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2,6,8-Trisubstituted 3-Hydroxychromone Derivatives as Fluorophores for Live-Cell Imaging

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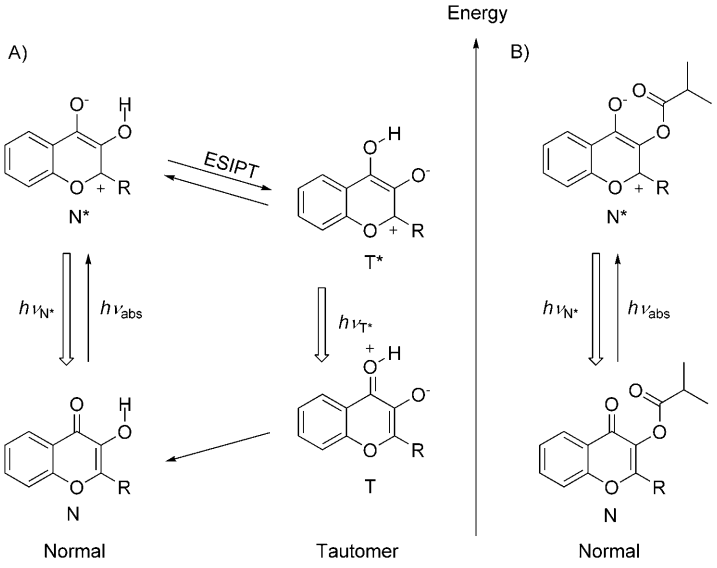
Abstract: We present the synthesis and photophysical characterisation of a series of structurally diverse, fluorescent 2,6,8-trisubstituted 3-hydroxychromone derivatives with high fluorescence quantum yields and molar extinction coefficients. Two of these derivatives (**9** and **10a**) have been studied as fluorophores for cellular imaging in HeLa cells and show excellent permeability and promising fluorescence properties in a cellular environment. In addition, we have demonstrated by photophysical characterisation of 3-isobutyroxychromone derivatives that esterification of the 3-hydroxyl group results in acceptable and useful fluorescence properties.

Keywords: cellular imaging · excited-state intramolecular proton transfer (ESIPT) · fluorescence · fluorophores · hydroxychromones

Introduction

The development of new, unique fluorophores for cellular studies is of significant importance in the field of chemical biology. Such compounds can provide information on a broad range of molecular processes and properties including binding interactions, DNA sequencing, conformational changes and cellular substructures.[1–3] In a project aimed at the development of novel chromone-based bioactive compounds, we came across a series of 3-hydroxychromone derivatives that showed interesting fluorescence properties. 3-Hydroxychromones are fluorophores that exhibit dual fluorescent behaviour originating from the normal excited form (N*) and from the phototautomer (T*) formed by means of an excited-state intramolecular proton transfer (ESIPT; Scheme 1A).[4–6] Due to their characteristic photophysical behaviour, 3-hydroxychromone derivatives have been used as biosensors,[7] hydrogen-bonding sensors,[8] fluorescent probes for dipotential (Ψ_D) measurements in lipid bilayers[9–13] and as photochemical dyes for protein labelling and apoptosis.[14–16] Recently, a spermine-conjugated 3-hydroxy-



Scheme 1. A) Photophysical cycle of 3-hydroxychromones. B) The ground and excited state of 3-isobutyroxychromones.

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chromone derivative was shown to change its fluorescence behaviour when interacting with double-stranded but not single-stranded DNA.[17] The 3-hydroxychromones have also been studied theoretically using semi-empirical calculations.[18] In addition, the chromone ring system has been defined as a privileged structure in drug discovery[19] due to its use in a wide variety of pharmacologically active compounds such as anti-cancer,[20] anti-HIV[21] and anti-inflammatory agents.[22]

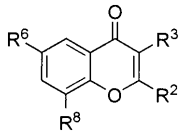
In our search for small organic compounds useful in studies of cellular processing, we are particularly interested in inherently fluorescent compounds. In the present study, we have therefore analysed the fluorescence properties of a series of 2-substituted 3-hydroxychromone derivatives (**1**–**10**, Table 1).[23,24] The compounds were chosen to allow an investigation of how various substituents in the 2-position will affect the ESIPT process (Scheme 1). The series therefore comprises compounds containing both powerful electron-withdrawing substituents such as in 2-(4-trifluoromethylphenyl)-3-hydroxychromone (**5**), and strongly electron-donating groups such as 2-(4-diethylaminophenyl)-3-hydroxychromone derivatives (e.g., **7a**,**b** and **10a**,**b**), earlier shown to have interesting fluorescence properties.[25] In addition, the photophysical properties for a series of 3-isobutyroxychromone derivatives (**11** and **12**) were characterised to compare the fluorescence behaviour in the absence of a free, unprotected hydroxyl group in the 3-position. Finally, two fluorescent compounds with a more favourable solubility profile (**9** and **10a**) containing a 3-amino-1-propynyl substituent in the 8-position have been used for live-cell imaging. Fluorescence microscopy in HeLa cells showed that these compounds exhibit excellent permeability properties resulting in a rapid uptake, no toxicity and good fluorescence properties in a cellular environment.

