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ORIGINAL PAPER

Purine Scaffold Effect on Fluorescence Properties of Purine-Hydroxyquinolinone Bisheterocycles

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Abstract The fluorescence properties of bisheterocyclic compounds that contain purine and the 3-hydroxyquinolin-4(1*H*)-one skeleton connected with an aliphatic spacer of a different length/structure (3HQP) were examined. It was found that the introducing of the spacer-purine scaffold led in the comparison to 3HQs themselves to (1) the possibility of the effectual excitation in the wider range of excitation wavelengths, moreover, some derivatives can be excited at relatively high wavelengths around 400 nm, (2) the lowering of the quantum yield and (3) the slight longer wavelength shift of the dual emission spectra. Tested organic solvents did not affect significantly the 3HQP fluorescence properties. The characters of emission spectra as well as the quantum yields of 3HQPs were notably influenced by the ratio of water and DMSO in their composed mixture applied as a solvent. With increasing water content in the mixture both I_1/I_2 and the quantum yield decreased.

Keywords 3-hydroxyquinolones · Purine-hydroxyquinolinone · Dual fluorescence probes

Introduction

The fluorescence properties of 3-hydroxyquinolin-4(1*H*)-ones (3HQ) has been already described and suggested as derivatives applicable in labelling of biomolecules [1–7]. Generally, 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. Because the emission spectra of 3HQs usually exhibit two well-separated local maxima, they are interesting as potential novel fluorescent labels with dual fluorescence. Because 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, studies of the fluorescent properties of the 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, 7]. The spacers were attached at two different locations: (1) at the carboxamide group at positions of the 6–8 of hydroxyquinolinone skeleton [6] and (2) *via* the phenyl ring at position 2 of 2-(4-aminosubstituted-3-nitrophenyl)-3HQs [7]. And it was found that the 3HQs can be appended to a biomolecule *via* a spacer attached to the carboxamide group at positions 7 or 6 (preferably 7) without loss of the two-band emission

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properties. In the second case, the presented results show that introducing substituted alkyl groups (with terminal amino or hydroxyl groups) led mostly to increased fluorescence quantum yields and the shape of the emission spectra (for compounds with aminoalkyl chains as the spacer) was influenced by the carbon chain length as well [7].

In current research we have turned our attention to labelling of nucleic acids components, which have a potential in development of new diagnostic kits. This work is the first one, which describes fluorescence properties of 3HQs directly connected to natural heterocyclic skeleton. This paper deals with the effect of variously substituted purines connected to 3HQ skeleton with aliphatic spacer on the fluorescences properties of such system (Scheme 1) and answers the question how can the spacer-purine scaffold influence the fluorescence properties of 3HQs.

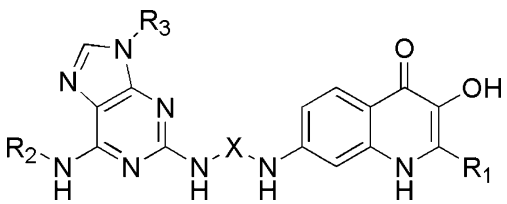
Experimental

The synthesis of examined 3HQPs (Table 1) was described elsewhere [8]. With a view to the investigation of the influence of the spacer-purine scaffold on fluorescence properties of 3HQP the spectroscopic data for appropriate 3HQs were measured as well (**1**, **6**, **13**, **18**, **22**, the synthesis of compounds **1** and **22** have been described elsewhere [9, 10], and derivatives **6**, **13** and **18** were prepared according to the previously described procedure [11, 12]).

The absorbance, excitation and emission fluorescence spectra of were measured by the spectrophotometer Helios alpha (Unicam) and the fluorescence spectrometer Cary Eclipse (Varian). The excitation wavelengths for individual substance are listed in Table 2. And 100 μg/mL solution of individual compound were measured.

