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Phenacyl ester derivatives bearing heterocycles as models for photocleavable linkers: synthesis and photolysis studies

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ABSTRACT

The synthesis of several phenacyl ester derivatives linked to oxygen and nitrogen heterocycles, benzoquinolone and (benzo)coumarins, was carried out in an effort to obtain systems that could be applied as photocleavable (bi)functional linkers for solid phase peptide synthesis. The heterocycles were attached to a spacer, with different lengths, followed by coupling to 2-bromo-1-phenylpropan-1-one, acting as a model for the solid support. Photolysis studies of the resulting phenacyl ester derivatives were carried out by irradiation in a photochemical reactor at different wavelengths (300, 350 and 419 nm), in methanol/HEPES buffer solution (80:20).

1. Introduction

The possibility of breaking bonds efficiently by irradiation with light of appropriate wavelength without the need of any other reagents is very appealing for solid phase peptide synthesis (SPPS) where the substrate is bound to a polymeric matrix, usually through a linker that can be cleaved by light in the very last step of the sequence, in order to liberate the desired product.[1] Additionally, photocleavable protecting groups can be used in the intermediate coupling cycles as temporary protection for the amino acids terminal function, since during the synthesis it is necessary that side chain protecting groups remain in place while those at the main chain terminal should be cleaved before starting a new cycle.[2] The use of photolabile linkers became widespread in the generation of combinatorial libraries of organic molecules because it allows a release of the library compounds under very mild conditions. Light-induced detachment is orthogonal to acid and basic reaction conditions and therefore affords additional flexibility in the synthesis on solid support. The prerequisites for a photochemically removable linker are the same as those described for photochemically removable protecting groups.[3]

One of the critical issues in protecting group chemistry is orthogonality, i.e., the possibility of selectively removing one group in the presence of others in any chronological sequence.[4] It has been proven the possibility of using different wavelengths of irradiation to achieve orthogonality within a set of photolabile protecting groups. Based on these studies, the concept of chromatic orthogonality has been coined as 'the possibility of transforming photochemically a specific chromophore at a specific wavelength, without affecting other photosensitive moieties'.[5]

In recent years, we have been involved in the design of heterocyclic photocleavable protecting groups, derived from oxygen and nitrogen such as (benzo)coumarins, (benzo)quinolones and their thionated analogues, and benzozaxoles.[6] These moieties have been used in the protection of amino acids, as models for bifunctional biomolecules, by coupling through ester and urethane bonds. Attempts were made to optimise the photolytic process at longer wavelengths, by tailoring of the heterocycle structure, and interesting results were obtained for some systems, resulting in fast cleavage of the bond between the amino acid and the heterocycle at 350 and 419 nm. Considering our previous experience in this field, we now report the synthesis of phenacyl ester derivatives linked to heterocycles, (benzo)coumarins and (benzo)quinolones, in order to obtain systems that could be applied as photocleavable (bi)functional linkers for solid phase synthesis, allowing photolysis at longer wavelengths with short irradiation times. In principle, a bifunctional linker would allow the selective cleavage of each bond (resin-linker, with λ_1, and linker-substrate, with λ_2) leaving the other bond intact. If the linker possessed a property like fluorescence, one could choose to cleave the resin-linker bond as a means to obtain a fluorescently labelled substrate (Scheme 1).

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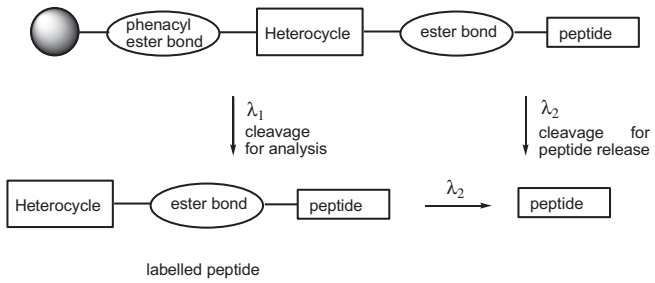


Scheme 1. Schematic representation of a photocleavable bifunctional linker for solid phase synthesis.

2. Results and discussion

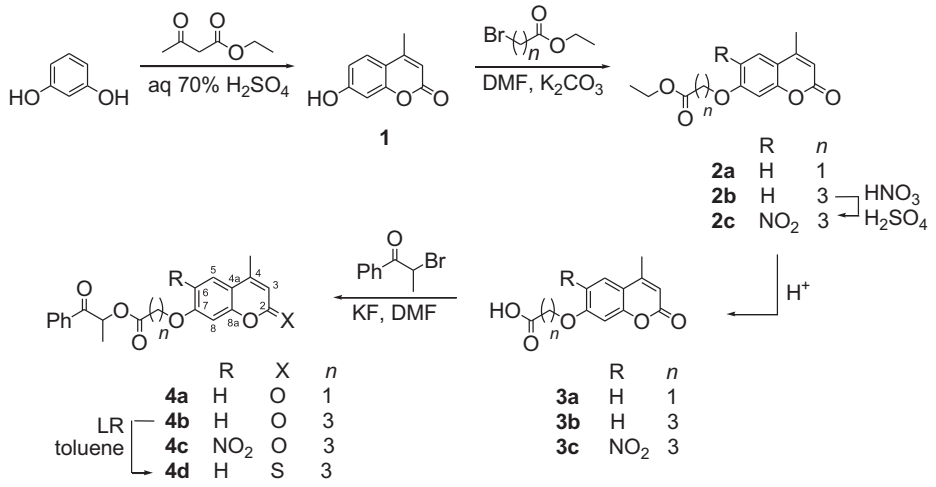
2.1. Synthesis

7-Hydroxy-4-methylcoumarin **1** was synthesised through a Pechmann reaction between 1,3-dihydroxybenzene and ethyl 3-oxobutanoate, catalysed by aqueous sulfuric acid at room temperature, through a method reported earlier (Scheme 2).[6c]

Benzocoumarins bearing a different ring fusion (angular and linear) were also obtained by a Pechmann reaction between 2, 7-dihydroxynaphthalene with ethyl 3-oxobutanoate, resulting a mixture of the two isomers that were very difficult to separate by chromatographic methods, and as a result, the mixture of the isomeric benzocoumarins was used in the subsequent synthetic steps, being only separated in the final product (Scheme 4).

The heterocycles bearing a hydroxyl group **1** and **6a**–**c** were coupled to ethyl bromoacetate (in the case of **1**) or ethyl 4-bromobutanoate (in the case of **1** and **6a**–**c**), in the presence of potassium carbonate in *N*,*N*-dimethylformamide,[9] in order to introduce a spacer of different length, resulting in the corresponding coumarins **2a**,**b**, benzoquinolone **7a** and benzocoumarins **7b**,**c**. Coumarin **2c** was obtained from **2b** by a classical nitration procedure in the presence of nitric acid and sulfuric acid. The ethyl esters were then hydrolysed to the corresponding carboxylic acids **3a**–**c**, **8a**–**c** (Schemes 2–4).[9]

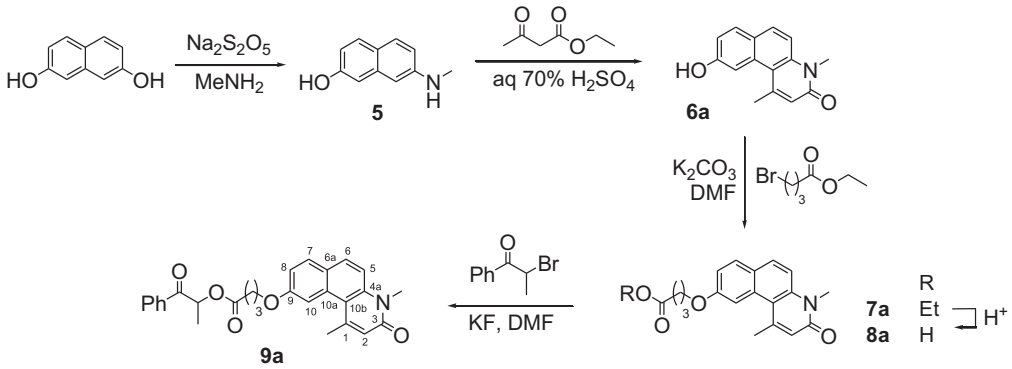
Finally, the phenacyl ester derivatives bearing different heterocycles were synthesised by coupling of 2-bromo-1-phenylpropan-1-one, acting as a model for the solid support (such as the brominated Wang resin), to the carboxylic acid terminal of heterocycle