Results and Discussion

In previous studies, we have developed an efficient strategy for the synthesis of a 3,6,8-trisubstituted chromone scaffold combined with a convenient introduction of different aromatic or conjugated substituents in the 2-position.[23,24] The synthetic strategy was based on the use of 6,8-dihalogenated 3-hydroxychromones, thereby allowing regioselective introduction of various substituents by means of palladium-mediated chemistry to further improve their properties as diverse ligands acting on different receptors/enzymes.

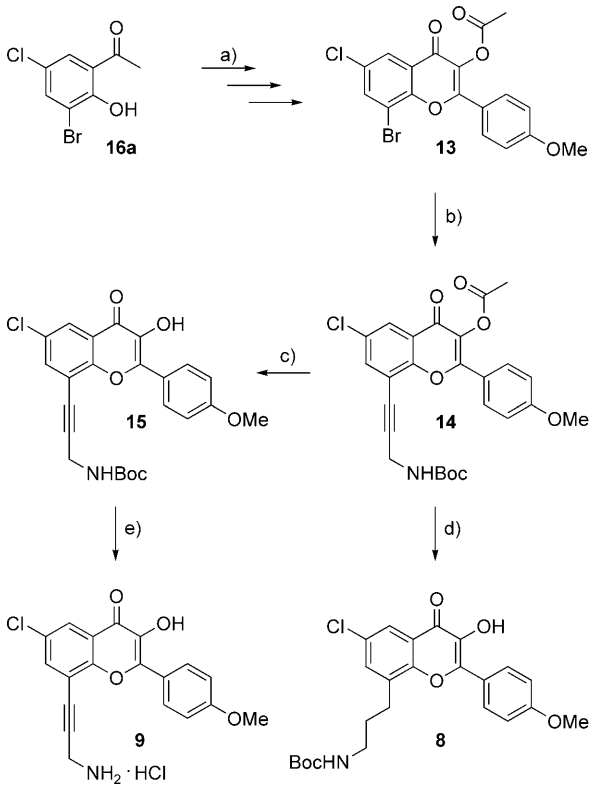
Synthesis: Our synthetic procedure to chromones **1**–**6** (Table 1) and **13** (Scheme 2) has been reported earlier.[23,24] Chalcones were prepared by means of an aldol condensation of commercially available 3-bromo-5-chloro-2-hydroxyacetophenone (**16a**, Scheme 2) and the appropriate aldehyde in high yields using KOH in EtOH. Efficient cyclisation with NaOH and H_2O_2 in THF/MeOH afforded the 3-hydroxychromone derivatives in yields between 57 and 88%. To facilitate the flash chromatographic purification on silica gel, the hydroxyl group was acetylated as in **13**. Compound **14** was synthesised in 78% yield from **13** by means of a Sonogashira cross-coupling reaction using $[PdCl_2(PPh_3)_2]$, CuI, NEt_3 and Boc-protected propargylamine in dry THF under microwave conditions at 120°C (Scheme 2). The acetyl ester in **14** was hydrolysed to provide **15** using NaOMe in MeOH followed by *tert*-butoxycarbonyl (Boc)-deprotection with 3N

Table 1. Photophysical data of 2,6,8-trisubstituted 3-hydroxychromones **1**–**10** and 2,6,8-trisubstituted 3-isobutyroxychromones **11**–**12**.[a]

