Tetrahedron Letters 51 (2010) 5060–5063

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Fluorescence properties of 2-aryl-3-hydroxyquinolin-4(1*H*)-one-carboxamides

Kamil Motyka *, Jan Hlaváč, Miroslav Soural, Petr Funk

Department of Organic Chemistry, Faculty of Science, Institute of Molecular and Translational Medicine, Palacký University, Tř. 17. listopadu 12, 771 46 Olomouc, Czech Republic

ARTICLE INFO

Article history:
Received 27 May 2010
Revised 7 July 2010
Accepted 16 July 2010
Available online 22 July 2010

Keywords:
3-Hydroxyquinolones
Absorption
Fluorescence
Dual fluorescence probes

ABSTRACT

The fluorescence properties of 2-aryl-3-hydroxyquinolin-4(1*H*)-one-carboxamides (3HQCs) with carboxylic alkylamide groups at positions 6, 7 or 8 (3HQ6Cs, 3HQ7Cs, and 3HQ8Cs) have been studied to evaluate their potential as molecular probes.

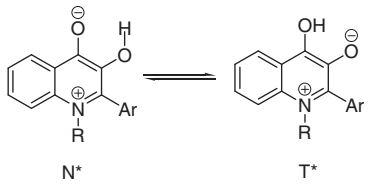
Fluorescent probes allow researchers to detect particular components of complex biomolecular assemblies, such as live cells, with exquisite sensitivity and selectivity. However, fluorescence techniques require suitable fluorescent labels with optimal properties. In the case of the common single-band 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 dye cannot be easily controlled and generally the label is not distributed homogenously.

The most interesting representatives of the dual fluorescence labels class are 3-hydroxyflavone derivatives which have been shown to be an effective tool for studies of polarity,[3,4] hydration and electronic polarizability,[4] electrostatic effects,[5–8] ionic nature, and ion concentration.[9,10] Despite the advantages of 3-hydroxyflavones as dual fluorescence probes in comparison with single-band probes, they have some limitations such as relatively low photostability and low quantum yields. These are the reasons behind the intensive development of new dual fluorescence labels with improved fluorescence properties.

Recently, several studies[1,2,11–13] on 2-aryl-3-hydroxyquinolin-4(1*H*)-ones (3HQs) have been published. 3HQs are structural analogs of 3-hydroxyflavones. 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[2,12] (Scheme 1, N*, normal form; T*, tautomeric form).

Different photophysical properties result in sufficiently separated emission bands.[2,11] 3-Hydroxybenzo[*g*]quinolones[4] (derivatives of 3-hydroxyquinolones with a fused benzene ring) also exhibit dual fluorescence. The dual emission of both groups of compounds is highly sensitive to solvent polarity and pH.[2,12,13] Furthermore, it was found that in comparison to 3-hydroxyflavones the 3-hydroxyquinolone dyes exhibited higher fluorescence quantum yields and a 10-fold increase in photostability.[2] Additionally, *N*-methyl substituted 3HQs demonstrate strong solvatochromic properties when the ratio of fluorescence maxima intensities is a function of the solvent polarity.[11] 3-Hydroxybenzo[*g*]quinolones also exhibit a red-shift absorption band[12] which allows their excitation by He/Cd and Ar-ion lasers.

The essential part of the fluorescence label-biomolecule system is the spacer between the two parts as it reduces potentially undesirable interactions between the fluorescence label and the biomolecule, especially steric hindrance and spatial interference. The 3HQs studied so far[1,2,11–13] do not contain any suitable functional group for a spacer connection, which of course can influence the fluorescence properties. This Letter is the first to offer a possible



N* T*

Scheme 1. Excited state tautomeric forms in ESIPT.

* Corresponding author. Tel.: +420 585 634 436; fax: +420 585 634 465.
E-mail address: motyka@orgchem.upol.cz (K. Motyka).

0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2010.07.103

solution to this problem and tries to give answers to the following questions: (1) which position is the most suitable for binding of the 3HQ skeleton to a spacer without the loss of the suitable emission properties and (2) what is the relationship between the spacer character and the resulting fluorescence.

These two simple questions hide a lot of systematic work based on finding the best combination of a large number of possible spacers, various positions on 3HQs and numerous combinations of different substituents on the quinolinone skeleton.