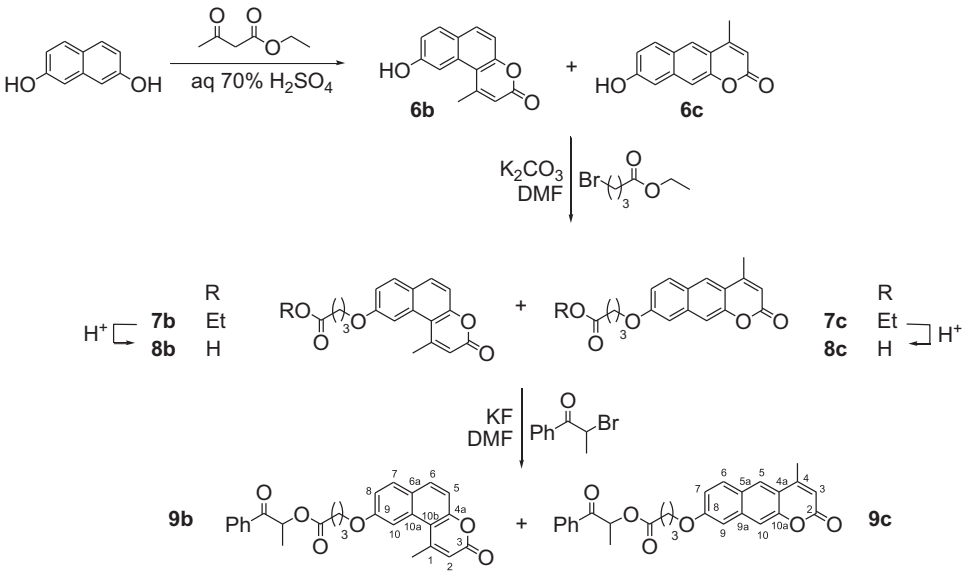
Scheme 2. Synthesis of coumarin phenacyl derivatives **4a**–**d**.

7-(*N*-Methylamino)naphthalen-2-ol **5** was obtained from 2, 7-dihydroxynaphthalene by reductive treatment with methylamine.[7] Through a modified Knorr synthesis[8] between **5** and ethyl 3-oxobutanoate, in acidic media, 9-hydroxy-1,4-dimethylbenzo[*f*] quinolin-3(4*H*)-one **6a** was obtained (Scheme 3).

derivatives **3a**–**c**, and **8a**–**c**, in *N*,*N*-dimethylformamide, at room temperature, in the presence of potassium fluoride.[10] The corresponding coumarin **4a**–**c**, benzoquinolone **9a** and benzocoumarin **9b**,**c** phenacyl esters were obtained as solids in moderate to good yields (Table 1). The coumarin phenacyl ester **4b** was further



Scheme 3. Synthesis of benzoquinolone phenacyl derivatives **9a**.



Scheme 4. Synthesis of benzocoumarin phenacyl derivatives **9b**,**c**.

Table 1
UV–vis absorption and emission data for phenacyl derivatives **4a**–**d** and **9a**–**c** in absolute ethanol

Compound	Absorption		Emission		
	λ_{abs} (nm)	log ε	λ_{em} (nm)	ϕ_F	Stokes' shift (nm)
4a	316	4.22	384	0.07	68
4b	320	4.28	381	0.13	61
4c	321	4.00	394	0.04	73
4d	397	4.28	433	0.01	36
9a	335	4.00	407	0.21	72
9b	347	4.05	441	0.82	94
9c	343	4.21	466	0.26	123

reacted with Lawesson's reagent (LR), by heating at reflux in dry toluene,[11] affording the expected thionated derivative **4d**. All final products were fully characterised by the usual techniques (^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, IR, UV–vis absorption and fluorescence spectroscopy) and the obtained data were in agreement with the structure of the compounds.

The photophysical properties (absorption and emission maxima, molar absorptivities and fluorescence quantum yields, ϕ_F) of the newly synthesised compounds **4a**–**d** and **9a**–**c** were evaluated by tracing the UV–vis absorption and emission spectra of degassed 10^{-5} M solutions in absolute ethanol (Table 1). Relative fluorescence quantum yields were calculated using 9,10-diphenylanthracene as standard (ϕ_F=0.95 in ethanol),[12] for all compounds, except for thionated coumarin **4d**, which required a 0.05 M solution of quinine in sulfuric acid as standard (ϕ_F=0.55).[13] For the ϕ_F determination, the fluorescence standard was excited at the wavelengths of maximum absorption found for each of the compounds to be tested and in all fluorimetric measurements, the absorbance of the solution did not exceed 0.1.

By analysis of the data in Table 1 for phenacyl esters **4** and **9**, it was found that the fluorescence quantum yield was influenced by the nature of the heterocycle: benzoquinolone **9a** and benzocoumarins **9b**,**c** had the highest quantum yields and, in the latter case, the fusion of the ring was also significant as the angular derivative **9b** showed higher quantum yield when compared to the linear derivative **9c**. Among coumarins **4**, nitration (in the case of **4c**) and thionation (in the case of **4d**) result in an expected decrease of the fluorescence quantum yield when compared to coumarin **4b**, due to the presence of the nitro group (a known fluorescence quencher) and the presence of a thiocarbonyl group, due to the heavy atom induced spin–orbit coupling by the sulfur atom that gives rise to an effective intersystem crossing mechanism that lowers the fluorescence emission.[14] As for the maximum wavelengths of absorption and emission, the most striking difference, a 77 and 52 nm bathochromic shift, occurred after conversion of the carbonyl (**4b**) to a thiocarbonyl group (**4d**), thus confirming the benefits of such change for shifting absorption and emission to longer wavelengths (see Fig. 1). Absorption at longer wavelengths could indicate that photolysis at higher wavelengths might be possible with shorter irradiation times.

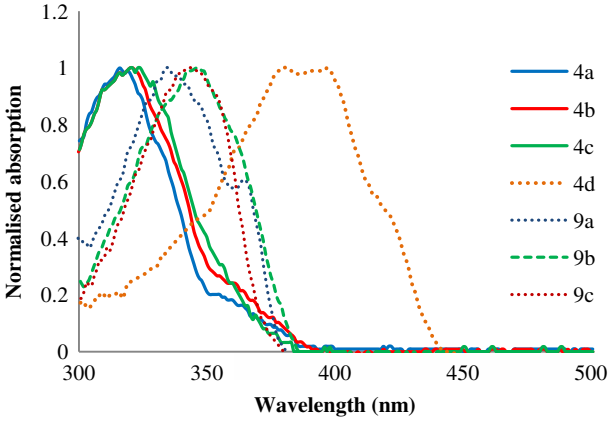


Fig. 1. Normalised UV–vis absorption spectra of coumarins **4a**–**d**, benzoquinolone **9a** and benzocoumarin **9b**,**c** conjugates.

2.2. Photolysis studies of phenacyl derivatives 4a–d and 9a–c

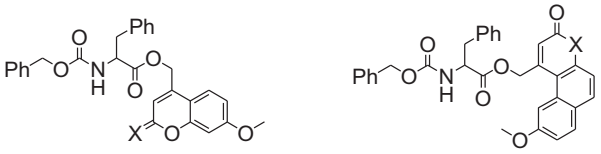
The evaluation of the various phenacyl ester derivatives **4a**–**d** and **9a**–**c** bearing different heterocycles as photocleavable linkers was carried out by photolysis under irradiation at different wavelengths (300, 350 and 419 nm). Solutions of the mentioned compounds (1×10^{-4} m) in an 80:20 mixture of methanol and HEPES

buffer, were irradiated in a Rayonet RPR-100 reactor in order to determine the most favourable cleavage conditions. The course of the photocleavage reaction was followed by reverse phase HPLC with UV detection. The determined irradiation time represents the time necessary for the consumption of the starting materials until less than 5% of the initial peak area (A) was detected. Based on HPLC data, the plot of ln A versus irradiation time showed a linear correlation for the disappearance of the starting material, which suggested a first order reaction, obtained by the linear least squares methodology for a straight line, with high correlation coefficients, and rate constants were calculated (Table 2).

Table 2
Irradiation times (t_{irr}, in min), photochemical quantum yields ($\Phi_P \times 10^{-3}$) and rate constants (in min^{-1}) for derivatives **4a**–**d**, **9a**–**c** and phenylalanine ester conjugates **4e**, **9d**,**e** at different wavelengths in methanol/HEPES buffer (80:20) solution

Compound	300 nm			350 nm			419 nm		
	t_{irr}	Φ_P	k	t_{irr}	Φ_P	k	t_{irr}	Φ_P	k
4a	30.8	0.187	0.097	3269	0.027	0.001	—	—	—
4b	28.4	0.189	0.105	1576	0.017	0.002	—	—	—
4c	260	0.018	0.012	784	0.015	0.004	—	—	—
4d	110	0.207	0.028	82.4	0.066	0.038	58.8	0.056	0.053
4e[6c]	33.7	0.235	0.090	253	0.054	0.013	—	—	—
4f[15]	101	0.272	0.032	45.7	0.106	0.072	36.6	0.071	0.084
9a	39.2	0.079	0.077	40.4	0.133	0.075	6003	0.027	0.001
9b	—	—	—	85.8	0.048	0.035	4989	0.050	0.001
9c	—	—	—	837	0.004	0.004	—	—	—
9d	44.2	0.489	0.067	43.0	0.619	0.070	2996	0.114	0.001
9e[6e]	33.6	0.851	0.089	38.1	0.441	0.083	301	0.659	0.010

Since the purpose of this work was to evaluate the possibility of using the above mentioned heterocycles as photocleavable linkers for solid phase synthesis, their behaviour towards irradiation was compared to that of the related coumarins **4e**,**f**, benzoquinolone **9d** and benzocoumarin **9e** phenylalanine conjugates, previously reported by us (Fig. 2).[6e,15]



4e X = O
4f X = S
9d X = NCH_3
9e X = O

Fig. 2. Structure of phenylalanine coumarin **4e**,**f**, benzoquinolone **9d** and benzocoumarin **9e** ester conjugates.