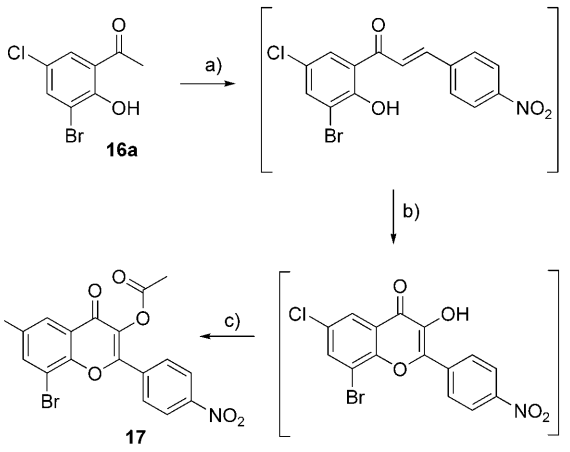


Compound	R^2	R^3	R^6	R^8	λ_{abs} [nm]	λ_{em} [nm][b] N*	T*	I_{N^*}/I_{T^*}	ε [$M^{-1}cm^{-1}$]	Φ_F
1	phenyl	OH	Cl	Br	355	435	550	0.05	12000	0.07
2	4-MeO-Ph	OH	Cl	Br	370	446	554	0.28	23000	0.06
3	2-thienyl	OH	Cl	Br	372	438	560	0.07	19000	0.09
4	3-thienyl	OH	Cl	Br	358	434	548	0.03	17000	0.13
5	4-CF_3-Ph	OH	Cl	Br	354	436	553	0.13	14000	0.03
6	CH=CHPh	OH	Cl	Br	381	446	544	0.24	12000	0.05
7a	4-NEt_2-Ph	OH	Cl	Br	438	564	–	–	27000	0.43
7b	4-NEt_2-Ph	OH	Br	Br	439	565	–	–	27000	0.42
8	4-MeO-Ph	OH	Cl	$(CH_2)_3NHBoc$	362	434	542	0.33	16000	0.07
9	4-MeO-Ph	OH	Cl	$C\equiv CCH_2NH_2$	372	444	556	0.14	14000	0.10
10a	4-NEt_2-Ph	OH	Cl	$C\equiv CCH_2NH_2$	441	568	–	–	21000	0.49
10b	4-NEt_2-Ph	OH	Br	$C\equiv CCH_2NH_2$	443	570	–	–	18000	0.48
11a	4-NEt_2-Ph	$OCOCH(CH_3)_2$	Cl	Br	417	556	–	–	36000	0.04
11b	4-NEt_2-Ph	$OCOCH(CH_3)_2$	Br	Br	418	560	–	–	32000	0.06
12a	4-NEt_2-Ph	$OCOCH(CH_3)_2$	Cl	$C\equiv CCH_2NHBoc$	420	554	–	–	33000	0.12
12b	4-NEt_2-Ph	$OCOCH(CH_3)_2$	Br	$C\equiv CCH_2NHBoc$	420	553	–	–	30000	0.12

[a] Photophysical data measured in 95% ethanol. [b] Emission values originating from the normal excited form (N*) and from the phototautomer (T*) formed by means of an excited-state intramolecular proton transfer (ESIPT) (see Scheme 1).



Scheme 2. Synthesis of 6,8-disubstituted 2-(4-methoxyphenyl)-3-hydroxychromones: a) i) 4-methoxybenzaldehyde, KOH, EtOH, 50 °C→RT, overnight; ii) NaOH (aq) (4 M), H_2O_2 (aq) (30 %), MeOH/THF (1:1), 0 °C→RT, overnight; iii) acetyl chloride, NEt_3, CH_2Cl_2, RT, overnight; b) $[PdCl_2(PPh_3)_2]$, NEt_3, CuI, *N*-Boc-propargylamine, THF, 120 °C, 20 min, microwave heating (78 %); c) NaOMe, MeOH, RT, 5 h (95 %); d) H_2, 5 % Pd/C, MeOH/dioxane, RT, 15 h (80 %); e) HCl, MeOH, RT, 6 h (97 %).



Scheme 3. Synthesis of 2-(4-nitrophenyl)-3-hydroxychromones: a) 4-nitrobenzaldehyde, KOH, EtOH, RT, overnight; b) NaOH (aq) (4 M), H_2O_2 (aq) (30 %), MeOH/THF (1:1), 0 °C→RT, overnight; c) acetyl chloride, Et_3N, DMF, RT, 24 h.

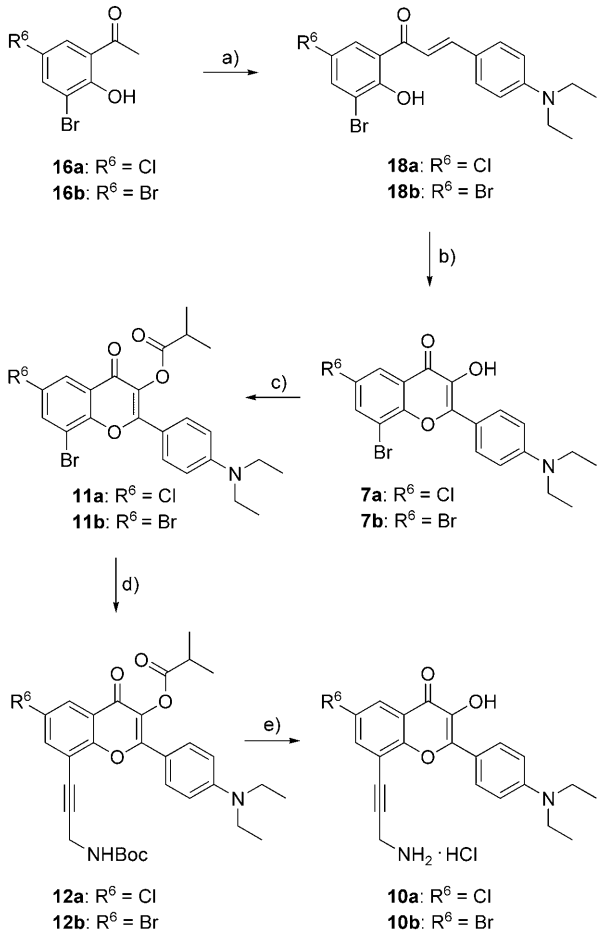
HCl in MeOH to afford **9** (Scheme 2). Interestingly, the propargyl group in **14** could be reduced using catalytic hydrogenation without disturbing the chromone system, thus producing **8** in 80 % yield.