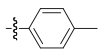
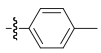
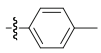
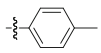
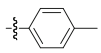
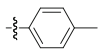
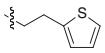
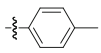
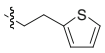
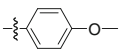
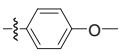
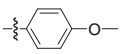
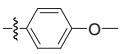
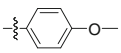
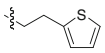
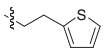
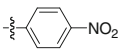
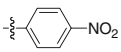
Results and Discussion

The excitation spectra (Fig. 1, Table 2) show that the excitation maximum wavelengths move in the range from 350 to 420 nm. The excitation spectra of the 3HQs show mostly several relatively narrow distinctive local maxima (Fig. 1).



Scheme 1 The general structure of the studied 3HQPs

Table 1 List of investigated 3HQPs

Compound	R_1	R_2	R_3	X
1	CH_3	-	-	-
2	CH_3		CH_2CH_3	$(CH_2)_4$
3	CH_3			$(CH_2)_2O(CH_2)_2$
4	CH_3	$(CH_2)_2CH_3$		$(CH_2)_4$
5	CH_3	$(CH_2)_2CH_3$		$(CH_2)_4$
6		-	-	-
7				$(CH_2)_4$
8			CH_2CH_3	$(CH_2)_3$
9		$(CH_2)_3OH$		$(CH_2)_2O(CH_2)_2$
10		$(CH_2)_2CH_3$	CH_2CH_3	$(CH_2)_3$
11			CH_2CH_3	$(CH_2)_3$
12			CH_2CH_3	$(CH_2)_4$
13		-	-	-
14				$(CH_2)_3$
15		$(CH_2)_3OH$		$(CH_2)_3$
16		$(CH_2)_2CH_3$		$(CH_2)_3$
17				$(CH_2)_3$
18		-	-	-
19			CH_2CH_3	$(CH_2)_3$
20		$(CH_2)_2CH_3$		$(CH_2)_3$
21				$(CH_2)_4$
22		-	-	-
23		$(CH_2)_2CH_3$		$(CH_2)_3$
24				$(CH_2)_4$

From the 3HQPs excitation spectra, in contrast to the 3HQs, it is evident that an effectual excitation is possible in the wider range of excitation wavelengths. And moreover, some derivatives can be excited at relatively high wavelengths around 400 nm. This fact is profitable with respect to the usability in biological applications when the fluorescence of intristic fluorophors as well as UV radiation effect on biosystems have to be taken into account.

