Benzoxazole derivatives as phototriggers for the release of butyric acid

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Abstract: With the aim of evaluating the application of new benzoxazole derivatives as photoactive moiety in butyric acid prodrugs, ester conjugates were synthesized by reaction of the chloromethylated heterocyclic precursors with the model drug. Photocleavage studies of the conjugates in methanol/HEPES buffer (80:20) solutions at different wavelengths of irradiation (254, 300 and 350 nm) confirmed the quantitative release of the butyric acid in short irradiation times.

Keywords: benzoxazoles; coumarins; oxo-benzopyrans; phototriggers; prodrugs; butyric acid.

1. Introdution

Photoremovable protecting groups find application in the photoactivation of organic, small inorganic species and ions in several areas including synthesis and bioapplications, namely in biochemistry, neurobiology and biomedicine.[1] In bioapplications these groups are more especially designated as phototriggers. As an extension, the use of an appropriate photolabile group could be an alternative to the molecular design of prodrugs, being the reactivity controlled by selecting the wavelength of the excitation light. Recent reports enclose the use of photoactive prodrugs in the improvement of drug delivery.[2-4]

Butyric acid is known to be related to the disruption of cell proliferation and induction of apoptosis; modification of cell morphology; and alteration of gene expression.[5,6] Nonetheless, poor absorption from the gastrointestinal tract due to the presence of a carboxylic acid group is a limitation to be circumvent.

Recent research by the authors includes synthesis and application of novel oxygen and nitrogen heterocycles as photolabile groups in the light triggered release of several biological relevant compounds, including butyric acid.[7-10] With the aim of improving the photophysical properties of oxo-benzopyranoxazole derivatives, and consequently the delivery of the drug, three new oxo-benzopyranoxazoles were synthesised and evaluated as photosensitive moieties. The stability to irradiation of the ester bond between butyric acid and the caging group was evaluated in a

photochemical reactor at 254, 300 and 350 nm using methanol with aqueous HEPES buffer in 80:20 solutions with collection of kinetic data.

2. Experimental

2.1. Typical procedure for the synthesis of oxo-benzopyranoxazoles 5-7 (described for 5). To a solution of 6-amino-4-(chloromethyl)-7-hydroxy-2-oxo-2*H*-benzopyran **1** (0.095 g, 0.42 mmol) in polyphosphoric acid (0.500 g), benzoic acid **3** (2 equiv, 0.101 g, 0.83 mmol) was added, and the mixture was stirred at 130 °C for 4 h. The reaction mixture was poured into iced water and stirred for 1 h to give a fine grey precipitate. The solid was collected by filtration, washed with cold water and dried in a vacuum oven. 8-(Chloromethyl)-2-phenyl-6-oxo-6*H*-benzopyrano[6,7-*d*]oxazole **5** was obtained as a grey solid (0.080 g, 62 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 5.11 (2H, s, CH_2), 6.69 (1H, s, H-7), 7.61-7-67 (3H, m, 3×Ar-H), 7.92 (1H, s, H-4), 8.21 (2H, dd, *J* 7.6 and 1.6 Hz, 2×Ar-H), 8.28 (1H, s, H-9) ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 41.47 (CH_2), 99.58 (C-4), 114.13 (C-7), 114.69 (C-8a), 115.46 (C-9), 125.65 (Ar-C), 127.28 (Ar-C), 127.35 (Ar-C), 129.20 (Ar-C), 132.27 (Ar-C), 138.36 (C-9a), 150.75 (C-8), 151.64 (C-4a), 151.93 (C-3a), 159.03 (C-6), 163.91 (C-2) ppm.

