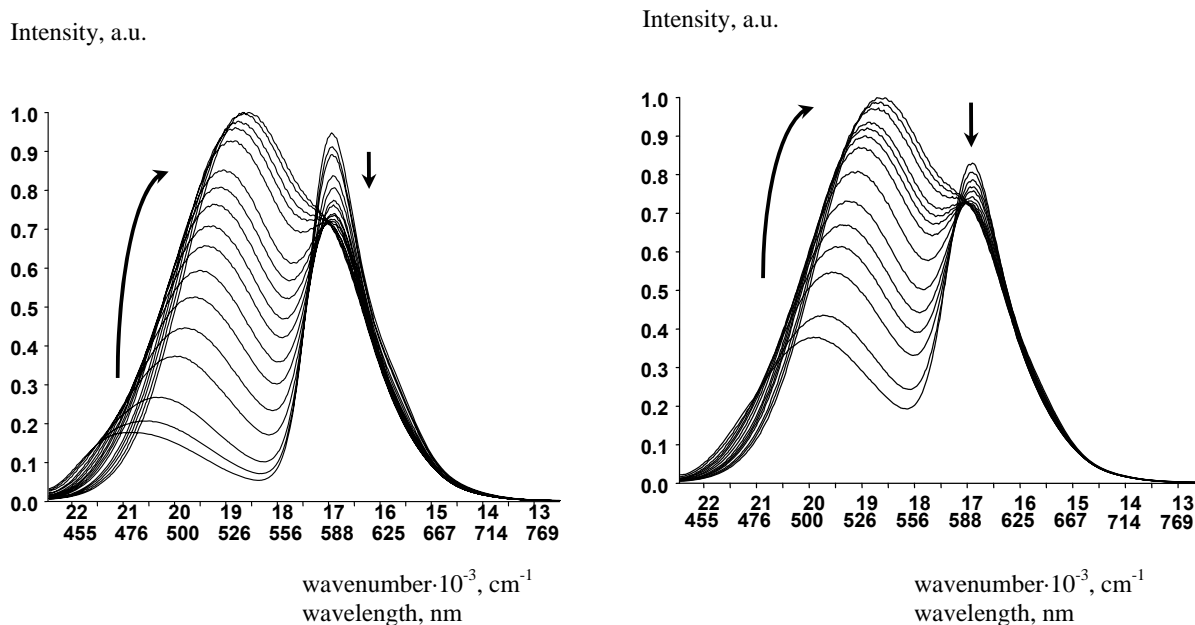


Figure 5. Fluorescence of **1d** in 1,4-dioxane (left) and acetone (right) at different water concentration (step 1% vol.).

Complex analysis of the “structure – property” dependences for the compounds under study led to the following conclusions. Combining the 3-hydroxychromone bicycle with electron donor fragments being good fluorophores allows to construct new multiband fluorescent dyes with high quantum yields of emission, good band separation, high solvatochromism and sensitivity of ratiometric response to a number of parameters of microenvironment. Further development of alternative ESIPT pathway theory can lead to the design of luminophores with increased number of emission bands, which will allow simultaneous external standard-free multiparametric probing of complex dependences, particularly in natural objects, as well as create probing systems with parallel self-controlled analysis of one parameter with different analytical signals.

Currently, investigations are carried out in the domain of implementation of new compounds in temperature sensing, complexation probing of heavy metals in water, fluorescence marker design, etc.