Initial attempts to synthesise the 2-(4-diethylaminophenyl)-3-hydroxychromone derivatives **7a**,**b** and **10a**,**b** (Table 1) were performed using the same synthetic route as described above for the synthesis of **1**–**6**.[23] However, formation of the chalcone intermediate failed. Other attempts using $Ba(OH)_2$ in MeOH[26] or NaOMe in DMF[27] were also performed but unfortunately did not result in any product formation. We therefore continued toward the 2-(4-diethylaminophenyl)-3-hydroxychromone derivatives using an alternative synthetic strategy starting from 4-nitrobenzaldehyde (Scheme 3) with the aim of later converting the nitro group to diethylamine by means of reduction and subsequent N-alkylation.

Thus, acetophenone **16a** (Scheme 3) was reacted with 4-nitrobenzaldehyde by means of an aldol condensation using KOH in EtOH. The chalcone intermediate was cyclised using H_2O_2 (30 %) and 4 M NaOH in MeOH/THF. The generated 3-hydroxychromone derivative was acetylated with acetyl chloride and triethylamine in DMF to give **17** in 15 % yield (over three steps). Compound **17** was prepared without any characterisation of the intermediates due to severe solubility problems. Unfortunately, the subsequent Sonogashira cross-coupling reaction in the 8-position did not result in any product formation, regardless of reaction conditions used. Based on this negative result together with the low solubility of the compounds, we decided to abandon the synthetic strategy by means of the nitro derivatives.

Instead a new route was adopted by which compounds **18a**,**b** were prepared successfully from 4-diethylaminobenzaldehyde and two different dihalogenated acetophenones (**16a**,**b**) by means of aldol condensation using aqueous sodium hydroxide (60 %)[28] in MeOH (Scheme 4).[26] Cyclisation to provide **7a**,**b** was accomplished using H_2O_2 (30 %) and 4 M NaOH in MeOH/THF. The 2-(4-diethylaminophenyl)-3-hydroxychromones were first protected as acetyl esters using acetyl chloride and triethylamine in DMF, but these compounds decomposed on silica gel during the chromatographic purification. Compounds **7a**,**b** were instead protected as the more stable 3-isobutyric ester derivatives, **11a**,**b**, using isobutyric anhydride in pyridine. Compounds **12a**,**b** were then prepared by means of Sonogashira cross-coupling under microwave conditions at 120 °C, using $[PdCl_2(PPh_3)_2]$, CuI, NEt_3 and Boc-protected propargylamine in dry THF followed by purification under neutral conditions (Al_2O_3) to avoid decomposition.[29] Boc-deprotection and cleavage of the isobutyric ester, **12a**,**b**, were performed in one step using 3 N HCl in MeOH to give the highly hygroscopic **10a**,**b**.

Formation of the 2-(4-diethylaminophenyl)-3-hydroxychromone derivatives **7a**,**b** and **10a**,**b** was shown to be an arduous challenge compared to the straightforward synthesis of the 2-(4-methoxyphenyl)-3-hydroxychromone derivatives **8**, **9** and **15**. However, we have been able to successfully complete the synthesis in moderate to high yields in every step. In addition, the purification procedure has been opti-



Scheme 4. Synthesis of 6,8-disubstituted 2-(4-diethylaminophenyl)-3-hydroxy-chromones: a) NaOH (aq) (60%), 4-diethylaminobenzaldehyde, MeOH, RT, overnight (**18a**,**b**: 62 and 78%, respectively); b) NaOH, H_2O_2 (aq) (30%), MeOH/THF (1:1), 0°C→RT, overnight (**7a**,**b**: 55 and 74%, respectively); c) isobutyric anhydride, pyridine, RT, 18–36 h (**11a**,**b**: 95% and 90%, respectively); d) $[PdCl_2(PPh_3)_2]$, NEt_3, CuI, *N*-Boc-propargylamine, THF, 120°C, 20 min, microwave heating (**12a**,**b**: 67 and 16%, respectively); e) HCl, MeOH, RT, 40 h (**10a**,**b**: 82 and 99%, respectively).

mised using recrystallisation instead of flash chromatography in four of the five synthetic steps.