2.2. Typical procedure for the synthesis of conjugates 8-10 (described for 8). The chloromethyl precursor **5** (0.090 g, 0.29 mmol) was dissolved in dry DMF (3 mL), potassium fluoride (3 equiv, 0.050 g, 0.86 mmol) and butyric acid (1 equiv, 0.026 mL, 0.29 mmol) were added. The reaction mixture was stirred at room temperature for 24 h. The solvent was removed by evaporation under reduced pressure and the crude residue was purified by column chromatography using chloroform/methanol (95:5) as eluent. (6-Oxo-2-phenyl-6*H*-benzopyrano[6,7-*d*]oxazol-8-yl)methyl butyrate **8** was obtained as a yellow solid (0.050 g, 48 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.01 (3H, t, *J* 7.6 Hz, $CH_2CH_2CH_3$), 1.73-1.78 (2H, m, $CH_2CH_2CH_2CH_3$), 2.48 (2H, t, *J* 7.2 Hz, $CH_2CH_2CH_3$), 5.38 (2H, s, CH_2), 6.52 (1H, s, H-7), 7.54-7.61 (4H, m, H4 and 3×Ar-H), 7.91 (1H, s, H-9), 8.25-8.28 (2H, m, 2×Ar-H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 13.20 ($CH_2CH_2CH_3$), 17.88 ($CH_2CH_2CH_3$), 35.44 ($CH_2CH_2CH_3$), 60.74 (CH_2), 99.37 (C-4), 111.85 (C-7), 113.51 (C-9), 114.36 (C-8a), 125.72 (Ar-C), 127.41 (Ar-C), 128.64 (Ar-C), 131.86 (Ar-C), 138.87 (C-9a), 148.99 (C-8), 151.48 (C-4a), 152.06 (C-3a), 159.79 (C-6), 164.49 (C-2), 172.24 (C=O ester) ppm.

2.3. General photolysis procedure

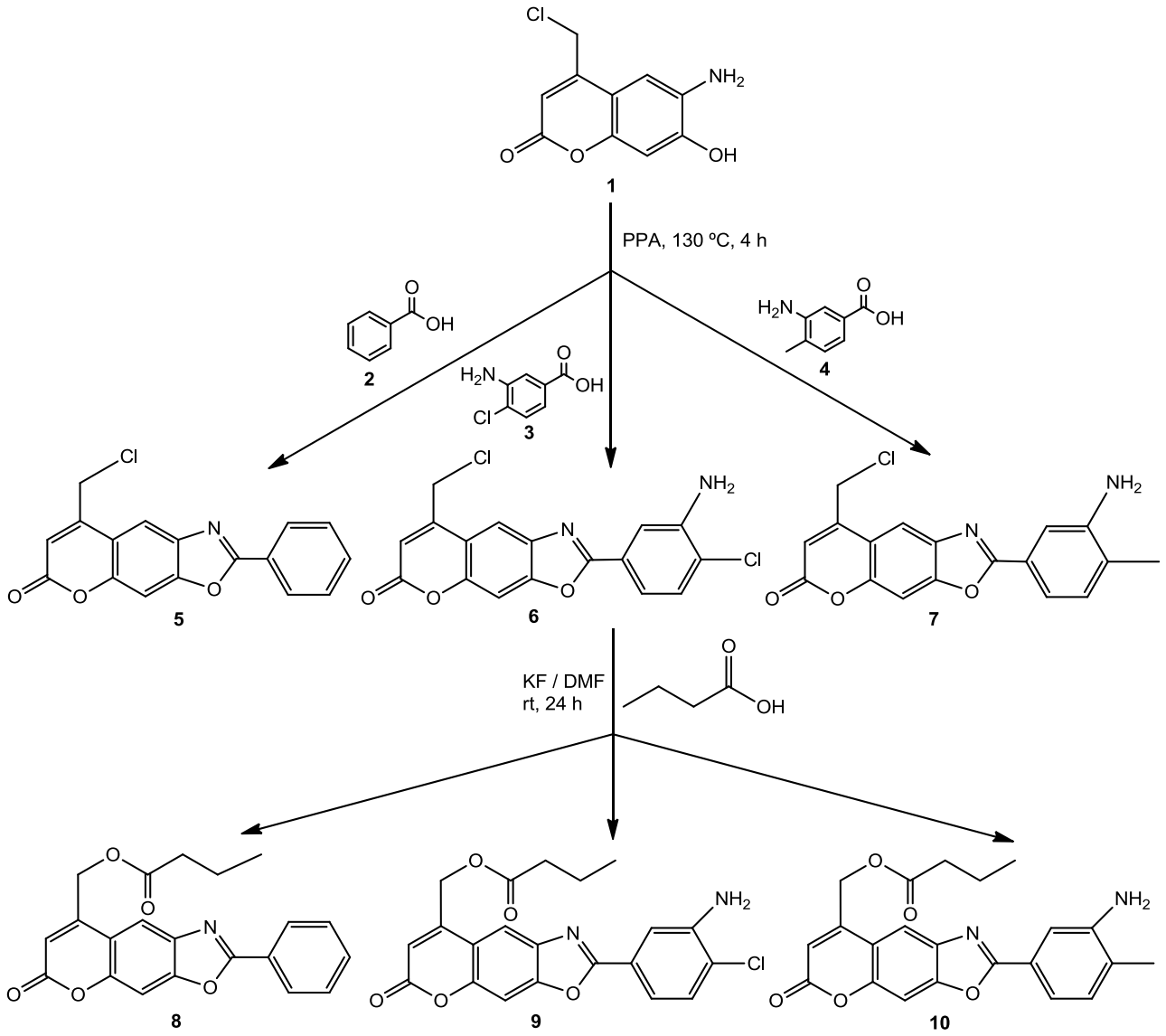
A 1×10^{-4} M methanol/HEPES buffer (80:20) solution of conjugates **8**-**10** (5 mL) was placed in a quartz tube and irradiated in a Rayonet RPR-100 reactor at the desired wavelength. The lamps used for irradiation were of 254, 300, and 350 ± 10 nm. HEPES buffer solution was prepared in distilled water 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 pH adjusted to 7.2. Aliquots of 100 µL were taken at regular intervals and analysed by RP-HPLC. The eluent was acetonitrile/water (3:1) at a flow rate of 0.8 mL/min for all compounds, previously filtered through a Millipore, type HN 0.45 µm filter and degassed by ultra-sound for 30 min. The chromatograms were traced by detecting UV absorption at the wavelength of maximum absorption (retention time: 6.6 min, **8**; 6.5 min, **9**; 6.7 min, **10**).