This study is focused on the evaluation of the fluorescence properties of 2-aryl-3-hydroxyquinolin-4(1*H*)-one-carboxamides (3HQCs) with the N-substituted carboxamide groups at positions 6 (3HQ6Cs), 7 (3HQ7Cs) or 8 (3HQ8Cs, Scheme 2).

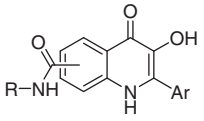
The carboxamide group was introduced into the dye molecule as a suitable functional group for attachment of the fluorescent label to the target biomolecule via a spacer of appropriate length and structure. The studied spacers contained alkyl chains of 3 and 5 carbons (to compare the effect of chain length), alcoholic and PEG moieties (to evaluate the effect of a polar spacer), and a benzyl group (to estimate the influence of an aromatic ring on the spacer). Compounds substituted with a carboxamide group at position 5 were not studied due to our failure to prepare them using the synthetic method[14] which we used for the preparation of 3HQ6Cs, 3HQ7Cs, and 3HQ8Cs.

First, the spectroscopic properties (UV–vis absorption and fluorescence) of 3HQ8Cs **2–8** with various substituted phenyl rings at position 2 (see Table 1) were investigated using methanol as the solvent (at a concentration of 100 mg/L). The syntheses of the studied 3HQ7Cs have been described elsewhere[14] and it was applied also for the preparation of 3HQ6Cs and 3HQ8Cs. The purity of the studied compounds was >95%. UV–vis absorption spectra were not modulated significantly by the phenyl substitution. From 300 to 450 nm, the absorption spectra exhibited two maxima at wavelengths around 335 and 380 nm. Surprisingly, the emission spectra of most of the 3HQ8Cs did not exhibit the expected two maxima. Only the emission maximum at a lower wavelength was detected (derivatives **2** and **4–8**) or the maximum at higher wavelength was indistinct (derivative **3**). When the quantum yields of derivatives **2–8** were compared (see Table 2), 2-(4-amino-3,5-dichlorophenyl) ligand (derivative **2**) with the highest quantum yield was chosen for further studies.

Subsequently, emission spectra of compounds **2** and **9–25** with the same substitution on the 2-phenyl ring but different N-substituents on the carboxamide group located at positions 6, 7 or 8 (see Table 2) were recorded to compare: (1) the influence of the spacer character on the fluorescence spectra and (2) the influence of the spacer position.

Generally, it was observed that compounds substituted at positions 6 and 7 exhibited two-band fluorescence spectra while compounds with the substitution at position 8 showed only single-band spectra with the emission maxima at lower wavelengths.

Additionally, with the 3HQ7Cs the lower wavelength maximum was not well-separated and coincided with the higher wavelength maximum, for example, the fluorescence spectra of compounds **14**, **20**, and **25** (Fig. 1). When comparing the same *N*-alkylamides at various positions, it is evident that the quantum yields decline in the order 3HQ7Cs, 3HQ6Cs, and 3HQ8Cs, respectively (except for compound **2**, Table 2). The significantly different emission parameters of 3HQ8Cs in comparison to 3HQ7Cs and 3HQ6Cs can be explained by the formation of an intramolecular hydrogen bond between the oxygen atom of the carboxamide group and the hydrogen atom at position 1 resulting in a stable six-membered ring.



Scheme 2. The general structure of the studied 3HQCs.

Table 1
List of the investigated 3HQCs

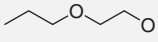
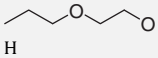
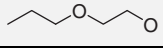
Compound	Ar	R	Carboxamide position
1	3,5-DiCl-4-NH_2	—	—
2	3,5-DiCl-4-NH_2	Propyl	8
3	3-NO_2-4-Cl	Propyl	8
4	3-NO_2-4-N(Pr)$_2$	Propyl	8
5	4-Me	Propyl	8
6	4-OMe	Propyl	8
7	4-F	Propyl	8
8	3-Br	Propyl	8
9	3,5-DiCl-4-NH_2	H	7
10	3,5-DiCl-4-NH_2	Propyl	7
11	3,5-DiCl-4-NH_2	Pentyl	7
12	3,5-DiCl-4-NH_2	Benzyl	7
13	3,5-DiCl-4-NH_2	Hydroxyethyl	7
14	3,5-DiCl-4-NH_2		7
15	3,5-DiCl-4-NH_2	H	6
16	3,5-DiCl-4-NH_2	Propyl	6
17	3,5-DiCl-4-NH_2	Pentyl	6
18	3,5-DiCl-4-NH_2	Benzyl	6
19	3,5-DiCl-4-NH_2	Hydroxyethyl	6
20	3,5-DiCl-4-NH_2		6
21	3,5-DiCl-4-NH_2	H	8
22	3,5-DiCl-4-NH_2	Pentyl	8
23	3,5-DiCl-4-NH_2	Benzyl	8
24	3,5-DiCl-4-NH_2	Hydroxyethyl	8
25	3,5-DiCl-4-NH_2		8