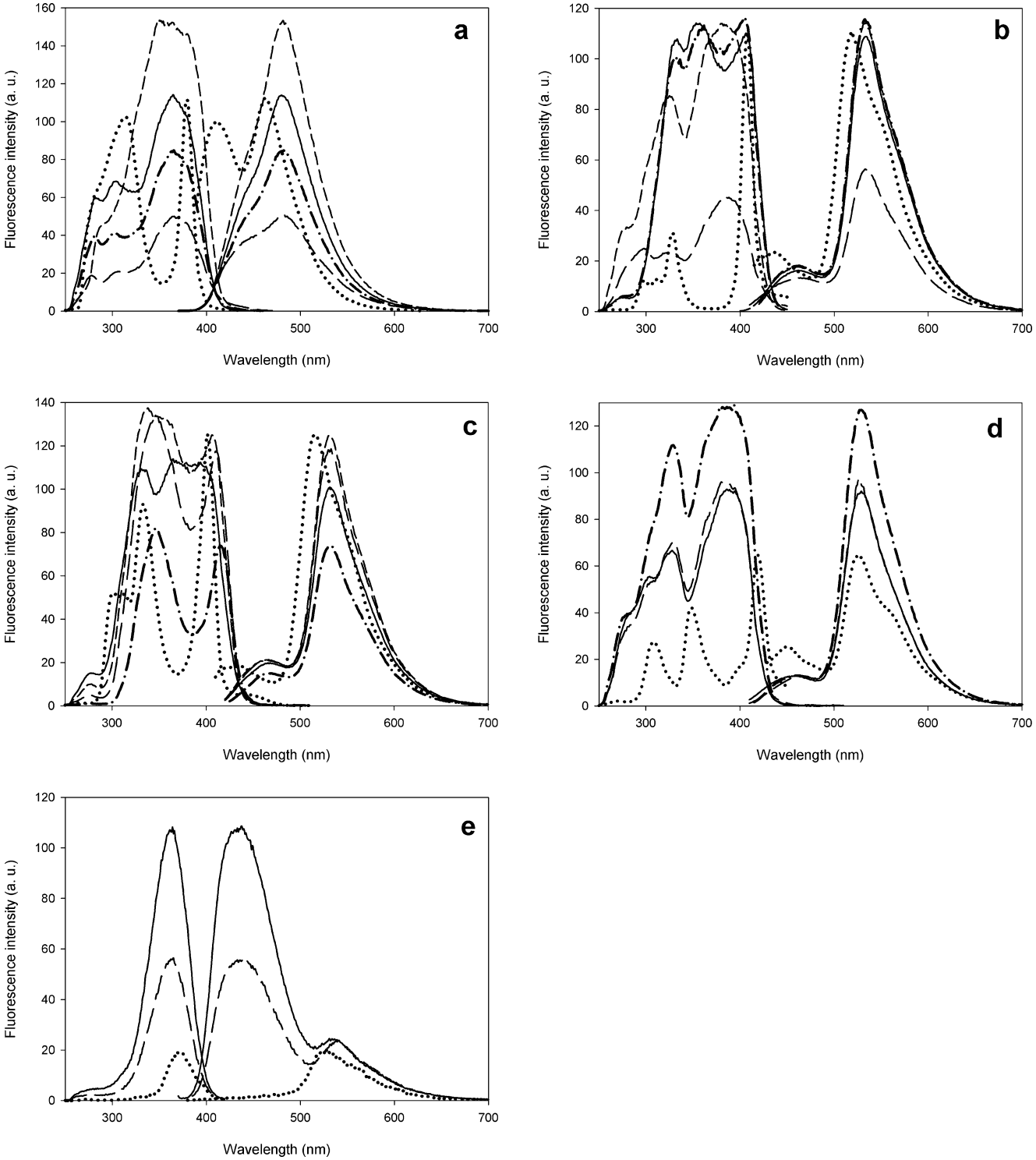


Fig. 1 Excitation and emission spectra of 3HQPs in DMSO. **a** (•••) 1; (—) 2; (– –) 3; (– • –) 4; (—) 5; **b** (•••) 6; (– • –) 7; (—) 8; (– –) 9; (—) 10; **c** (•••) 13; (– –) 14; (—) 15; (—) 16; (– • –) 17; **d** (•••) 18; (—) 19; (– • –) 20; (– –) 21; **e** (•••) 22; (– –) 23; (—) 24

The difference (Stokes shift) between the longer excitation wavelength and the closer emission maxima wavelength remains still sufficient for the measurement of fluorescence intensity at lower wavelength maximum of the emission spectrum.

Generally, the introducing of the spacer-purine scaffold to 3HQ skeleton in the comparison to appropriate single 3HQ led to a slight longer wavelength shift of the dual emission spectra (approximately 15 nm, Fig. 1). In the case of the 2-methyl 3HQ derivatives (**2–5**, **9**) the emission spectra lost the dual

Table 2 Spectroscopic properties of the 3HQPs in DMSO

Compound	$\lambda_{ex,max}$(nm)[a]	$\lambda_{em,1}$ (nm)[b]	$\lambda_{em,2}$ (nm)[c]	I_1/I_2[d]	φ (%)[e]
1	380	412	462	0.89	49.73
2	364	–	480	–	22.98
3	364	–	440	–	17.80
4	365	–	441	–	22.93
5	350	–	481	–	53.27
6	407	438	518	0.21	35.97
7	406	461	533	0.16	37.53
8	381	461	531	0.16	20.94
9	384	–	533	–	11.39
10	353	462	534	0.15	34.28
11	363	462	533	0.15	27.52
12	358	463	532	0.17	33.19
13	402	433	515	0.14	43.00
14	347	466	531	0.18	47.53
15	365	467	531	0.21	28.99
16	338	464	531	0.17	39.77
17	347	463	531	0.20	31.46
18	420	450	525	0.38	24.75
19	385	462	529	0.14	18.73
20	394	462	529	0.11	18.60
21	380	464	529	0.14	22.04
22	371	–	528	–	0.01
23	365	439	538	2.33	0.45
24	364	437	536	4.50	0.74