In these conjugates, the model amino acid is linked by an ester bond to the heterocycle at the ring containing the heteroatom. In the new reported phenacyl derivatives, the link is at the fused benzene ring. With this in mind, and the different type of link to the amino acid (ester) and to the model of the solid support (phenacyl ester), it was expected to have some differentiation on the photocleavage behaviour. The photolysis data of conjugates **4e**,**f** and **9d**,**e**, previously reported by us, is presented in Table 2 for easier comparison.

Comparison of the data for phenacyl coumarin derivatives **4a**–**d** revealed the influence of the structure of the compound on the photocleavage rates: the unsubstituted coumarins **4a** and **4b** cleaved with similar rates at 300 nm and much faster than derivatives **4c**,**d**. The length of the spacer was not critical for the photolytic behaviour in the case of compounds **4a**,**b**. At 350 nm, the trend was reversed and the thionated coumarin derivative **4d** showed the shortest irradiation time of all compounds (82 min), with the irradiation times for **4a** and **4b** being impractical (Fig. 3). Due to this promising result, irradiation at 419 nm was carried out for **4d** and the result improved with an irradiation time of about 59 min.

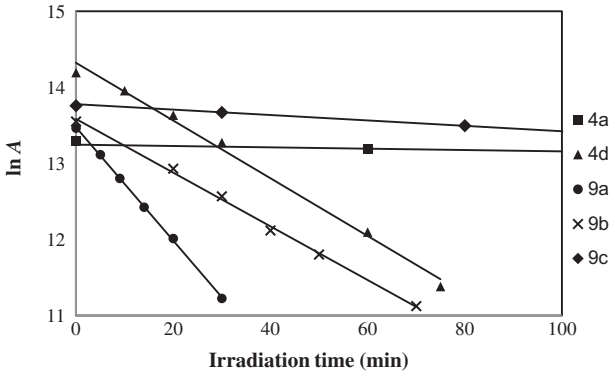


Fig. 3. Plot of ln A versus irradiation time for the photolysis of conjugates **4a** (■), **4d** (▲), **9a** (●), **9b** (×) and **9c** (◆) at 350 nm in methanol/HEPES (80:20) solution.

As for compounds **9a** and **9b**, it was found that both cleaved in similar rates at 350 and 419 nm, which were faster for irradiation at 350 nm (between 40 and 86 min). Benzoquinolone **9a** cleaved two times faster than benzocoumarin **9b** at 350, while at 419 nm the difference in the irradiation times was minimal.

The photochemical quantum yields (Φ_P) were calculated based on half-lives ($t_{1/2}$), molar absorptivities at the irradiation wavelength (ε) and the incident photon flux (I_0), which was determined by potassium ferrioxalate actinometry.[16] The photocleavage process was not as efficient as desirable (see Φ_P in Table 2), possibly by deactivation via fluorescence pathways that compete with the photochemical reaction, as well as the type of reactor used (open chamber reactor).

As for the benzocoumarin derivatives **9b** and **9c**, their structural difference being the ring fusion, the angular **9b** cleaved faster than the linear **9c** at 350 nm (at about 10 times faster). For this reason, photolysis at 419 nm was only done for derivative **9b**, but it resulted in a very long and unfeasible irradiation time.

As a result of the above comments, the most promising heterocyclic linker would be the thionated coumarin **4d** at 419 nm if its application was considered in connection with a short-wavelength and short t_{irr} photocleavable protecting group: in this scenario, the protecting group would be cleaved without meaningful cleavage of the bond to the linker and in the end the linker would be cleaved by irradiation at 419 nm. On the other hand, if a substrate was to be cleaved from the solid support bearing its temporary protecting groups, compound **4d** would allow fast cleavage at 419 nm, with the remaining structure of the substrate being unaffected.

We envisaged the application of coumarin, benzoquinolone and benzocoumarins as bifunctional linkers (with a phenacyl ester bond to the solid support by the fused benzene ring and an ester bond to the substrate by the ring containing the heteroatom). For this to be feasible, the photolytic behaviour of the ester and phenacyl ester bonds should be very distinct. Thus, a comparison between the obtained photolysis results with previously reported structurally related coumarins **4e**,**f**, benzoquinolone **9d** and benzocoumarin **9e** phenylalanine conjugates (Fig. 2) was carried out.

Based on the data presented in Table 2, the behaviour of coumarins **4a** and **4e** was similar at 300 nm but at 350 nm a distinction was possible with the ester bond in **4e** cleaving more than 10 times faster than the phenacyl ester bond in **4a** (ca. 4 h vs 54 h). For the

thionated coumarins **4d** and **4f**, cleavage rates of the ester and phenacyl ester bonds were very similar and the difference seen at 350 nm was not high enough for a clearly distinct performance. For the benzoquinolones **9a** and **9d**, also no clear difference was seen between the cleavage rates of the ester and phenacyl ester bonds. Comparison of the results for benzocoumarins **9b** and **9e** revealed that at 419 nm the ester bond cleaved in much shorter irradiation times (ca. 5 h) when compared to the phenacyl ester bond (ca. 83 h).

Phenacyl esters have been widely used as caging groups for biological applications and the cleavage mechanism is thought to involve the release of a carboxylic acid and a ketone as the by-product.[17] It is reasonable to assume a related pathway yielding the same photoproducts, carboxylic acids **3a**–**d** and **8a**–**c** and 1-phenylpropan-1-one. Esters, on the other hand, are cleaved by a homolytical/heterolytical fission of the O–CH_2 bond, resulting in the release of a carboxylic acid and an alcohol as the by-product.[6c]

3. Conclusions

The synthesis of several phenacyl ester derivatives linked to oxygen and nitrogen heterocycles, benzoquinolone and (benzo) coumarins, was described by reaction of 2-bromo-1-phenylpropan-1-one, acting as a model for a solid support, with a carboxylic acid group. The resulting compounds were tested as photocleavable linkers with a spacer, with different lengths, by irradiation in a photochemical reactor at different wavelengths (300, 350 and 419 nm), in methanol/HEPES buffer solution (80:20). It was found that the most promising heterocyclic linker would be the thionated coumarin **4d** at 419 nm if it was considered in connection with a fast-cleaving short-wavelength photocleavable protecting group. As for the use of the synthesised phenacyl esters as bifunctional linkers, involving the simultaneous presence of an ester and a phenacyl ester bond in the same molecule, it was possible to confirm a different photolytic behaviour for these two types of bond, when comparing the results for the phenacyl ester bond in coumarin **4a** and benzocoumarin **9b** with the corresponding ester bond conjugates **4e** and **9e**.

4. Experimental section

4.1. General

All melting points were measured on a Stuart SMP3 melting point apparatus. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel 60F_{254}) and spots were visualised under UV light. Chromatography on silica gel was carried out on Merck Kieselgel (230–240 mesh). IR spectra were determined on a BOMEM MB 104 spectrophotometer. UV–vis absorption spectra (200–700 nm) were obtained using a Shimadzu UV/2501PC spectrophotometer. NMR spectra were obtained on a Varian Unity Plus Spectrometer at an operating frequency of 300 MHz for ^{1}H and 75.4 MHz for ^{13}C or a Bruker Avance III 400 at an operating frequency of 400 MHz for ^{1}H and 100.6 MHz for ^{13}C using the solvent peak as internal reference at 25 °C. All chemical shifts are given in parts per million using δ_H Me_4Si=0 ppm as reference and *J* values are given in hertz. Assignments were made by comparison of chemical shifts, peak multiplicities and *J* values and were supported by spin decoupling-double resonance and bidimensional heteronuclear correlation techniques. Low and high resolution mass spectrometry analyses were performed at the 'C.A.C.T.I.—Unidad de Espectrometria de Masas', at University of Vigo, Spain. Fluorescence spectra were collected using a FluoroMax-4 spectrofluorometer. Photolyses were carried out using a Rayonet RPR-100 chamber reactor equipped with 10 lamps of 300 (21 W), 350 (24 W) and 419 (14 W) nm. HPLC analyses were performed using a Licrospher 100 RP18 (5 μm) column in an HPLC system composed by a Jasco PU-980 pump, a Shimadzu SPD-GAV UV/Vis detector and a Shimadzu C-RGA Chromatopac register. All commercial reagents were used as received.