Photophysical characterisation: Two sets of compounds, 3-hydroxychromone derivatives (**1**–**10**, Table 1) with electron-withdrawing and -donating aromatic or conjugated substituents in the 2-position and 2-(4-diethylaminophenyl)-3-isobutyroxychromone derivatives (**11a**,**b** and **12a**,**b**, Table 1), were characterised for their photophysical properties. The majority of the 3-hydroxychromone derivatives (**1**–**6**, **8** and **9**) showed low-energy absorption maxima centred around 360 nm and two defined emission maxima representing the normal excited species (N*) and the excited tautomer (T*) centred at ≈440 and ≈555 nm, respectively. Unlike these analogues, the 2-(4-dietylaminophenyl)chromone derivatives (**7a**,**b** and **10a**,**b**) shifted the absorption (≈440 nm) and emission maxima to longer wavelengths and gave a single defined emission profile assigned to N* centred at ≈564–570 nm. This kind of single emission band has been reported earlier for 2-(4-diethylaminophenyl)-3-hydroxychromone derivatives when using ethanol as a solvent due to strong charge-transfer properties, perturbation and solvatochromism.[25] In that study, the emission maximum for 4′-diethylamino-3-hydroxyflavone was found to have much shorter wavelength (ca. 505 nm) than those observed for **7a**,**b** and **10a**,**b**. However, in studies where electron-withdrawing groups (e.g., methoxycarbonylvinyl) are attached to the chromone, both the absorption and emission maxima are significantly redshifted,[30] therefore the shift to longer wavelengths observed for our compounds was expected since Cl, Br and 3-amino-1-propynyl groups are all electron-withdrawing. The redshifted single emission band detected for **7a**,**b** and **10a**,**b** originates from the N* excited state and is a result of a solvent stabilisation of the charge-separated excited state with a positive charge on the 4′-diethylamino group and the negative charge on the carbonyl oxygen. The stabilisation has been suggested to be an effect of the highly polar and protic properties of ethanol.[25] Furthermore, the photophysical properties for the 2-(4-diethylaminophenyl)-3-hydroxychromone derivatives change with solvent polarity.[27] The excitation of the 2-(4-diethylaminophenyl)-3-hydroxychromone derivatives show lower solvent polarity dependence compared with Nile Red, a known solvatochromic dye, whereas the emission shows higher solvent polarity dependence.[31,32]

A comparison of how different substituents in the 2-position affected the fluorescence quantum yield shows that the introduction of an electron-withdrawing 4′-CF_3-phenyl group (**5**) results in the lowest quantum efficiency (Φ_F = 0.03) in the series. Introduction of a phenyl, 4′-MeO-phenyl, 2-thienyl, 3-thienyl or styryl group (**1**–**4**, **6**) shows only minor improvements (Φ_F = 0.05–0.13) of the quantum yield. However, introduction of a 4′-diethylaminophenyl substituent (**7a**,**b**) increases the quantum yield dramatically (Φ_F = 0.42–0.43) due to the strong electron-donating properties from the *para*-diethylamino group, thus facilitating charge transfer and dielectric stabilisation. The *para*-positioned methoxy group, as in **2**, does not show the same electron-donating ability, thereby leading to a considerably lower quantum yield (Φ_F = 0.06).

In comparison with the 8-bromo-analogues **2** and **7a**,**b**, the introduction of an electron-withdrawing 3-amino-1-propynyl substituent in the 8-position as in **9** and **10a**,**b** resulted in only a minor variation in absorption and emission values and in quantum yields. Similar results were also observed for the reduced 3-(*tert*-butoxycarbonylamino)propyl derivative **8**. As in previous studies, it can be noted that the intensity ratio of the dual emission bands (I_{N^*}/I_{T^*}) is tuned (from 0.03 for **4** to considerably higher values for **7** and **10** that virtually exhibit no T* emission; Table 1) by the substituent pattern on the 3-hydroxychromone system.[30,33]

To investigate the importance of the free hydroxyl group in the 3-position for the fluorescence behaviour, the 3-isobu-

tyroxychromone derivatives **11a,b** and **12a,b** were also photophysically characterised (Table 1). These derivatives are not able to exhibit dual fluorescence, ESIPT-mediated formation of T*, since the protected hydroxyl group in the 3-position lacks the intermolecular proton-transfer ability (Scheme 1B). As expected, the spectra of **11a,b** and **12a,b** exhibit only one emission band, assigned to the normal excited species (N*), in this case centred around 555 nm. The slight displacement of these emission maxima (ca. 10 nm) compared with **7a,b** and **10a,b** can be explained by the difference in electronic structure when attaching the 3-isobutyroxy group to the chromone system. Thus, the measurements on **11a,b** and **12a,b** confirm the conclusion about the emission of **7a,b** and **10a,b** originating from the N* state. In addition, when comparing **11a,b** and **12a,b** with the unprotected derivatives **7a,b** and **10a,b**, we observe higher molecular extinction values and dramatically decreased quantum yields (from Φ_F of 0.4–0.5 to 0.1) (Table 1). In spite of this, the results indicate that esterification of the 3-hydroxyl group results in compounds with acceptable and useful fluorescence properties.