[a] λ_{ex}, excitation maximum wavelength

[b] $\lambda_{em,1}$, the fluorescence lower wavelength emission maximum

[c] $\lambda_{em,2}$, the fluorescence higher wavelength emission maximum

[d] I_1/I_2, the ratio of fluorescence maxima intensities

[e] φ, fluorescence quantum yield (determined with quinine sulphate in 0.5 M H_2SO_4 (φ=0.577 [13]), taken as a reference fluorescence standard)

fluorescence character that means the lower wavelength maxima merged in the second local maxima and became extinguished (Fig. 1a). The opposite effect was observed for 2-(4-nitrophenyl) quinolinones—the compound **22** exhibited only single-band emission spectrum with the maximum at 528 nm whereas for the 3HQPs **23** and **24** dual fluorescence were recorded (Fig. 1e).

Mostly, a decrease in the quantum yield after the introducing of the spacer-purine scaffold (with some exceptions such as compounds **5**, **7**, **14**, **23** and **24**) was observed. An interesting positive influence of the connection of spacer-purine scaffold on the quantum yield was recorded for compounds with 2-(4-nitrophenyl) as R_1 (**22**–**24**). Despite of the fact that the quantum yield of **22**–**24** grew markedly (more than 40 times) the value of the quantum yield remained all along too low (up to 0.74%). Although the fluorescence data only for two compounds with ethoxyethyl as the spacer (**3** and **9**) are at the disposal it seems that its presence in the 3HQP molecule led to the decrease in quantum yield. This observation has been also proved earlier in the work dealing with the effect of the spacer on 3HQ fluorescence properties [6].

The effect of solvents such as methanol, acetonitrile, dimethyl sulfoxide, ethylacetate and toluene on the emission spectrum of compounds **10**, **14, 20** and **24** was not significant and any obvious relationship between polarity and the ratio of maxima intensities was not found (Table 3). Nevertheless, water/DMSO 1:1 used as a solvent significantly changed the character of the emission spectra (see the emission spectra for the compound **10** in Fig. 2). The emission spectra lost the dual character and exhibit only one maximum at longer wavelengths. Therefore the relationship between the fluorescence properties of selected representatives (**10**, **14, 20** and **24**) and water content in solvent was studied in detail.

The ratio of fluorescence intensity at local maxima I_1/I_2 decreased with increasing water content (expressed as a ratio of volumes of water and DMSO, V_{aq}/V_{DMSO}). The dependence between the ratio I_1/I_2 and V_{aq}/V_{DMSO} reached a plateau at V_{aq}/V_{DMSO} of 0.46 and with increasing V_{aq}/V_{DMSO} the emission maxima ratio did not change notably (Fig. 3). The quantum yields of these representatives were meaningfully affected by solvents (Table 3). Generally, the highest quantum yield was observed for DMSO (**10**, **24**) and/or DMSO:water V_{aq}/V_{DMSO} of 0.07 (**14**, **20**) and the lowest for methanol and/or other DMSO: water solutions. With increasing water content, i. e. with increasing V_{aq}/V_{DMSO} ratio, the quantum yields of all selected representatives gradually decreased and in the case of compound **10** its quantum yield lowered even almost

Table 3 Spectroscopic properties of compounds **10**, **14**, **20** and **24** in various solvents

