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Fluorescence properties of some 2-(4-amino-substituted-3-nitrophenyl)-3-hydroxyquinolin-4(1*H*)-ones

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ABSTRACT

2-(4-Amino-substituted-3-nitrophenyl)-3-hydroxyquinolin-4(1*H*)-ones have been studied to evaluate their fluorescence properties and possible use as molecular fluorescent probes. The amino group was substituted with various alkyl moieties possessing a suitable terminal functional group (such as hydroxy or amino group) that could serve to bind a 3-hydroxyquinolin-4(1*H*)-one (3HQ) fluorescence label to a biomolecule. Besides simple hydrocarbon chains, ligands containing ethylenoxy units as optimal spacers were also tested. The structure–fluorescence properties and theoretical applicability of the studied molecules are discussed.

Fluorescent probes allow researchers to detect particular components of complex biomolecular assemblies with exquisite sensitivity and selectivity, as well as to observe interesting properties of the biosystem or biological processes. Nevertheless, fluorescence techniques require a suitable fluorescent label with optimal properties. In the case of common single-band fluorescent labels the fluorescence intensity depends on the label concentration which can vary because of various biological processes in a sample.[1] Dual fluorescence labels that exhibit two well-separated emission bands are not dependent on the concentration because the ratio of the intensities of the two bands can be applied as a signal.[1,2] This possibility is an advantage in complex biological systems such as, cells or tissues where the local concentration of the label cannot be controlled easily and generally, the label is not distributed homogenously.

These are reasons behind the intensive development of new dual fluorescence labels with improved fluorescence properties. Recently, studies[1–6] dealing with 2-aryl-3-hydroxyquinolin-4(1*H*)-ones (3HQs) have been published. The dual fluorescence spectrum of 3HQs is a result of an excited state intramolecular proton transfer (ESIPT) reaction leading to the formation of two excited state tautomeric forms of the probe.[2,4] Different photophysical properties result in sufficiently separated emission bands.[2,3]

The essential part of the fluorescence label-biomolecule system is the spacer between both these parts as it reduces potential undesirable interactions between the fluorescence label and the biomolecule, especially steric hindrance and spatial interference. A study of the fluorescent properties of a system composed of an appropriately substituted 3HQ as the fluorescent label bearing a suitable spacer for attachment of the label to the target biomolecule was described.[6] The effect of spacer location at the carboxamide group at positions 6–8 of the hydroxyquinolinone skeleton was described in the Letter mentioned above.[6] This study is focused on evaluation of the fluorescence properties of 2-(4-amino-substituted-3-nitrophenyl)-3HQs (Scheme 1) with the aim to evaluate the possibility of binding the 3HQ fluorescence label to a biomolecule via the phenyl ring at position 2. The studied derivatives were chosen because of their simple preparation via nucleophilic substitution of the chlorine with various amines[7] which leads to a large number of diverse derivatives which may be suitable for an attachment to the target biomolecule (Scheme 1).

In this Letter we examined a set of derivatives **2**–**17** were prepared using aliphatic diamines (**3**–**5**, **16**) or amino alcohols (**6**–**14**, **17**). To investigate the influence of amino group substitution on the resulting fluorescent properties, the prepared ligands contained alkyl chains of 2–5 carbons (to compare the effect of chain length), and ligands with ethyleneoxy chains (used as hydrophilic spacers to avoid convolution) were studied (Table 1). The syntheses of these 3HQs have been described elsewhere[7] and the purity was >98% as verified by HPLC–MS and NMR analysis.[8]

The excitation spectra of the studied compounds showed two maxima, around 305 nm and 350 nm (for compound **16** as an example, see Fig. 1). From a comparison of the emission spectra excited at the excitation wavelengths of both the maxima it is apparent that the emission intensity of the first maximum at 419 nm remains the same, and the intensity of the second maximum at 518 nm is diminished when a shorter excitation wavelength is used (Fig. 1). For this reason, as well as for improved

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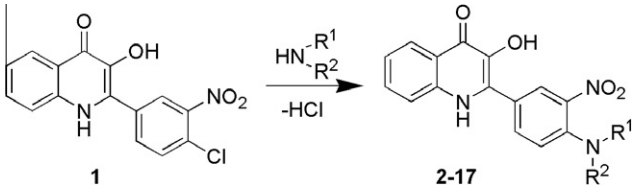
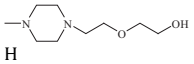


Scheme 1. General method for the preparation of the target compounds.