All the 3-hydroxychromone derivatives (**1**–**10**) studied here display high extinction coefficient values ($\varepsilon > 12000\,M^{-1}cm^{-1}$) with the highest values for **7a,b** ($\varepsilon = 27000\,M^{-1}cm^{-1}$; see Table 1). On the contrary, as has been discussed above, the difference in fluorescence quantum yields between the derivatives is more pronounced (Table 1). The extinction coefficients for the 3-isobutyroxy-substituted derivatives (**11**, **12**) were even higher ($\varepsilon = 32000$–$36000\,M^{-1}cm^{-1}$) than those for **7a,b**, but again the quantum yields are considerably lower. Thus, the 3-hydroxy group is important for obtaining high quantum yields, but it is evidentially possible to use esterified derivatives for fluorescence studies.

To investigate the utility of 3-hydroxychromones as fluorophores for cellular imaging, the fluorescence quantum yield of two compounds, **10a,b**, which showed a more hydrophilic character, was established in water, and was found to be virtually zero. It has been shown that the fluorescence of a solution of 4′-diethylamino-3-hydroxyflavones in ethyl acetate changes with the addition of water,[34] and becomes completely quenched in pure water.[35] Instead, 2-(2-furyl)- and 2-(2-benzofuryl)-3-hydroxychromones have shown that ESIPT in water and the increase in quantum yields was especially pronounced for derivatives containing an electron-donating substituent in the 7-position.[35] However, we reasoned that the quenching in water for **10a,b** could be used as an advantage since they would not be detectable in a hydrophilic cellular environment but instead exhibit fluorescence properties when moving into more hydrophobic areas, for example, into a hydrophobic active site, a hydrophobic receptor binding pocket or into membrane structures. We therefore decided to investigate how these compounds would behave as fluorophores for live-cell imaging.

Cellular studies: Fluorescence microscopy, including live-cell imaging, is an essential tool in chemical biology and has become widely used to study cellular processes and diseases in several biomedical research areas.[2]

To explore the utility of 3-hydroxychromone derivatives as fluorophores for cellular imaging, HeLa cells were incubated with either compound **9** or **10a** in two separate experiments. The fluorescence was imaged by multiphoton laser scanning microscopy (MPLSM) using two-photon excitation (2PE). 2PE was employed as these compounds are normally excited in the UV range. 2PE will also increase cell viability and allow the use of an optimal excitation wavelength. Live-cell imaging at 37 °C showed that both compounds penetrated rapidly into the cells. The uptake was already observed within a minute with an accumulation localised between the cellular membrane and the nucleus. In Figure 1, the uptake is shown at two different time points. In