Compound	**10**		**14**		**20**		**24**	
Solvent	I_1/I_2	φ (%)	I_1/I_2	φ (%)	I_1/I_2	φ (%)	I_1/I_2	φ (%)
MeCN	0.1075	21.80	0.0884	24.20	0.0735	14.46	3.48	0.52
DMSO	0.1500	34.28	0.1793	47.53	0.1055	18.60	4.46	0.74
EtOAc	0.1159	24.69	0.0939	26.53	0.0895	13.98	2.97	0.55
MeOH	0.1701	25.97	0.01312	28.55	0.1167	15.80	4.67	0.60
Toluene	0.1611	32.34	0.1276	36.56	0.1402	16.16	3.50	0.69
0.07[a]	0.1359	30.71	0.1100	49.74	0.1014	18.94	6.25	0.72
0.23[a]	0.0749	26.58	0.0743	45.20	0.0738	17.31	5.39	0.62
0.45[a]	0.0618	20.89	0.0656	39.47	0.0645	15.62	4.66	0.46
0.78[a]	0.0592	15.84	0.0628	32.25	0.0605	12.57	4.06	0.37
1.29[a]	0.0618	10.30	0.0662	23.68	0.0639	9.50	3.83	0.32

[a] the volumetric ratio of water and DMSO V_{aq}/V_{DMSO}

three times (from 34.28 to 10.30%). Possible explanations of described facts relating to the effect of the presence of water in solvent can consist of (1) hydrophobic interactions of the spacer resulting in the convolution of the 3HQP molecule and/or (2) a protic disruption of intra as well as inter molecular hydrogen bonds of 3HQs. The presence of hydrogen bonds has been already confirmed by X-ray diffraction analysis [14]. This eventual processes can affected the ratio of tautomeric forms of 3HQs [1, 3] responsible for the dual character of the emission spectra for the benefit of that causing the fluorescence emission at higher wavelengths. Unfortunately, compounds **3** and **9** that contain ethoxyethyl chain as the spacer and they should not be prone to the molecule convolution in aqueous environment exhibited only single-band emission spectra. And therefore a comparison of fluorescence properties of compounds with hydrophobic and hydrophilic spacers cannot be performed. The 3HQPs with these fluorescence properties, i. e. the variable ratio I_1/I_2 and the quantum yield depending on water content, could be utilized as the water-presence sensitive indicator, nevertheless, such application would require extensive research in some complex samples exceeding the intention of this work.

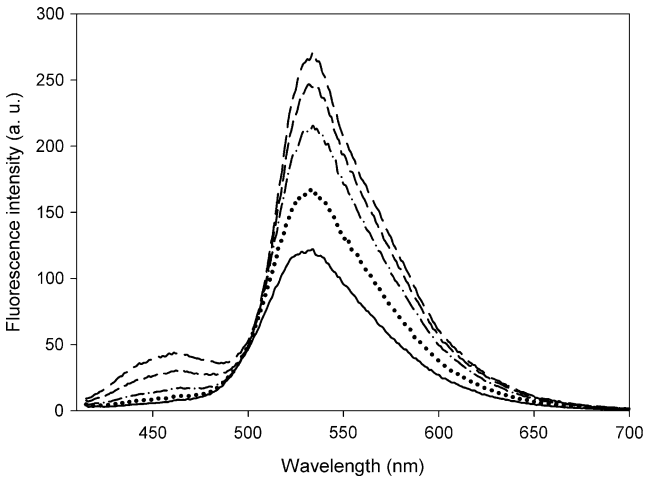


Fig. 2 Fluorescence emission spectra of compound 10 in water-DMSO mixture of various composition. (– –) V_{aq}/V_{DMSO}=0; (—) V_{aq}/V_{DMSO}=0.07; (– • –) V_{aq}/V_{DMSO}=0.23; (•••) V_{aq}/V_{DMSO}=0.45; (—) V_{aq}/V_{DMSO}=0.78

Conclusion

The next step of the biomolecule-fluorescent label system development has been performed (the initial study of the spacer location effect has been described earlier [6, 7]). The fluorescence properties of variously substituted purines connected to 3HQ skeleton by aliphatic spacer were studied in order to investigate how the spacer-purine scaffold influences the fluorescence of the eventual labelled nucleotide. It was found that the introducing of the spacer-purine scaffold led to (1) the possibility of the effectual excitation in the wider range of excitation wavelengths, moreover, some derivatives can be excited at relatively high wavelengths around 400 nm, (2) the lowering of the quantum