Table 1
List of investigated 3HQs

Compound	R^1	R^2
2	H	H
3	H	$(CH_2)_2NH_2$
4	H	$(CH_2)_3NH_2$
5	H	$(CH_2)_4NH_2$
6	CH_3	$(CH_2)_2OH$
7	CH_3CH_2	$(CH_2)_2OH$
8	$CH_3(CH_2)_2$	$(CH_2)_2OH$
9	$(CH_2)_2OH$	$(CH_2)_2OH$
10		
11	H	$(CH_2)_2OH$
12	H	$(CH_2)_3OH$
13	H	$(CH_2)_4OH$
14	H	$(CH_2)_5OH$
15	H	$(CH_2)_3OCH_2CH_3$
16	H	$(CH_2)_2O(CH_2)_2NH_2$
17	H	$(CH_2)_2O(CH_2)_2OH$

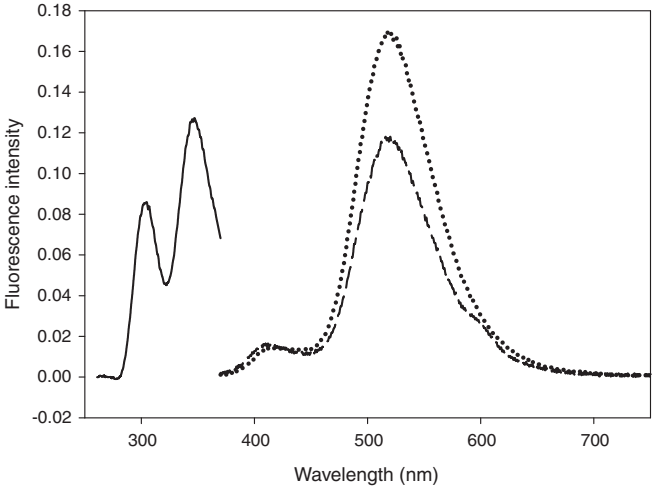


Figure 1. Excitation and emission spectra of compound **16** in methanol. (—), excitation spectrum; (– –), emission spectrum at λ_{ex} = 303 nm; (•••), emission spectrum at λ_{ex} = 347 nm.

utility for biological applications, the higher excitation wavelength was applied in all further measurements.

It was observed that the nature of the amino group influenced significantly the quantum yields of the compounds. The quantum yields depended on the chain length (Fig. 2) and terminal substituents on the alkyl chain. When the amino group was unsubstituted the quantum yield was very low (compound **2**, $\varphi = 1.6 \times 10^{-3}$%). In the case of aminoalkyl derivatives **3**–**5** the quantum yield was influenced by the number of carbon atoms (but unfortunately, did not exceed 0.36%).

A similar dependence was observed with hydroxyalkyl derivatives **11**–**14**. On the other hand, an interesting fact was observed when the corresponding hydroxyalkyl and aminoalkyl derivatives

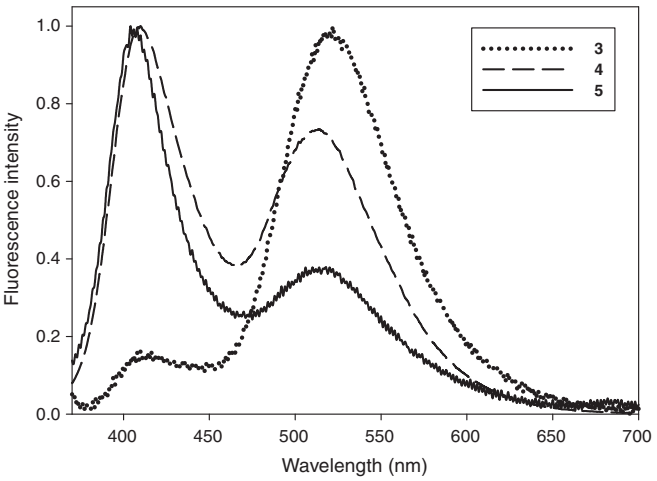


Figure 2. Normalized fluorescence emission spectra of compounds **3**,**4** and **5** in methanol.

of the same carbon chain length were compared. The quantum yields of both hydroxyethyl and hydroxybutyl derivatives were approximately 500 times higher compared to the analogous amino derivatives (compare compounds **3**, **5** and **11**, **13**) whereas the difference in the quantum yield of propylene derivatives was very low (compare compounds **4** and **12**). Compound **11** exhibited the highest quantum yield of those studied (φ = 10.21%). Compounds **10**, **16** and **17** which contain ethyleneoxy units as spacers (polyethyleneglycols are often used as spacers due to their water solubility, biocompatibility and ready availability in a variety of lengths) exhibited similar quantum yields that were not influenced by the nature of the terminal substituent (hydroxy group vs amino group, compare compounds **16** and **17**).