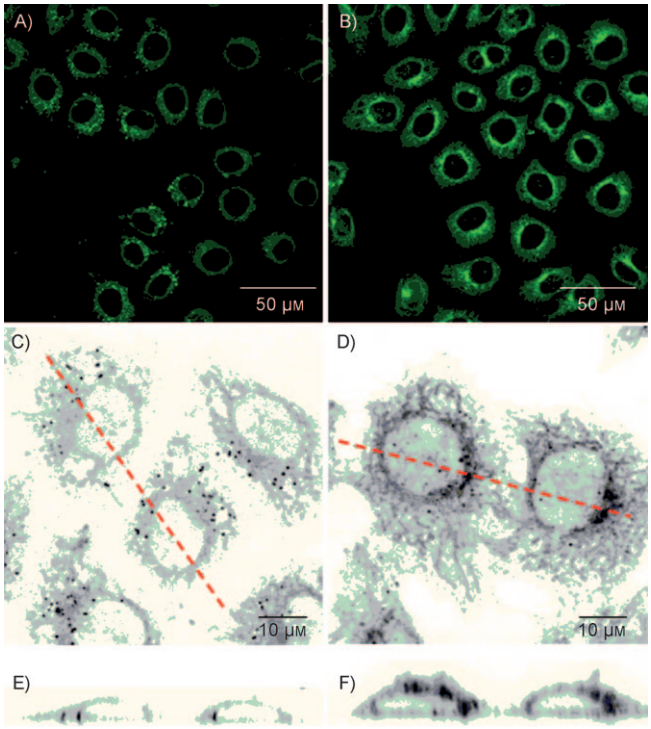


Figure 1. Two-photon excitation (2PE), live-cell imaging showing uptake of compound **9** (A, C, E) and **10a** (B, D, F). Panels A and B: Single plane, larger field of view ($215 \times 215\,\mu m^2$), after approximately 20 min. Scale bar = 50 μM. Panels C and D: Uptake after 12 min, single planes ($74 \times 74\,\mu m^2$) from the middle of 9 μm thick z stacks. Scale bar = 10 μM. Panels E and F: Cross-sections through the z stacks along the dashed lines in C and D. Panels C–F have been modified for clarity (negative picture mode).

Figure 1A and B, larger fields of view of the uptake after approximately 20 min can be seen, whereas the uptake after 12 min is shown for a few cells in Figure 1C–F. Both compounds seem to be taken up by endosomal structures, indicated by the bright punctuate structures in the images, and by weaker fluorescent membrane-network structures that resemble the endoplasmic reticulum. Furthermore, experiments at 4 °C showed no cellular uptake, thereby supporting

the hypothesis of an endocytotic mechanism.[36] Importantly, there were no signs of cytotoxic effects after incubation for two hours. Differences in photophysical properties such as extinction coefficients (ε) and quantum yields (Φ_F), indicate that the brightness ($\varepsilon\Phi_F$) of **10a** is superior in comparison with **9** (10000 vs. $1400\,M^{-1}cm^{-1}$) (cross-section values: 12 and 0.13 GM, respectively (1 GM = $10^{-50}\,cm^4\,s\,photon^{-1}\,molecule^{-1}$); see the Supporting information). Although **10a** appears more adequate as a fluorescent probe in cellular microscopy studies than **9**, both derivatives show excellent fluorescence properties in a cellular environment with high permeabilities and non-toxic profiles.

Conclusion

We have in the present study demonstrated that multifunctionalised chromone derivatives constitute a new promising class of fluorophores useful for cellular imaging. Through efficient synthetic methods we have access to a series of differently functionalised chromone derivatives in which the structure can be tuned to generate compounds with useful fluorescent properties. Two derivatives (**9** and **10a**) with acceptable and high fluorescence quantum yields demonstrate rapid permeabilities into living cells and non-toxic profiles in a cellular environment. The fluorescence quenching observed in water for **10a**,**b** gives opportunities to use these derivatives as indicators/probes for, for example, protein interactions and receptor-binding studies. Although the fluorescence properties of 3-hydroxychromone derivatives are characterised by the ability to participate in an ESIPT process, esterified 3-isobutyroxychromone derivatives also demonstrate useful fluorescence behaviour and high molar extinction coefficients.

We are presently working on establishing the exact cellular localisation (e.g., specific organelles or hydrophobic compartments) of 3-hydroxychromone derivatives **9** and **10a** with the aim of exploring their further applications in more advanced cellular studies. In addition, we currently explore the use of chromone derivatives as peptidomimetics.[37] Such studies could involve protein labelling, examination of specific binding interactions to target proteins, and/or the cellular localisation of chromone derivatives coupled to other compounds targeted for specific organelles.