Table 2
Spectroscopic properties of HQCs in methanol

Compound	λ_{ex}[a] (nm)	$\lambda_{em,1}$[b] (nm)	$\lambda_{em,2}$[c] (nm)	I_1/I_2[d]	φ[e] (%)
1	345	417	503	0.10	7.05
2	341	444	—	—	0.39
3	337	426	—	—	0.026
4	340	436	—	—	0.32
5	333	428	—	—	$2.80.10^{-3}$
6	344	424	—	—	$7.29.10^{-3}$
7	331	421	—	—	0.029
8	339	440	—	—	0.071
9	333	442	534	0.35	50.64
10	332	401	523	0.070	26.69
11	333	451	534	0.11	49.66
12	332	449	534	0.11	49.69
13	334	445	534	0.090	50.05
14	348	448	534	0.11	41.07
15	343	443	528	0.10	7.06
16	342	432	520	0.13	44.07
17	344	445	528	0.12	48.29
18	343	441	530	0.11	48.54
19	340	440	528	0.13	42.54
20	348	448	534	0.11	41.07
21	340	436	—	—	3.61
22	341	482	—	—	0.30
23	340	442	—	—	0.53
24	340	444	—	—	0.58
25	343	454	—	—	0.24

[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 sulfuric acid (φ = 0.577[15]), taken as a reference fluorescence standard).

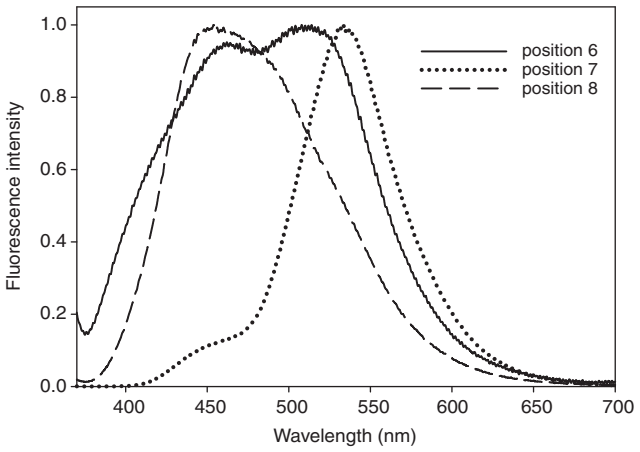


Figure 1. Fluorescence spectra of compounds **14**, **20**, and **25**

The variation of *N*-alkyl carboxamide substitution did not reveal any influence upon the fluorescence properties of 3HQCs, that is, no significant differences among derivatives **9**–**14**, **15**–**20**, and **21**–**25** were observed, which indicates the independent nature of the fluorescence on the spacer constitution (with respect to the tested ligands character). From a comparison of the quantum yields of compounds **1** and **9–25** (Table 2) it is evident that the introduction of the N-substituted carboxamide groups at positions 6 and 7 increased significantly the quantum yield of the corresponding derivatives while carboxamide substitution at position 8 led to a decrease in the quantum yield.

The effect of pH (concentration of compound **14** in a phosphate buffer was 2.5 mg/L) and solvent (aqueous phosphate buffer, methanol, ethanol, acetonitrile, dimethyl sulfoxide, and toluene) on the fluorescence properties were also studied. For this study, compound **14** was chosen due to the good fluorescence properties of 3HQ7Cs and suitable R substitution, that is, ethyleneoxy units. Ethyleneoxy units (polyethyleneglycols) are often used as spacers due to their water solubility, biocompatibility, and ready availability in a variety of lengths.