4.2. Synthesis of compounds 1–9

*4.2.1. General procedure for the synthesis of compounds **1** and **6b**,**c**.* Compounds **1** and **6b**,**c** were prepared by reaction of 1, 3-dihydroxybenzene or 2,7-dihydroxynaphthalene (1 equiv), respectively, with ethyl 3-oxobutanoate (1.5 equiv), and aqueous 70% sulfuric acid (5 mL) was added. The reaction mixture was stirred at room temperature until reaction completion. The reaction mixture was poured into ice, the formed precipitate was filtered, washed with water and dried at 50 °C.

*4.2.1.1. 7-Hydroxy-4-methylcoumarin, **1**.* After stirring at room temperature for 17 h, compound **1** was obtained as a white solid (2.135 g, 12.1 mmol, 89%). Mp=185.3–186.8 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.34 (3H, s, CH_3), 6.10 (1H, s, H3), 6.68 (1H, d, *J* 2.7 Hz, H8), 6.78 (1H, dd, *J* 2.7 and 8.7 Hz, H6), 7.56 (1H, d, *J* 8.7 Hz, H5), 10.51 (1H, s, OH). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ=18.12 (CH_3), 102.18 (C8), 110.25 (C3), 112.01 (C4a), 112.87 (C6), 126.62 (C5), 153.56 (C4), 154.85 (C8a), 160.31 (C2), 161.18 (C7). FTIR (KBr 1%, cm^{-1}): ν=3190, 1679, 1652, 1600, 1515, 1451, 1390, 1369, 1336, 1274, 1239, 1213, 1160, 1134, 1068, 1018, 983, 898, 864, 846, 806, 761, 747, 693, 641, 583, 510. UV–vis (ethanol, nm): λ_{max} (log ε)=323 (4.12). MS *m*/*z* (ESI, %): 176 ([M+H]$^+$, 68). HRMS: *m*/*z* (ESI) calcd for $C_{10}H_8O_3$ 176.0473, found 176.0479.

*4.2.1.2. Benzocoumarins **6b** and **6c**.* After stirring for 5 days, a mixture of compounds **6b**,**c** was obtained as a light pink solid (0.367 g, 74%). The solid mixture was used in the next reaction without further purification or separation.

*4.2.1.2.1. 1-Methyl-9-hydroxybenzo[f]coumarin, **6b**.* ^{1}H NMR (400 MHz, DMSO-d_6): δ=2.86 (3H, d, *J* 0.8 Hz, CH_3), 6.41 (1H, d, *J* 1.2 Hz, H2), 7.13 (1H, dd, *J* 8.8 and 2.0 Hz, H8), 7.28 (1H, d, *J* 8.8 Hz, H5), 7.90 (1H, d, *J* 8.8 Hz, H7), 8.00 (1H, d, *J* 2.0 Hz, H10), 8.04 (1H, d, *J* 8.8 Hz, H6). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ=18.17 (CH_3), 108.66 (C10), 110.67 (C10b), 113.90 (C5), 115.77 (C2), 117.25 (C8), 125.22 (C6a), 130.31 (C10a), 131.33 (C7), 134.35 (C6), 152.27 (C1), 155.35 (C4a), 157.80 (C9), 159.38 (C3).

*4.2.1.2.2. 4-Methyl-8-hydroxybenzo[g]coumarin, **6c**.* ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.50 (3H, s, CH_3), 6.33 (1H, s, H3), 7.11 (1H, dd, *J* 2.4 and 8.8 Hz, H7), 7.15 (1H, d, *J* 2.0 Hz, H9), 7.61 (1H, s, H10), 7.94 (1H, d, *J* 8.8 Hz, H6), 8.27 (1H, s, H5), 10.19 (1H, s, OH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ=18.71 (CH_3), 105.22 (C9), 110.46 (C10), 114.50 (C3), 117.90 (C4a), 119.63 (C7), 125.02 (C5), 125.28 (C5a), 130.43 (C6), 136.79 (C9a), 151.00 (C4), 152.08 (C10a), 158.72 (C8), 161.15 (C2).

*4.2.2. Synthesis of 7-(N-methylamino)naphthalen-2-ol, **5**.* 2,7-Dihydroxynaphthalene (1 equiv, 0.500 g, 3.12 mmol) was mixed to sodium metabisulfite (2.44 equiv, 1.448 g, 7.62 mmol), sodium hydroxide (5.2 equiv, 0.650 g, 16.20 mmol), water (12 mL) and methylamine hydrochloride (5.2 equiv, 1.094 g, 16.20 mmol). The reaction mixture was stirred, in an oil bath, at 90 °C for 3 days. The reaction mixture was extracted with ethyl acetate (3×10 mL), and the organic layer was dried with anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The resulting crude was purified by silica gel column chromatography using dichloromethane/methanol (98:2) as eluent, yielding compound **5** as dark yellow solid (0.300 g, 1.73 mmol, 56%). Mp=101.4–103.3 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.72 (3H, d, *J* 4.8 Hz, CH_3), 5.76–5.81 (1H, m, NH), 6.42 (1H, d, *J* 2.1 Hz, H8), 6.62–6.68 (2H, m, H3 and H6), 6.80 (1H, d, *J* 2.7 Hz, H1), 7.40–7.45 (2H, m, H4 and H5),

9.32 (1H, s, OH). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ=29.70 (CH_3), 100.48 (C8), 106.96 (C1), 113.16 (C3), 114.92 (C6), 121.09 (C8a), 128.04 (C5), 128.84 (C4), 136.79 (C4a), 147.94 (C7), 155.52 (C2). FTIR (liquid film, cm^{-1}): ν=3335, 1634, 1616, 1525, 1418, 1351, 1241, 1205, 1120, 1045, 965, 951, 877, 830, 613. MS *m*/*z* (EI, %): 173 (M^+, 100). HRMS: *m*/*z* (EI) calcd for $C_{11}H_{11}ON$ 173.0841, found 173.0850.

4.2.3. Synthesis of 9-hydroxy-1,4-dimethylbenzo[f]quinolin-3(4H)-one, ***6a****.* Ethyl 3-oxobutanoate (1.5 equiv, 0.33 mL, 2.60 mmol) was heated at 180 °C and compound **5** (0.300 g, 1.73 mmol) was added. The reaction mixture was heated at reflux for 45 min. After evaporation of the excess ethyl 3-oxobutanoate under vacuum, the residue was stirred with aqueous 70% sulfuric acid (5 mL) at 95 °C for 45 min. After cooling to room temperature, water (10 mL) was added to the mixture, followed by extraction with ethyl acetate (3×15 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The resulting solid was purified by silica gel column chromatography using chloroform/methanol (100:1) of increasing polarity as eluent, yielding compound **6a** as a dark solid (0.269 g, mmol, 65%). Mp=269.1–271.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ=2.84 (3H, s, CH_3), 3.72 (3H, s, NCH_3), 6.57 (1H, d, *J* 0.4 Hz, H2), 7.07 (1H, dd, *J* 8.8 and 2.0 Hz, H8), 7.54 (1H, d, *J* 9.2 Hz, H5), 7.86 (1H, d, *J* 8.8 Hz, H7), 8.01 (1H, d, *J* 9.6 Hz, H6), 8.02 (1H, d, *J* 2.0 Hz, H10), 9.96 (1H, s, OH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ=26.31 (CH_3), 29.96 (NCH_3), 108.63 (C10), 112.44 (C5), 113.90 (C10b), 116.12 (C8), 121.22 (C2), 123.89 (C6a), 130.73 (C7), 132.14 (C10a), 132.24 (C6), 140.64 (C4a), 147.59 (C1), 156.78 (C9), 160.16 (C3). FTIR (KBr 1%, cm^{-1}): ν=3165, 1625, 1614, 1590, 1522, 1495, 1470, 1428, 1416, 1291, 1234, 1100, 1069, 1009, 921, 887, 847, 666. MS *m*/*z* (EI, %): 239 (M^+, 81). HRMS: *m*/*z* (EI) calcd for $C_{15}H_{13}O_2N$ 239.0947, found 239.0893.

4.2.4. General procedure for the synthesis of compounds ***2a,b*** *and* ***7a–c****.* Compounds **1** and **6a–c** (1 equiv) were added to a suspension of potassium carbonate (1.5 equiv) in dry *N*,*N*-dimethylformamide. Ethyl bromoacetate (in the case of **1**) or ethyl 4-bromobutanoate (in the case of compounds **1** and **6a–c**) (1.1 equiv) was added dropwise to the mixture. The reaction mixture was stirred at room temperature for 1 day. Water (10 mL) was added to the mixture, followed by extraction with ethyl acetate (3×15 mL). The organic layer was washed with aqueous 5% HCl, dried with anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The resulting oil was purified by silica gel column chromatography using chloroform/methanol (100:1) as eluent, yielding compounds **2a**,**b** and **7a–c**.