3. Results and Discussion

Previous work reported by the authors includes the use of fused oxazole systems, namely an oxo-benzopyranoxazole, as phototriggers in the release of butyric acid.[9,10] In order to improve the photophysical properties and the photorelease times of the considered drug from this type of heterocycles, compounds **5**-**7** bearing the phenyl ring without substituents and with chlorine atom or methyl group, in addition to the amine function, were synthesized.

The synthesis of these new chloromethylated oxo-benzopyran[6,7-*d*]oxazoles **5**-**7** was achieved by a condensation reaction between 6-amino-4-(chloromethyl)-7-hydroxy-2-oxo-2*H*-benzopyrane **1**[9] and benzoic acid **2**, 3-amino-4-chlorobenzoic acid **3** or 3-amino-4-methylbenzoic acid **4** mediated by polyphosphoric acid at 130º C according to a known procedure.[11] Compounds **5**-**7** were used in the derivatisation of butyric acid in the presence of potassium fluoride in *N*,*N*-dimethylformamide at room temperature,[12] resulting in the ester prodrugs **8**-**10** in moderate to good yields (Table 1, Scheme 1).

UV-visible spectroscopic characterization was carried out to obtain the required parameters for monitoring during photolysis. Absorption and emission spectra of degassed 10^{-5} M solutions in methanol/HEPES buffer (80:20) solution of conjugates **8**-**10** were measured and the corresponding data are presented in Table 1.



Scheme 1. Synthesis of oxo-benzopyranoxazoles **5-7** and the corresponding butyric acid conjugates **8-10**.

Relative fluorescence quantum yields (Φ_F) were calculated using 9,10-diphenylanthracene in ethanol (Φ_F 0.95)[13] as standard. For the Φ_F determination, the fluorescence standard was excited at the wavelengths of maximum absorption found for each compound to be tested and in all fluorimetric measurements the absorbance of the solution did not exceed 0.1.