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[1] J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd ed., Springer, Baltimore, **2006**.
[2] P. Lang, K. Yeow, A. Nichols, A. Scheer, *Nat. Rev. Drug Discovery* **2006**, *5*, 343–356.
[3] J. Zhang, R. E. Campbell, A. Y. Ting, R. Y. Tsien, *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 906–918.
[4] P. K. Sengupta, M. Kasha, *Chem. Phys. Lett.* **1979**, *68*, 382–385.
[5] M. Itoh, Y. Fujiwara, M. Sumitani, K. Yoshihara, *J. Phys. Chem.* **1986**, *90*, 5672–5678.
[6] M. Itoh, Y. Tanimoto, K. Tokumura, *J. Am. Chem. Soc.* **1983**, *105*, 3339–3340.
[7] K. Enander, L. Choulier, A. L. Olsson, D. A. Yushchenko, D. Kanmert, A. S. Klymchenko, A. P. Demchenko, Y. Mély, D. Altschuh, *Bioconjugate Chem.* **2008**, *19*, 1864–1870.
[8] V. V. Shynkar, A. S. Klymchenko, E. Piemont, A. P. Demchenko, Y. Mély, *J. Phys. Chem. A* **2004**, *108*, 8151–8159.
[9] A. S. Klymchenko, G. Duportail, Y. Mély, A. P. Demchenko, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 11219–11224.
[10] A. S. Klymchenko, G. Duportail, T. Ozturk, V. G. Pivovarenko, Y. Mély, A. P. Demchenko, *Chem. Biol.* **2002**, *9*, 1199–1208.
[11] G. M′Baye, V. V. Shynkar, A. S. Klymchenko, Y. Mély, G. Duportail, *J. Fluoresc.* **2006**, *16*, 35–42.
[12] G. Duportail, A. Klymchenko, Y. Mely, A. Demchenko, *FEBS Lett.* **2001**, *508*, 196–200.
[13] V. V. Shynkar, A. S. Klymchenko, G. Duportail, A. P. Demchenko, Y. Mély, *Biochim. Biophys. Acta Biomembr.* **2005**, *1712*, 128–136.
[14] S. V. Avilov, C. Bode, F. G. Tolgyesi, A. S. Klymchenko, J. Fidy, A. P. Demchenko, *Biopolymers* **2005**, *78*, 340–348.
[15] S. Ercelen, A. S. Klymchenko, A. P. Demchenko, *FEBS Lett.* **2003**, *538*, 25–28.
[16] V. V. Shynkar, A. S. Klymchenko, C. Kunzelmann, G. Duportail, C. D. Muller, A. P. Demchenko, J.-M. Freyssinet, Y. Mely, *J. Am. Chem. Soc.* **2007**, *129*, 2187–2193.
[17] A. S. Klymchenko, V. V. Shvadchak, D. A. Yushchenko, N. Jain, Y. Mély, *J. Phys. Chem. B* **2008**, *112*, 12050–12055.
[18] S. O. Yesylevskyy, A. S. Klymchenko, A. P. Demchenko, *J. Mol. Struct.* **2005**, *755*, 229–239.
[19] D. A. Horton, G. T. Bourne, M. L. Smythe, *Chem. Rev.* **2003**, *103*, 893–930.
[20] V. S. Parmar, M. E. Bracke, J. Philippe, J. Wengel, S. C. Jain, C. E. Olsen, K. S. Bisht, N. K. Sharma, A. Courtens, S. K. Sharma, K. Vennekens, V. Van Marck, S. K. Singh, N. Kumar, A. Kumar, S. Malhotra, R. Kumar, V. K. Rajwanshi, R. Jain, M. M. Mareel, *Bioorg. Med. Chem.* **1997**, *5*, 1609–1619.
[21] D. Yu, C.-H. Chen, A. Brossi, K.-H. Lee, *J. Med. Chem.* **2004**, *47*, 4072–4082.
[22] J. A. Hutter, M. Salman, W. B. Stavinoha, N. Satsangi, R. F. Williams, R. T. Streeper, S. T. Weintraub, *J. Nat. Prod.* **1996**, *59*, 541–543.
[23] K. Dahlén, E. A. A. Wallén, M. Grøtli, K. Luthman, *J. Org. Chem.* **2006**, *71*, 6863–6871.
[24] K. Dahlén, M. Grøtli, K. Luthman, *Synlett* **2006**, 897–900.
[25] P. T. Chou, M. L. Martinez, J. H. Clements, *J. Phys. Chem.* **1993**, *97*, 2618–2622.
[26] N. Desideri, C. Conti, P. Mastromarino, F. Mastropaolo, *Antiviral Chem. Chemother.* **2000**, *11*, 373–381.
[27] A. S. Klymchenko, T. Ozturk, A. P. Demchenko, *Tetrahedron Lett.* **2002**, *43*, 7079–7082.
[28] The use of a strong aqueous base (60%) is essential for the reaction.
[29] The Sonogashira coupling with **11a**,**b** requires at least 4 equiv of propargylamine since attempts using 2 equiv were performed without any product formation. Further, compound **12b** was formed from the 6,8-dibromochromone **11b** in low yields (16%) due to formation of the undesired disubstituted product.
[30] A. S. Klymchenko, Y. Mély, *Tetrahedron Lett.* **2004**, *45*, 8391–8394.
[31] M. M. Davis, H. B. Helzer, *Anal. Chem.* **1966**, *38*, 451–461.
[32] D. L. Sackett, J. Wolff, *Anal. Biochem.* **1987**, *167*, 228–234.
[33] A. S. Klymchenko, V. G. Pivovarenko, T. Ozturk, A. P. Demchenko, *New J. Chem.* **2003**, *27*, 1336–1343.
[34] G. Duportail, A. Klymchenko, Y. Mély, A. P. Demchenko, *J. Fluoresc.* **2002**, *12*, 181–185.

[35] A. S. Klymchenko, A. P. Demchenko, *New J. Chem.* **2004**, *28*, 687–692.
[36] K. L. Goldenthal, I. Pastan, M. C. Willingham, *Exp. Cell Res.* **1984**, *152*, 558–564.
[37] E. A. A. Wallén, K. Dahlén, M. Grøtli, K. Luthman, *Org. Lett.* **2007**, *9*, 389–391.

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