4.2.4.1. Ethyl 2-(4-methylcoumarin-7-yloxy)acetate, ***2a****.* Compound **2a** was obtained as colourless oil (0.100 g, 0.38 mmol, 67%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.32 (3H, t, *J* 6.9 Hz, CH_2CH_3), 2.41 (3H, d, *J* 0.9 Hz, CH_3), 4.26–4.33 (2H, m, CH_2CH_3), 4.69 (2H, s, CH_2), 6.16 (1H, d, *J* 0.9 Hz, H3), 6.78 (1H, d, *J* 2.7 Hz, H8), 6.92 (1H, dd, *J* 8.7 and 2.4 Hz, H6), 7.53 (1H, d, *J* 9 Hz, H5). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ=14.13 (CH_2CH_3), 18.65 (CH_3), 61.70 (CH_2CH_3), 65.33 (CH_2), 101.67 (C8), 112.49 (C3), 112.55 (C6), 114.39 (C4a), 125.76 (C5), 152.36 (C4), 155.05 (C8a), 160.61 (C7), 161.03 (C2), 167.94 (C=O ester). FTIR (liquid film, cm^{-1}): ν=3064, 2982, 2938, 1756, 1723, 1615, 1561, 1506, 1444, 1424, 1389, 1370, 1301, 1268, 1212, 1154, 1083, 1029, 981, 853, 818, 736, 703, 666, 634. MS *m*/*z* (EI, %): 265 (M^+, 100). HRMS: *m*/*z* (EI) calcd for $C_{14}H_{14}O_5$ 265.0841, found 265.0827.

4.2.4.2. Ethyl 4-(4-methyl-coumarin-7-yloxy)butanoate, ***2b****.* Compound **2b** was obtained as white solid (0.130 g, 0.45 mmol, 82%), Mp=81.3–82.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.27 (3H, t, *J* 7.2 Hz, CH_2CH_3), 2.12–2.19 (2H, m, βCH_2), 2.40 (3H, d, *J* 1.2 Hz, CH_3), 2.53 (2H, t, *J* 7.2 Hz, αCH_2), 4.08 (2H, t, *J* 6.4 Hz, γCH_2), 4.16 (2H, q, *J* 7.2 Hz, CH_2CH_3), 6.13 (1H, t, *J* 1.3 Hz, H3), 6.80 (1H, d, *J* 2.4 Hz, H8), 6.85 (1H, dd, *J* 8.8 and 2.4 Hz, H6), 7.49 (1H, d, *J* 8.8 Hz, H5). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=14.20 (CH_2CH_3), 18.63 (CH_3), 24.35 (βCH_2), 30.62 (αCH_2), 60.52 (CH_2CH_3), 67.30 (γCH_2), 101.46 (C8), 111.96 (C3), 112.44 (C6), 113.60 (C4a), 125.50 (C5), 152.48 (C4), 155.23 (C8a), 161.16 (C2), 161.84 (C7), 172.92 (C=O ester). FTIR (KBr 1%, cm^{-1}): ν=3017, 1744, 1615, 1561, 1513, 1478, 1457, 1419, 1393, 1368, 1346, 1281, 1264, 1204, 1182, 1157, 1135, 1070, 1019, 1118, 1100, 995, 951, 811, 781, 665, 650. MS *m*/*z* (EI, %): 290 (M^+, 79). HRMS: *m*/*z* (EI) calcd for $C_{16}H_{18}O_5$ 290.1154, found 290.1160.

4.2.4.3. Ethyl 4-(1,4-dimethylbenzo[f]quinolin-3(4H)-one-9-yloxy)butanoate, ***7a****.* Compound **7a** was obtained as beige solid (0.040 g, 1.13 mmol, 55%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.28 (3H, t, *J* 7.2 Hz, CH_2CH_3), 2.20–2.23 (2H, m, βCH_2), 2.59 (2H, t, *J* 7.2 Hz, αCH_2), 2.94 (3H, s, CH_3), 3.86 (3H, s, CH_3), 4.15–4.20 (4H, m, γCH_2 and CH_2CH_3), 6.71 (1H, s, H2), 7.18 (1H, dd, *J* 8.8 and 2.4 Hz, H8), 7.46 (1H, d, *J* 9.2 Hz, H5), 7.82 (1H, d, *J* 8.8 Hz, H7), 7.91 (1H, d, *J* 9.2 Hz, H6), 8.00 (1H, d, *J* 2.0 Hz, H10). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=14.23 (CH_2CH_3), 24.65 (βCH_2), 26.77 (CH_3), 30.48 (NCH_3), 30.81 (αCH_2), 60.50 (CH_2CH_3), 66.96 (γCH_2), 107.65 (C10), 112.84 (C5), 115.63 (C10b), 115.87 (C8), 122.46 (C2), 125.17 (C6a), 130.53 (C7), 131.98 (C6), 132.43 (C10a), 140.80 (C4a), 147.51 (C1), 157.98 (C9), 161.51 (C3), 173.13 (C=O ester). FTIR (KBr 1%, cm^{-1}): ν=3021, 2995, 1718, 1620, 1535, 1517, 1458, 1367, 1233, 1200, 1151, 1102, 1048, 1003, 993, 940, 777. MS *m*/*z* (EI, %): 353 (M^+, 88). HRMS: *m*/*z* (EI) calcd for $C_{21}H_{23}O_4N$ 353.1628, found 353.1661.

4.2.4.4. Benzocoumarins ***7b*** *and* ***7c****.* The compounds were obtained as a beige solid mixture (0.260 g, 89%). The mixture was used in the next reaction without further purification.

4.2.4.4.1. Ethyl 4-(1-methyl-benzo[f]coumarin-9-yloxy)butanoate, ***7b****.* ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.24–1.29 (3H, t, *J* 7.2 Hz, CH_2CH_3), 2.17–2.20 (2H, m, βCH_2), 2.57–2.59 (2H, m, αCH_2), 2.90 (3H, s, CH_3), 4.13–4.19 (4H, γCH_2 and CH_2CH_3), 6.31 (1H, s, H2), 7.17 (1H, dd, *J* 7.2 and 2.4 Hz, H8), 7.26 (1H, d, *J* 8.8 Hz, H5), 7.73–7.77 (1H, m, H7), 7.87 (1H, d, *J* 2.4 Hz, H10), 8.02 (1H, d, *J* 8.6 Hz, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=14.14 (CH_2CH_3), 18.17 (CH_3), 24.61 (βCH_2), 30.41 (αCH_2), 61.05 (CH_2CH_3), 66.70 (γCH_2), 108.66 (C10), 110.67 (C10b), 113.90 (C5), 115.77 (C2), 117.25 (C8), 125.22 (C6a), 130.31 (C10a), 131.33 (C7), 134.35 (C6), 152.27 (C1), 155.35 (C4a), 157.80 (C9), 159.38 (C3).

4.2.4.4.2. Ethyl 4-(4-methyl-benzo[g]coumarin-8-yloxy)butanoate, ***7c****.* ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.26–1.28 (3H, t, *J* 7.2 Hz, CH_2CH_3), 2.20–2.24 (2H, m, βCH_2), 2.54–2.60 (5H, m, αCH_2 and CH_3), 4.15–4.21 (4H, m, γCH_2 and CH_2CH_3), 6.30 (1H, d, *J* 1.2 Hz, H3), 7.12–7.16 (2H, m, H7 and H9), 7.59 (1H, s, H10), 7.82 (1H, d, *J* 9.0 Hz, H6), 8.01 (1H, s, H5). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=14.15 (CH_2CH_3), 18.45 (CH_3), 24.54 (βCH_2), 30.42 (αCH_2), 61.03 (CH_2CH_3), 66.74 (γCH_2), 105.25 (C9), 110.45 (C10), 114.51 (C3), 117.89 (C4a), 119.90 (C7), 125.11 (C5), 125.31 (C5a), 130.44 (C6), 136.83 (C9a), 151.04 (C4), 152.09 (C10a), 158.70 (C8), 161.19 (C2).

4.2.4.5. Ethyl 4-(4-methyl-6-nitrocoumarin-7-yloxy)butanoate, ***2c****.* Compound **2b** (0.072 g, 0.25 mmol) was dissolved in 96% sulfuric acid (0.5 mL), in an ice bath, and 65% nitric acid (0.1 mL) was added. The reaction mixture was stirred at room temperature for 30 min and then poured into ice, the formed precipitate was filtered and dried. The solid was purified by silica gel column chromatography using dichloromethane/methanol (98:2) as eluent, yielding compound **2c** as yellow solid (0.033 g, 0.09 mmol, 36%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.25–1.30 (3H, m, CH_2CH_3), 2.17–2.22 (2H, m, βCH_2), 2.42 (3H, d, *J* 1.2 Hz, CH_3), 2.57–2.62 (2H, m, αCH_2), 3.01–3.40 (2H, m, γCH_2), 4.22–4.26 (2H, m, CH_2CH_3), 6.26 (1H, d, *J* 1.2 Hz, H3), 6.96 (1H, s, H8), 8.19 (1H, s, H5). FTIR (KBr 1%, cm^{-1}): ν=3054, 2987, 2868, 1745, 1714, 1623, 1546, 1422, 1385, 1363, 1301,

1265, 1163, 1094, 1064, 896, 842, 738, 705, 666. MS *m*/*z* (EI, %): 335 (M^+, 100). HRMS: *m*/*z* (EI) calcd for $C_{16}H_{17}O_7N$ 335.1005, found 335.1019.