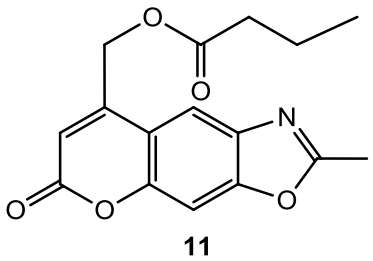
Regarding the maximum absorption wavelengths (λ_{abs}) in methanol/HEPES buffer (80:20) solutions the new conjugates **8-10** displayed a bathochromic shift from 13 to 19 nm (λ_{abs} 339-345 nm) in comparison with compound **11**, previously obtained by us (Figure 1).[9]

The fluorescence spectra in the same solvent revealed that emission maxima (λ_{em}) of conjugates **8-10** occurred in the range 418-456 nm, with relative fluorescent quantum yields inferior to the analogue **11**, and good Stokes' shifts (79 -112 nm).

Table 1. Yields, UV/visible absorption and fluorescence data for compounds **5-11** in methanol/HEPES buffer (80:20) solutions.

Cpd	Yield (%)	Absorption		Fluorescence		
		λ_{abs} (nm)	log ε	λ_{em} (nm)	Φ_F	Stokes' shift (nm)
5	62	341	3.98	397	0.06	56
6	70	341	3.85	418	0.01	77
7	54	342	3.46	423	0.02	81
8	48	339	3.86	418	0.07	79
9	70	344	3.78	456	0.07	112
10	69	345	4.04	430	0.01	85
11[9]	95	326	3.83	424	0.10	98

The release of butyric acid from conjugates **8**-**10** was carried out by photolysis at different wavelengths. Solutions of the mentioned compounds (1×10^{-4} M) in methanol/HEPES buffer (80:20) solutions were irradiated in a Rayonet RPR-100 reactor at 254, 300 and 350 nm, in order to determine the most favourable cleavage conditions. The course of the photocleavage reactions 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 area was detected (Table 2).



11

Figure 1. Structure of conjugate **11**.[9]

Table 2. Irradiation times (t_{irr}, in min) and k ($\times 10^{-2}$ min^{-1}) for the complete photolysis (95%) of compounds **8-11** at different wavelengths in methanol/HEPES buffer (80:20) solutions.

Compound	254 nm		300 nm		350 nm	
	t_{irr}	k	t_{irr}	k	t_{irr}	k
8	13	23.1	46	6.05	55	5.38
9	58	5.14	195	1.56	193	1.56
10	35	8.62	127	2.32	160	1.87
11[9]	45	6.59	33	6.59	285	1.04

The results at various wavelengths of irradiation revealed the significant influence of the aromatic substitution on the oxazole ring, at position 2 of the oxo-benzopyranoxazole, in the irradiation time (t_{irr}) necessary to release butyric acid (Table 2). In comparison with conjugate **11**, bearing a methyl group, the most relevant result is related to the decrease of irradiation times at 350 nm, more than five times in the case of compound **8** (t_{irr} 55 mim), which is advantageous for biological purposes. On the other hand, by comparing conjugates **9** and **10**, the presence at the benzene ring of a methyl group promotes faster photolysis than chlorine atom in all wavelengths of irradiation. Considering practical applications of the present compounds, although they cleaved readily at 254 nm (the fastest being **8** with 13 min) and also at 300 nm (the fastest being **8** with 46 min), photolysis at these wavelengths can be damaging to biological media. Therefore, photolysis at 350 nm and longer wavelengths is always preferable, and encouraged by the obtained results, we will continue the development of new oxo-benzopyranoxazoles.

4. Conclusions

Three new fluorescent ester conjugates were synthesised in moderated to good yields by a straightforward procedure, between chloromethylated oxo-benzopyranoxazole derivatives and butyric acid. Evaluation of these conjugates as photoactive prodrugs revealed that quantitative release of butyric acid was possible under irradiation at 254, 300 and 350 nm in short irradiation times. This preliminary study also suggests that aromatic substitution at the oxazole ring decreased the time necessary for delivering the drug. The presence and nature of substituents at phenyl could also influence the photolysis of conjugates and future studies will be carried out to clarify these effects and increase the conjugation in the aromatic system.

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