However, due to the low quantum yields of compounds **10**, **16** and **17** (0.01–0.05%, Table 2), they appear to be ineligible candidates for further fluorescence label-biomolecule system development. For compounds **3**–**5**, an interesting dependency between length of the alkyl chain and the spectral properties was observed. With increasing chain length, the ratio of emission maxima I_1/I_2 increased which shows that the ratio of the excited state tauto-

Table 2
Spectroscopic properties of the 3HQs in methanol

Compound	λ_{ex}[a]	$\lambda_{em,1}$[b] (nm)	$\lambda_{em,2}$[c] (nm)	I_1/I_2[d]	φ[e] (%)
1	348	428	508	0.21	0.04
2	349	408	516	0.12	1.6×10^{-3}
3	360	410	522	0.14	0.02
4	354	410	511	1.38	0.36
5	345	404	514	2.73	5.7×10^{-3}
6	352	430	511	0.091	0.03
7	356	430	510	0.10	0.03
8	355	429	515	0.10	0.01
9	355	416	510	0.15	0.94
10	352	429	513	0.13	0.01
11	348	426	502	0.11	10.21
12	345	415	516	0.05	0.14
13	347	417	506	0.10	2.74
14	347	413	514	0.05	0.07
15	350	411	510	0.11	3.34
16	347	419	518	0.08	0.05
17	351	416	518	0.06	0.01

[a] λ_{ex}, excitation wavelength.
[b] $\lambda_{em,1}$, the fluorescence emission maximum at lower wavelengths.
[c] $\lambda_{em,2}$, the fluorescence emission maximum at higher wavelengths.
[d] I_1/I_2, the ratio of fluorescence maxima intensities.
[e] φ, fluorescence quantum yield (determined with quinine sulfate in 0.5 M H_2SO_4 (φ = 0.577[9]), taken as a reference fluorescence standard).

Table 3
Spectroscopic properties of compounds **11**, **16** and **17** in various solvents

Compound	11		16		17	
	I_1/I_2	φ (%)	I_1/I_2	φ (%)	I_1/I_2	φ (%)
MeCN	0.01	13.28	0.15	0.05	0.04	0.04
DMSO	0.08	18.77	0.33	0.07	0.11	0.04
EtOAc	0.01	15.28	0.15	0.07	0.04	0.03
MeOH	0.08	10.21	0.08	0.05	0.06	0.01
MeOH/H_2O 1:1	0.08	4.91	0.16	0.06	0.11	0.01
Toluene	0.02	21.30	0.18	0.09	0.05	0.05

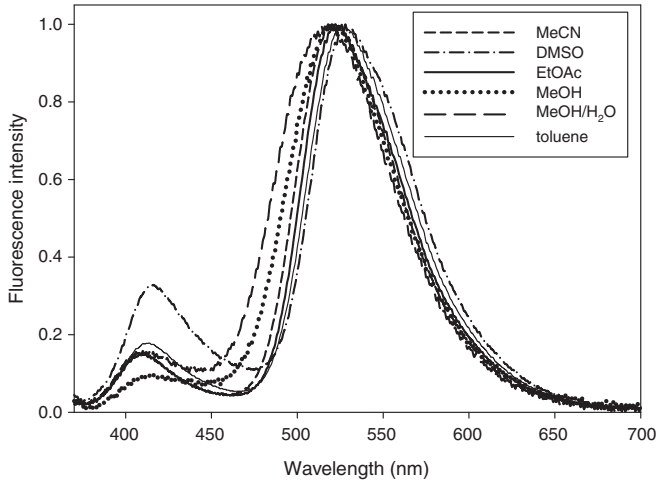


Figure 3. Normalized fluorescence emission spectra of compound **16** in various solvents.

meric forms[2,4] grew to the benefit of those with fluorescence emission at lower wavelength. Surprisingly, a similar dependence between length of alkyl chain and the spectral properties was not observed for compounds containing hydroxyalkyl chains (entries **11**–**14**).

The effect of solvents (Table 3, Fig. 3) on the emission spectra of compounds **11**, **16** and **17** (representative compounds with higher and lower quantum yields) was not significant and any obvious relationship between polarity and the ratio of maxima intensities was not found (see Fig. 3 with the emission spectra of compound **16** as an example). On the other hand, the quantum yields of these representatives were meaningfully affected by solvents (Table 3). Generally, the highest quantum yield was observed for toluene and the lowest for methanol (**16**) and/or methanol/water 1:1 (**11**, **17**). In the case of compound **11** the quantum yield of a toluene solution (21.30%) was twice than that of a methanolic solution (10.21%).