*4.2.5. General procedure for the synthesis of compounds **3a**–**c** and **8a**–**c**.* Compounds **2a**–**c** and **7a**–**c** (1 equiv) were added to a solution of water/acetic acid/hydrochloric acid (3:1:0.1, 5 mL/equiv) and the mixture was heated at reflux for 30 min. The solution was allowed to cool slowly and the precipitate was filtered off. Compounds **3a**–**c** and **8a**–**c** were obtained as solids.

*4.2.5.1. 2-(4-Methylcoumarin-7-yloxy)acetic acid, **3a**.* The compound was obtained as white solid (0.065 g, 0.28 mmol, 73%). Mp=201.2–202.8 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.38 (3H, d, *J* 0.9 Hz, CH_3), 4.82 (2H, s, CH_2), 6.21 (1H, d, *J* 1.2 Hz, H3), 6.94–6.98 (2H, m, H6 and H8), 6.68 (1H, d, *J* 9.0 Hz, H5). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ=18.14 (CH_3), 64.82 (CH_2), 101.51 (C8), 111.39 (C3), 112.30 (C6), 113.55 (C4a), 126.52 (C5), 153.40 (C4), 154.55 (C8a), 160.11 (C2), 160.79 (C7), 169.67 (C=O acid). FTIR (KBr 1%, cm^{-1}): ν=2923, 1765, 1674, 1660, 1621, 1606, 1557, 1511, 1428, 1392, 1374, 1326, 1299, 1206, 1169, 1153, 1085, 1020, 977, 921, 857, 848, 813, 752, 738, 655. MS *m*/*z* (EI, %): 234 (M^+, 100). HRMS: *m*/*z* (EI) calcd for $C_{12}H_{10}O_5$ 234.0528, found 234.0559.

*4.2.5.2. 4-(4-Methyl-coumarin-7-yloxy)butanoic acid, **3b**.* The compound was obtained as white solid (0.100 g, 0.38 mmol, 96%). Mp=170.3–171.4 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ=1.90–2.00 (2H, m, βCH_2), 2.36–2.41 (5H, m, αCH_2 and CH_3), 4.09 (2H, t, *J* 6.6 Hz, γCH_2), 6.19 (1H, d, *J* 1.2 Hz, H3), 6.93–6.96 (2H, m, H6 and H8), 6.67 (1H, d, *J* 9.3 Hz, H5). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ=18.14 (CH_3), 23.99 (βCH_2), 29.99 (αCH_2), 67.41 (γCH_2), 101.20 (C8), 111.13 (C3), 112.42 (C6), 113.12 (C4a), 126.50 (C5), 153.46 (C4), 154.76 (C8a), 160.19 (C2), 161.60 (C7), 174.04 (C=O acid). FTIR (KBr 1%, cm^{-1}): ν=3095, 1700, 1687, 1607, 1558, 1512, 1480, 1427, 1392, 1366, 1720, 1211, 1162, 1073, 1020, 986, 891, 872, 848, 828, 810, 770, 753, 713, 666. MS *m*/*z* (EI, %): 262 (M^+, 100). HRMS: *m*/*z* (EI) calcd for $C_{14}H_{14}O_5$ 262.0841, found 262.0888.

*4.2.5.3. 4-(4-Methyl-6-nitrocoumarin-7-yloxy)butanoic acid, **3c**.* The compound was obtained as yellow solid (0.030 g, 9.77 mmol, 98%). Mp=190.1–193.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ=1.90–1.98 (2H, m, βCH_2), 2.38–2.42 (5H, m, αCH_2 and CH_3), 4.27 (2H, t, *J* 6.4 Hz, γCH_2), 6.38 (1H, d, *J* 1.4 Hz, H3), 7.39 (1H, s, H8), 8.31 (1H, s, H5). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ=18.00 (CH_3), 23.68 (βCH_2), 29.66 (αCH_2), 69.23 (γCH_2), 102.66 (C8), 112.53 (C4a), 113.10 (C3), 122.84 (C5), 136.09 (C6), 152.76 (C4), 153.97 (C7), 156.61 (C8a), 159.11 (C2), 173.86 (C=O acid). FTIR (KBr 1%, cm^{-1}): ν=3046, 1732, 1624, 1537, 1382, 1356, 1309, 1207, 1162, 1062, 991, 904, 845, 666. MS *m*/*z* (ESI, %): 308 ($[M+H]^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{14}H_{14}O_7N$ 308.07648; found 308.07650.

*4.2.5.4. 4-(1,4-Dimethylbenzo[f]quinolin-3(4H)-one-9-yloxy)butanoic acid, **8a**.* The compound was obtained as beige solid (0.035 g, 1.08 mmol, 95%). Mp=114.8–116.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ=2.00–2.07 (2H, m, βCH_2), 2.42–2.55 (2H, m, αCH_2), 2.91 (3H, s, CH_3), 3.73 (3H, s, NCH_3), 4.18 (2H, t, *J* 6.0 Hz, γCH_2), 6.61 (1H, s, H2), 7.21 (1H, dd, *J* 8.8 and 2.4 Hz, H8), 7.62 (1H, d, *J* 9.2 Hz, H5), 7.94 (1H, d, *J* 9.2 Hz, H7), 7.80 (1H, d, *J* 2.0 Hz, H10), 8.01 (1H, d, *J* 9.2 Hz, H6). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ=24.32 (αCH_2), 26.03 (CH_3), 30.07 (βCH_2), 30.21 (NCH_3), 66.67 (γCH_2), 107.11 (C10), 113.43 (C5), 114.39 (C10b), 115.85 (C8), 121.61 (C2), 124.67 (C6a), 130.61 (C7), 131.76 (C6), 132.05 (C10a), 140.69 (C4a), 147.52 (C1), 157.50 (C9), 160.16 (C3), 174.10 (C=O acid). FTIR (KBr 1%, cm^{-1}): ν=3020, 2994, 1728, 1622, 1542, 1519, 1461, 1366, 1233, 1200, 1150, 1100, 1050, 1000, 993, 938, 776, 717, 677. MS *m*/*z* (EI, %): 325 (M^+, 100). HRMS: *m*/*z* (EI) calcd for $C_{19}H_{19}O_4N$ 325.1315, found 325.1306.

*4.2.5.5. Benzocoumarins **8b** and **8c**.* The compounds were obtained as a light pink solid mixture (0.075 g, 75%). The mixture was used in the next reaction without further purification.

*4.2.5.5.1. 4-(1-Methyl-benzo[f]coumarin-9-yloxy)butanoic acid, **8b**.* ^{1}H NMR (400 MHz, DMSO-d_6): δ=2.02 (2H, t, *J* 6.3 Hz, βCH_2), 2.41–2.50 (2H, m, αCH_2), 2.93 (3H, s, CH_3), 4.19 (2H, t, *J* 6.3 Hz, γCH_2), 6.46 (1H,s, H2), 7.28 (1H, dd, *J* 9.0 and 2.1 Hz, H-8), 7.37 (1H, d, *J* 8.7 Hz, H5), 7.97–8.00 (2H, m, H7 and H10), 8.11 (1H, d, *J* 9.0 Hz, H6), 12.1 (1H, s, OH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ=18.25 (CH_3), 24.66 (βCH_2), 30.32 (αCH_2), 66.75 (γCH_2), 108.69 (C10), 110.70 (C10b), 114.01 (C5), 115.73 (C2), 117.34 (C8), 125.24 (C6a), 130.32 (C10a), 131.39 (C7), 134.40 (C6), 152.22 (C1), 155.36 (C4a), 157.82 (C9), 159.36 (C3).

*4.2.5.5.2. Ethyl 4-(4-methyl-benzo[g]coumarin-8-yloxy)butanoic acid, **8c**.* ^{1}H NMR (400 MHz, DMSO-d_6): δ=1.98–2.07 (2H, m, βCH_2), 2.41–2.45 (2H, t, *J* 7.2 Hz, αCH_2), 2.52 (3H, d, *J* 1.2 Hz, CH_3), 4.13 (2H, t, *J* 6.4 Hz, γCH_2), 6.38 (1H, d, *J* 1.2 Hz, H3), 7.19 (1H, dd, *J* 9.2 and 2.4 Hz, H7), 7.38 (1H, d, *J* 2.4 Hz, H9), 7.13 (1H, s, H10), 8.00 (1H, d, *J* 9.2 Hz, H6), 8.33 (1H, s, H5), 12.12 (1H, s, OH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ=18.44 (CH_3), 24.55 (βCH_2), 30.42 (αCH_2), 66.73 (γCH_2), 105.27 (C9), 110.44 (C10), 114.58 (C3), 117.94 (C4a), 119.92 (C7), 125.08 (C5), 125.36 (C5a), 130.45 (C6), 136.92 (C9a), 151.08 (C4), 152.11 (C10a), 158.61 (C8), 161.23 (C2).