The ratio I_2/I_1 decreased with increasing pH (Table 2 and Fig. 2), and at pH 10.5 only the emission maximum at lower wavelengths appeared which corresponds to the formation of the tautomeric form with the hydroxy group at position 3 (Scheme 1, N*). With increasing pH the short wavelength band intensity increased as well. This fact corresponds reasonably to the observation with 3HQ8Cs, where the N–H bond is weak due to an intramolecular H-bond.

The effect of solvents (Fig. 3) on both the emission spectrum and quantum yield of compound **14** was not significant and any obvious relationship between the polarity and the ratio of maxima intensities was not found.

This fact is in agreement with previous observations[2] where compounds with a hydrogen atom at position 1 showed low intensity of short wavelength bands and limited sensitivity to solvent polarity in contrast to *N*-Me 3HQs. As a possible explanation, the decrease in acidity of the 3-OH group in the excited state of the *N*-Me 3HQs which leads to the formation of the N* form of the 3HQs can be suggested (Scheme 1).

In conclusion, the fluorescence properties of 2-aryl-3-hydroxyquinolin-4(1*H*)-one-carboxamides with different N-substituted carboxamide groups at positions 6, 7, and 8 have been studied. It was found that the phenyl substituents at position 2 of the 3HQCs affected only marginally the shape of the emission spectra, but significantly influenced the quantum yield, which varied by three orders of magnitude (compare derivatives 2–8).

Further, the effect of the carboxamide group position on 3HQ was studied. It was observed that the carboxamide position influenced the quantum yield as well as the emission spectra. The 3HQ8Cs exhibited only single-band spectra with the maxima at lower wavelengths, while 3HQ7Cs and 3HQ6Cs exhibited spectra with two maxima.

Generally, the quantum yield declined in the order HQ7Cs, HQ6Cs, and HQ8Cs, respectively (except compounds **2**, **10**, and

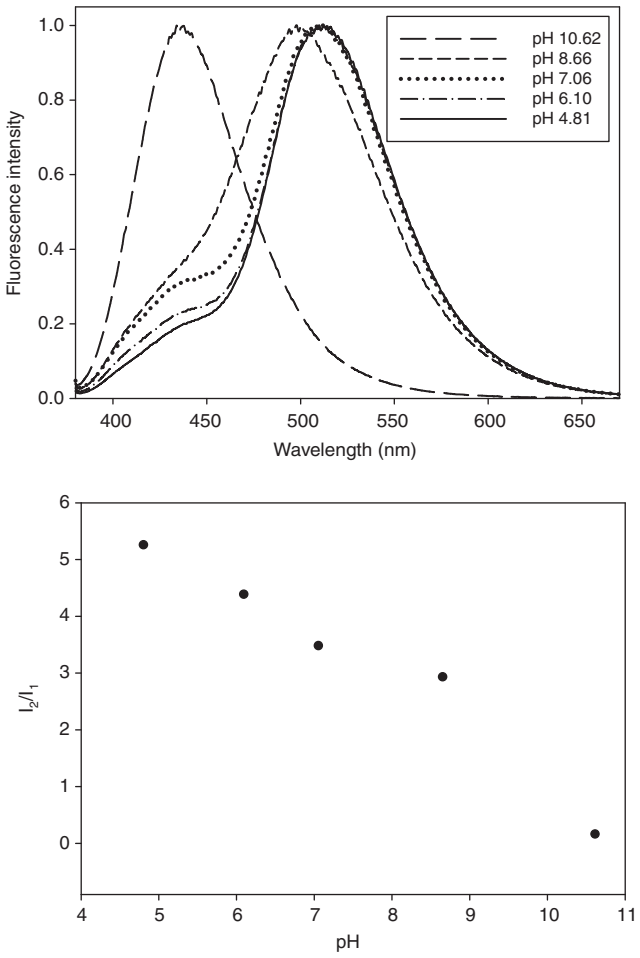


Figure 2. Emission spectra of compound **14** at different pH values (above) and the dependence of I_2/I_1 on pH (below).