In conclusion, the fluorescence properties of 2-phenyl-substituted-3HQs were studied from the point of view of their application as a part of a biomolecule-fluorescence probe system. It was found that the 2-phenyl substituents of the 3HQs affected significantly the fluorescence quantum yield, which varied from 1.6×10^{-3} (**2**) to 10.21% (**11**). Moreover, in the case of compounds **3**–**5** the shape of the emission spectra was influenced by the carbon chain length. However, this was not the case for compounds **11**–**14** with hydroxyalkyl chains of different length.

The results show that introducing substituted alkyl groups (with terminal amino or hydroxy groups) to compound **1** led mostly to increased fluorescence quantum yields.

Although compounds **11** and **13** can, in theory, be used for attachment directly or via any other spacer to a biomolecule through the terminal hydroxy group, elongation of the hydroxyethyl ligand in compound **11** with an aminoethyl group (**16**) or hydroxyethyl group (**17**) led to a crucial decrease of the fluorescence quantum yields. A relatively small change in the structure of the studied molecules affected significantly the quantum yields (up to five orders of magnitude) which indicates the possibility to find improved, substituted 3HQs, as dual fluorescent labels bound to a biomolecule via the phenyl ring at position 2 and having suitable spectral parameters. The value of the quantum yield after binding of such systems to biomolecules is also an interesting question, which could provide information on the actual use of such derivatives as fluorescent probes.

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Supplementary data

Supplementary data (analytical data for compounds **2** and **4**–**16**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.12.013.

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7. Krejčí, P.; Hradil, P.; Hlaváč, J.; Hajdúch, M. Patent WO 2008028427, 2008; *Chem. Abstr.* **2008**, *148*, 331,570. Compounds **1** and **2** were prepared by the following procedure: 2-(4-chloro-3-nitrophenyl)-2-oxoethyl 2-aminobenzoate or 2-(4-amino-3-nitrophenyl)-2-oxoethyl 2-aminobenzoate (44.8 mmol) was suspended in polyphosphoric acid (167.3 g). The reaction mixture was heated to 100 °C and stirred for 90 min. The mixture was then poured into H_2O/crushed ice (700 ml). The precipitated product was filtered, washed with H_2O, dried and recrystallized from 2-methoxyethanol. Compounds **3**–**17** were prepared by the following general procedure: quinolinone **1** (200 mg, 0.63 mmol) was added to a solution of amine (6.3 mmol) and *N*-methylpyrrolidone (1.0 ml) and the mixture was stirred at 110 °C for 2 h. After cooling to room temperature, H_2O (20 ml) was added and the pH adjusted to 7 with dilute HCl (1:3). The precipitated solid was collected by suction, washed thoroughly with H_2O and dried at 80 °C. The crude product was recrystallized from 2-methoxyethanol.
8. Yields and analytical data of selected compounds (the remainder are provided as Supplementary data): *2-(4-Chloro-3-nitrophenyl)-3-hydroxyquinoline-4(1H)-one* (**1**): Yellow powder, 11.93 g (84%), mp 284–287 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.29 (t, J = 7.9 Hz, 1H, ArH); 7.63 (t, J = 5.3 Hz, 1H, ArH); 7.69 (d, J = 8.4 Hz, 1H, ArH); 7.99 (d, J = 8.4 Hz, 1H, ArH); 8.14 (d, J = 2.4 Hz, 1H, ArH); 8.17 (d, J = 2.3 Hz, 1H, ArH); 8.52 (s, 1H, ArH); 11.67 (br s, 1H, OH). MS: m/z 319.9 [M(^{37}Cl)+H]$^+$, $C_{15}H_9ClN_2O_4$ calcd 316.70. *3-Hydroxy-2-(4-amino-3-nitrophenyl)quinolin-4(1H)-one* (**3**): Dark red powder, 170 mg (75%), mp 150–156 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.88 (t, J = 5.85 Hz, 2H); 3.38–3.53 (t, 2H); 7.18–7.33 (m, 2H); 7.58 (t, J = 6.9 Hz, 1H); 7.72 (d, J = 7.8 Hz, 1H); 7.97–8.18 (m, 3H); 8.63 (br s, 2H). MS: m/z 341.1 [M+H]$^+$, $C_{17}H_{16}N_4O_4$ calcd 340.33.
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