*4.2.6. General procedure for the synthesis of compounds **4a**–**c** and **9a**–**c**.* Compounds **3a**–**c** and **8a**–**c** (1 equiv) were dissolved in dry *N*,*N*-dimethylformamide. 2-Bromo-1-phenylpropan-1-one (1 equiv) and potassium fluoride (3 equiv) were added to the mixture. The mixture was stirred, in an oil bath, at 50 °C for 1 day. The reaction mixture was filtered and the solvent was removed in a rotary evaporator. The crude residue was purified by silica gel column chromatography using chloroform/methanol (100:1) as eluent.

*4.2.6.1. 1-Oxo-1-phenylpropan-2-yl 2-(4-methylcoumarin-7-yloxy)acetate, **4a**.* The compound was obtained as white solid (0.065 g, 0.18 mmol, 83%). Mp=178.1–179.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.59 (3H, d, *J* 7.2 Hz, βCH_3), 2.39 (3H, d, *J* 1.2 Hz, CH_3), 4.79–4.88 (2H, m, CH_2), 6.08–6.13 (1H, m, αH), 6.15 (1H, d, *J* 1.2 Hz, H3), 6.82 (1H, d, *J* 2.8 Hz, H8), 6.92 (1H, dd, *J* 8.4 and 2.8 Hz, H6), 7.47–7.53 (3H, m, H5, H3′ and H5′), 7.59–7.63 (1H, m, H4′), 7.92 (2H, dd, *J* 8.4 and 1.2 Hz, H2′ and H6′). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=17.10 (βCH_3), 18.61 (CH_3), 65.04 (CH_2), 72.58 (αC), 102.06 (C8), 112.19 (C6), 112.50 (C3), 114.46 (C4a), 125.76 (C5), 128.41 (C2′ and C6′), 128.85 (C3′ and C5′), 133.83 (C4′), 133.97 (C1′), 152.31 (C4), 154.97 (C8a), 160.49 (C7), 161.01 (C2), 167.55 (C=O ester), 195.77 (C=O ketone). FTIR (KBr 1%, cm^{-1}): ν=1769, 1697, 1609, 1601, 1560, 1513, 1499, 1449, 1425, 1387, 1365, 1347, 1286, 1271, 1228, 1218, 1200, 1166, 1140, 1091, 1062, 1027, 1017, 990, 974, 948, 898, 864, 847, 703. UV–vis (ethanol, nm): λ_{max} (log ε)=316 (4.22). MS *m*/*z* (ESI, %): 367 ($[M+H]^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{21}H_{19}O_6$ 367.11761; found 367.11748.

*4.2.6.2. 1-Oxo-1-phenylpropan-2-yl 4-(4-methylcoumarin-7-yloxy)butanoate, **4b**.* The compound was obtained as a colourless oil (0.025 g, 0.08 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.54 (2H, d, *J* 6.8 Hz, βCH_3), 2.16–2.19 (2H, m, βCH_2), 2.39 (3H, s, CH_3), 2.63–2.67 (2H, m, αCH_2), 4.08 (2H, t, *J* 6.4 Hz, γCH_2), 5.97–6.02 (1H, m, αH), 6.12 (1H, d, *J* 1.2 Hz, H3), 6.80 (1H, d, *J* 2.4 Hz, H8), 6.85 (1H, dd, *J* 8.4 and 2.8 Hz, H6), 7.46–7.49 (3H, m, H5, H3′ and H5′), 7.56–7.61 (1H, m, H4′), 7.94 (2H, dd, *J* 8.0 and 3.2 Hz, H2′ and H6′). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=17.10 (βCH_3), 18.61 (CH_3), 24.28 (βCH_2), 30.28 (αCH_2), 67.07 (γCH_2), 71.56 (αC), 101.49 (C8), 111.90 (C3), 112.45 (C6), 113.57 (C4a), 125.48 (C5), 128.38 (C2′ and C6′),

128.78 (C3′ and C5′), 133.60 (C4′), 134.27 (C1′), 152.48 (C4), 155.19 (C8a), 161.26 (C2), 161.82 (C7), 172.36 (C=O ester), 196.76 (C=O ketone). FTIR (liquid film, cm^{-1}): ν=1732, 1699, 1615, 1558, 1510, 1472, 1449, 1426, 1388, 1369, 1294, 1265, 1229, 1202, 1091, 1071, 1036, 1016, 973, 879, 849, 792, 702, 666. UV–vis (ethanol, nm): λ_{max} (log ε)=320 (4.28). MS *m*/*z* (ESI, %): 395 ($[M+H]^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{23}H_{23}O_6$ 395.14891, found 395.14885.

4.2.6.3. 1-Oxo-1-phenylpropan-2-yl 4-(4-methyl-6-nitrocoumarin-7-yloxy)butanoate, ***4c****.* The compound was obtained as a colourless oil (0.030 g, 0.07 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.54 (2H, d, *J* 7.2 Hz, βCH_3), 2.17–2.27 (2H, m, βCH_2), 2.44 (3H, d, *J* 1.2 Hz, CH_3), 2.69–2.73 (2H, m, αCH_2), 4.24–4.27 (2H, m, γCH_2), 5.96–6.01 (1H, m, αH), 6.26 (1H, d, *J* 1.2 Hz, H3), 6.97 (1H, s, H8), 7.46–7.50 (2H, m, H3′ and H5′), 7.56–7.60 (1H, m, H4′), 7.93 (2H, dd, *J* 8.4 and 1.6 Hz, H2′ and H6′), 8.18 (1H, s, H5). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=17.10 (βCH_3), 18.55 (CH_3), 24.08 (βCH_2), 29.95 (αCH_2), 68.89 (γCH_2), 71.77 (αC), 102.39 (C8), 112.73 (C4a), 113.99 (C3), 122.94 (C5), 128.40 (C2′ and C6′), 128.78 (C3′ and C5′), 133.60 (C4′), 134.25 (C1′), 151.49 (C4), 155.17 (C7), 157.25 (C8a), 159.40 (C2), 172.24 (C=O ester), 196.82 (C=O ketone). FTIR (liquid film, cm^{-1}) ν=3037, 2937, 1737, 1698, 1622, 1598, 1533, 1501, 1449, 1384, 1363, 1316, 1291, 1256, 1229, 1207, 1161, 1135, 1095, 1064, 1035, 1001, 973, 909, 846, 736, 702, 666. UV–vis (ethanol, nm): λ_{max} (log ε)=321 (4.00). ES *m*/*z* (ESI, %): 440 ($[M+H]^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{23}H_{22}O_8N$ 440.13399, found 440.13407.

4.2.6.4. 1-Oxo-1-phenylpropan-2-yl 4-(1,4-dimethylbenzo[f]quinolin-3(4H)-one-9-yloxy)butanoate, ***9a****.* The compound was obtained as beige oil (0.020 g, 4.37 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.54 (2H, d, *J* 7.2 Hz, βCH_3), 2.05–2.27 (2H, m, βCH_2), 2.69–2.73 (2H, m, αCH_2), 2.92 (3H, s, CH_3), 3.85 (3H, s, NCH_3), 4.19 (2H, t, *J* 6.0 Hz, γCH_2), 5.98–6.03 (1H, m, αH), 6.71 (1H, s, H2), 7.18 (1H, d, *J* 8.8 and 2.4 Hz, H8), 7.43–7.49 (3H, m, H3′, H5′ and H5), 7.55–7.59 (1H, m, H4′), 7.80 (1H, d, *J* 9.2 Hz, H7), 7.90 (1H, d, *J* 9.2 Hz, H6), 7.92–7.95 (2H, m, H2′ and H6′), 7.99 (1H, d, *J* 2.4 Hz, H10). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=17.11 (βCH_3), 24.49 (βCH_2), 26.76 (CH_3), 30.46 (αCH_2), 30.50 (NCH_3), 66.73 (γCH_2), 71.53 (αC), 107.64 (C10), 112.76 (C5), 115.67 (C10b), 115.96 (C8), 122.28 (C2), 125.14 (C6a), 128.37 (C2′ and C6′), 128.77 (C3′ and C5′), 130.47 (C7), 131.99 (C6), 132.38 (C10a), 133.60 (C4′), 134.29 (C1′), 140.70 (C4a), 147.71 (C1), 157.96 (C9), 161.50 (C3), 172.55 (C=O ester), 196.79 (C=O ketone). FTIR (liquid film, cm^{-1}): ν=1737, 1698, 1652, 1652, 1603, 1598, 1576, 1548, 1519, 1449, 1441, 1416, 1366, 1323, 1226, 1172, 1133, 1096, 1041, 1001, 973, 859, 832, 793, 735, 701, 666. UV–vis (ethanol, nm): λ_{max} (log ε)=335 (4.00). MS *m*/*z* (ESI, %): 458 ($[M+H]^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{23}H_{23}O_5S$ 458.19620, found 458.19623.