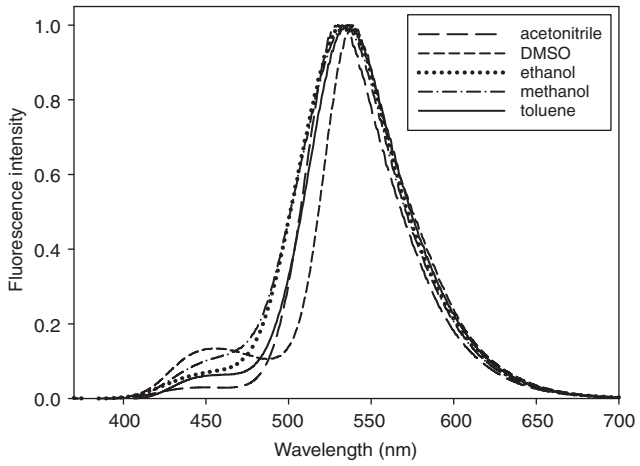


Figure 3. Emission spectra of compound **14** in different solvents.

16). The pH experiments with a selected compound **14** proved the sensitivity to the pH environment. The ratio of maxima intensities (I_2/I_1) for compound **14** decreased with increasing pH. The final conclusion in terms of possible practical uses of 3HQCs as fluorescent labels is as follows: (1) 3HQs can be appended to a biomolecule via a spacer attached to the carboxamide group at positions 7 or 6 (preferably 7) without the loss of the two-band emission properties, (2) the spacer character does not influence the fluorescence properties, and (3) 3HQ7Cs have potential as pH probes.

Next, efforts will be invested in the studies of suitable substitution of the phenyl at position 2 to find fluorescent labels with better spectral properties and their evaluation with respect to all necessary parameters required for a good fluorescent probe.

Acknowledgment

The authors are grateful to the Ministry of Education, Youth and Sport of the Czech Republic for the Grant MSM6198959216 and to the EEA/Norway grant A/CZ0046/1/0022. Infrastructural part of this project (Institute of Molecular and Translational Medicine) was supported from the Operational Programme Research and Development for Innovations (project CZ.1.05/2.1.00/01.0030).

References and notes

1. Yushchenko, D. A.; Bilokin', M. D.; Pyvovarenko, O. V.; Duportail, G.; Mely, Y.; Pivovarenko, V. G. *Tetrahedron Lett.* **2006**, *47*, 905–908.
2. Yushchenko, D. A.; Shvadchak, V. V.; Klymchenko, A. S.; Duportail, G.; Mely, Y.; Pivovarenko, V. G. *New J. Chem.* **2006**, *30*, 774–778.
3. Klymchenko, A. S.; Pivovarenko, V. G.; Ozturk, T.; Demchenko, A. P. *New J. Chem.* **2003**, *27*, 1336–1343.
4. Klymchenko, A. S.; Demchenko, A. P. *Phys. Chem. Chem. Phys.* **2003**, *5*, 461–468.
5. Klymchenko, A. S.; Duportail, G.; Mely, Y.; Demchenko, A. P. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 11219–11224.
6. Shynkar, V. V.; Klymchenko, A. S.; Duportail, G.; Demchenko, A. P.; Mely, Y. *Biochim. Biophys. Acta* **2005**, *1712*, 128–136.
7. Klymchenko, A. S.; Avilov, S. V.; Demchenko, A. P. *Anal. Biochem.* **2004**, *329*, 43–57.
8. Sytnik, A.; Gormin, D.; Kasha, M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11968–11972.
9. Poteau, X.; Saroja, G.; Spies, C.; Brown, R. G. *J. Photochem. Photobiol.* **2004**, *162*, 431–439.
10. Pivovarenko, V. G.; Valdzyuk, O. B.; Kosterin, S. O. *J. Fluoresc.* **2006**, *16*, 9–15.
11. Yushchenko, D. A.; Shvadchak, V. V.; Klymchenko, A. S.; Duportail, G.; Pivovarenko, V. G.; Mely, Y. *J. Phys. Chem. A* **2007**, *111*, 8986–8992.
12. Bilokin, M. D.; Shvadchak, V. V.; Yushchenko, D. A.; Klymchenko, A. S.; Duportail, G.; Mely, Y.; Pivovarenko, V. G. *Tetrahedron Lett.* **2009**, *50*, 4714–4719.
13. Bilokin, M. D.; Shvadchak, V. V.; Yushchenko, D. A.; Duportail, G.; Mely, Y.; Pivovarenko, V. G. *J. Fluoresc.* **2009**, *19*, 545–553.
14. Soural, M.; Krchnak, V. *J. Comb. Chem.* **2007**, *9*, 793–796.
15. Melhuish, W. H. *J. Phys. Chem.* **1961**, *65*, 229–235.