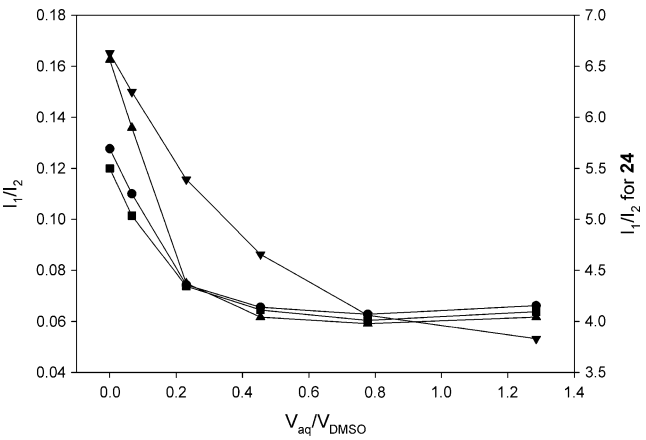


Fig. 3 Emission maxima intensity ratio I_1/I_2 depending on V_{aq}/V_{DMSO}. ▲10; ● 14; ■ 20; ▼24

yield (with some exceptions such as compounds **5**, **7**, **14**, **23** and **24**) and (3) the slight longer wavelength shift of the dual emission spectra. Although tested organic solvents did not affect significantly the 3HQP fluorescence properties, the characters of emission spectra as well as the quantum yields of 3HQPs were notably influenced by the composition of water-DMSO mixture applied as a solvent. With increasing water content in the mixture both I_1/I_2 and the quantum yield decreased. The measured data show the potential of 3HQs to be applied for the fluorescent labelling of compounds containing the purine skeleton. Moreover, the sensitivity of 3HQPs to protic environment could be utilized in some biological applications where the character of microenvironment needs to be evaluated.

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12. Analytical data of compounds **6**, **13** and **18**: **6**: ms $[M+H]^+$= 268.3. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.84 (s, 3 H) 7.12 (d, *J*=8.78 Hz, 2 H) 7.26 (t, *J*=7.14 Hz, 1 H) 7.57 (t, *J*=7.68 Hz, 1 H) 7.72 (d, *J*=8.42 Hz, 1 H) 7.78 (d, *J*=8.97 Hz, 2 H) 8.14 (dd, *J*=8.14 Hz, 1 H) 11.49 (br. s., 1 H). ^{13}C NMR (DMSO-d_6) δ ppm 55.9, 114.3, 118.9, 122.2, 122.3, 124.9, 130.9, 131.2, 131.9, 138.1, 138.5, 160.5, 170.3; **13**: ms $[M+H]^+$=252.3. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.91 (s, 3 H) 7.26 (t, *J*=7.50 Hz, 1 H) 7.37 (d, *J*=8.05 Hz, 2 H) 7.58 (t, *J*=7.59 Hz, 1 H) 7.72 (m, 3 H) 8.15 (d, *J*=8.05 Hz, 1 H) 11.52 (br. s., 1 H). ^{13}C NMR (DMSO-d_6) δ ppm 21.6, 118.9, 122.2, 122.3, 124.9, 129.4, 130.0, 131.0, 132.0, 138.2, 138.5, 139.4, 170.5; **18**: ms $[M+H]^+$=244.3. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.19–7.36 (m, 2 H) 7.60 (t, *J*=7.32 Hz, 1 H) 7.73–7.91 (m, 2 H) 8.04 (d, *J*=3.29 Hz, 1 H) 8.12 (d, *J*=7.87 Hz, 1 H) 11.27 (br. s., 1 H). ^{13}C NMR (DMSO-d_6) δ ppm 118.8, 122.2, 122.3, 124.9, 126.6, 127.3, 127.9, 130.9, 131.4, 133.4, 137.6, 138.7, 170.5
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