4.2.6.5. Benzocoumarins ***9b*** *and* ***9c****.* The resulting crude oil was purified by silica gel column chromatography using chloroform/methanol (100:1) as eluent. Fractions containing the separated products were combined and evaporated, yielding compound **9b** as colourless oil and compound **9c** as white solid.

4.2.6.5.1. 1-Oxo-1-phenylpropan-2-yl 4-(1-methyl-benzo[f]coumarin-9-yloxy)butanoate, ***9b****.* Yield 0.030 g, 0.07 mmol, 78%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.54 (2H, d, *J* 6.8 Hz, βCH_3), 2.21–2.27 (2H, m, βCH_2), 2.67–2.77 (2H, m, αCH_2), 2.91 (3H, s, CH_3), 4.19 (2H, t, *J* 6.0 Hz, γCH_2), 5.98–6.03 (1H, m, αH), 6.32 (1H, d, *J* 0.8 Hz, H2), 7.21 (1H, d, *J* 8.8 and 2.4 Hz, H8), 7.31 (1H, dd, *J* 8.8 Hz, H5), 7.47 (3H, dt, *J* 6.4 and 1.6 Hz, H3′ and H5′), 7.58 (1H, dt, *J* 7.6 and 1.2 Hz, H4′), 7.81 (1H, d, *J* 8.8 Hz, H7), 7.88 (1H, d, *J* 8.8 Hz, H6), 7.92–7.95 (3H, m, H10, H2′ and H6′). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=17.13 (βCH_3), 24.55 (βCH_2), 26.22 (CH_3), 30.39 (αCH_2), 66.75 (γCH_2), 71.56 (αCH), 107.08 (C10), 113.74 (C10b), 115.31 (C5), 115.89 (C2), 116.45 (C8), 126.45 (C10a), 128.37 (C2′ and C6′), 128.79 (C3′ and C5′), 131.01 (C7), 131.69 (C6a), 133.30 (C6), 134.27 (C1′), 154.17 (C1), 155.29 (C4a), 158.36 (C9), 160.45 (C3), 172.53 (C=O ester), 196.79 (C=O ketone). FTIR (KBr 1%, cm^{-1}): ν=3063, 2984, 2938, 1730, 1698, 1624, 1596, 1552, 1518, 1449, 1434, 1401, 1375, 1357, 1312, 1281, 1251, 1227, 1175, 1137, 1090, 1050, 973, 931, 838, 735, 702, 666, 580. UV–vis (ethanol, nm): λ_{max} (log ε)=347 (4.05). MS *m*/*z* (ESI, %): 445 ($[M+H]^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{27}H_{25}O_6$ 445.16456, found 445.16459.

4.2.6.5.2. 1-Oxo-1-phenylpropan-2-yl 4-(4-methyl-benzo[g]coumarin-8-yloxy)butanoate, ***9c****.* Yield 0.045 g, 0.10 mmol, 66%. Mp=136.2–137.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.55 (2H, d, *J* 6.8 Hz, βCH_3), 2.18–2.26 (2H, m, βCH_2), 2.53 (3H, d, *J* 1.2 Hz, CH_3), 2.68–2.72 (2H, m, αCH_2), 4.17 (2H, t, *J* 6.4 Hz, γCH_2), 5.99–6.04 (1H, m, αH), 6.28 (1H, d, *J* 1.2 Hz, H3), 7.11 (1H, d, *J* 2.4 Hz, H9), 7.14 (1H, dd, *J* 8.8 and 2.4 Hz, H7), 7.48 (3H, dt, *J* 6.0, 1.6 Hz, H3′ and H5′), 7.56–7.60 (2H, m, H4′ and H10), 7.81 (2H, dd, *J* 9.2 Hz, H6), 7.95 (2H, dd, *J* 8.4 and 1.2 Hz, H2′ and H6′), 7.99 (1H, s, H5). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=17.13 (βCH_3), 18.65 (CH_3), 24.43 (βCH_2), 30.46 (αCH_2), 66.74 (γCH_2), 71.55 (αC), 105.40 (C9), 111.64 (C10), 114.56 (C3), 117.92 (C4a), 119.57 (C7), 124.64 (C5), 125.58 (C5a), 128.41 (C2′ and C6′), 128.79 (C3′ and C5′), 130.14 (C6), 133.62 (C4′), 134.32 (C1′), 136.42 (C9a), 150.84 (C4), 152.12 (C10a), 158.72 (C8), 160.98 (C2), 172.53 (C=O ester), 196.85 (C=O ketone). FTIR (KBr 1%, cm^{-1}): ν=3427, 3058, 2934, 1279, 1729, 1632, 1597, 1484, 1464, 1449, 1389, 1358, 1323, 1255, 1228, 1188, 1148, 1052, 1034, 977, 942, 891, 841, 810, 694. UV–vis (ethanol, nm): λ_{max} (log ε)=343 (4.21). MS *m*/*z* (ESI, %): 445 ($[M+H]^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{27}H_{25}O_6$ 445.16456, found 445.16467.

4.2.7. Synthesis of 1-oxo-1-phenylpropan-2-yl 2-(4-methylcoumarin-2-thione-7-yloxy)butanoate, ***4d****.* Compound **4b** (0.093 g, 0.23 mmol) was dissolved in dry toluene (5 mL) and Lawesson's reagent was added (0.8 equiv, 0.076 g, 0.18 mmol). The reaction mixture was heated at reflux for 1 day. The mixture was filtered hot and the solvent removed by rotary evaporation. The crude residue was purified by silica gel column chromatography using chloroform as eluent, yielding compound **4d** as a yellow solid (0.060 g, 0.15 mmol, 65%). Mp=115.3–118.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.55 (3H, d, *J* 6.8 Hz, βCH_3), 2.12–2.23 (2H, m, βCH_2), 2.35 (3H, d, *J* 1.2 Hz, CH_3), 2.60–2.73 (2H, m, αCH_2), 4.11 (2H, t, *J* 6.4 Hz, γCH_2), 5.98–6.03 (1H, m, αH), 6.93 (1H, dd, *J* 8.8 and 2.4 Hz, H6), 6.96 (1H, d, *J* 2.4 Hz, H8), 7.07 (1H, d, *J* 1.2 Hz, H3), 7.47–7.51 (2H, m, H3′ and H5′), 7.54 (1H, d, *J* 8.8 Hz, H5), 7.58–7.62 (1H, m, H4′), 7.95 (2H, dd, *J* 8.4 and 1.2 Hz, H2′ and H6′). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=17.13 (βCH_3), 18.00 (CH_3), 24.26 (βCH_2), 30.30 (αCH_2), 67.29 (γCH_2), 71.60 (αC), 101.04 (C8), 114.32 (C6), 115.29 (C4a), 125.46 (C5), 126.58 (C3), 128.41 (C2′ and C6′), 128.80 (C3′ and C5′), 133.64 (C4′), 134.29 (C1′), 144.92 (C4), 157.92 (C8a), 162.26 (C7), 172.30 (C=O ester), 196.75 (C=O ketone), 195.35 (C2). FTIR (KBr 1%, cm^{-1}): ν=3043, 2985, 2944, 1731, 1699, 1625, 1603, 1546, 1467, 1449, 1390, 1348, 1302, 1286, 1250, 1228, 1212, 1175, 1156, 1135, 1072, 1017, 971, 935, 842, 697, 666. UV–vis (ethanol, nm): λ_{max} (log ε)=397 (4.28). MS *m*/*z* (ESI, %): 411 ($[M+H]^+$, 100). HRMS: *m*/*z* (ESI) calcd for $C_{23}H_{23}O_5S$ 411.12607, found 411.12585.

4.3. General photolysis procedure

A 1×10^{-4} m methanol/HEPES buffer (80:20) solution of conjugates **4** and **9** (5 mL) was placed in a quartz tube and irradiated in a Rayonet RPR-100 reactor at the desired wavelength. The lamps used for irradiation were of 300, 350 and 419±10 nm. HEPES buffer solution was prepared in distilled water with HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) (10 mM), sodium chloride (120 mM), potassium chloride (3 mM), calcium chloride (1 mM) and magnesium chloride (1 mM) and the pH adjusted to 7.2 with aqueous 1 M sodium hydroxide.

Aliquots of 100 μL were taken at regular intervals and analysed by RP-HPLC. The eluent was acetonitrile/water (3:1) previously filtered through a Millipore, type HN 0.45 μm filter and degassed by

ultra-sound for 30 min, at a flow rate of 0.8 mL/min for all compounds. The chromatograms were traced by detecting UV absorption at the wavelength of maximum absorption for each conjugate (retention time: **4a**, 5.6; **4b**, 4.5; **4c**, 5.3; **4d**, 7.8; **9a**, 8.3; **9b**, 7.7; **9c**, 8.4 min).

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