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Photoactivatable prodrugs of butyric acid based on new coumarin fused oxazole heterocycles

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ABSTRACT

New coumarin fused oxazoles were investigated as photosensitive units for carboxylic acid groups using butyric acid as a model compound. 6-Oxo-6*H*-benzopyrano[6,7-*d*]oxazol-8-yl)methyl derivatives possessing various (hetero)aromatic substituents at position 2 of the heterocyclic system were used in the synthesis of ester conjugates of butyric acid. Photolysis at selected wavelengths in methanol/HEPES buffer (80:20) solutions, monitored by HPLC/UV and ^{1}H NMR, resulted in the complete release of butyric acid. The shorter irradiation times for cleavage at longer wavelengths occurred for the conjugate with a 4-oxo-4*H*-benzopyran-2-yl substituent and thus (6-oxo-2-(4-oxo-4*H*-benzopyran-2-yl)-6*H*-benzopyrano[6,7-*d*]oxazol-8-yl)methyl has potential as a candidate photosensitive moiety for butyric acid prodrugs.

1. Introduction

A diversity of light-sensitive moieties have been reported in recent years to target molecules including amines and amino acid neurotransmitters, nucleic acids, enzyme substrates and inhibitors, proteins, biochemical sensors, as well as to be used as triggers for the preparation of biomaterials. The use of light in combination with these moieties enables the behavioural manipulation of organisms, control of cell biochemistry and the treatment of a variety of anomalous physiological conditions and manifestations of disease [1–6]. Moreover, "phototherapeutics" possesses wide-ranging potential applications in cancer therapy, tissue engineering and surgery [7]. The preparation of light-sensitive species usually involves the covalent modification of a functional group essential for the biological activity of the compound of interest. This modification results from the use of a photocleavable protecting group, also designated as a phototrigger or caging group. Carboxylates, amines and alcohols are typical reactive sites for substitution by photocleavable units, usually through ester, carbonates, carbamate and anhydride linkages [3,8,9]. The implementation of a caging strategy provides both spatial and temporal control over the release of molecules triggered by ultraviolet and visible light.

The occurrence of severe side effects, drug resistance, along with other diverse factors that influence the efficacy of drug activity are responsible for the development of innovative methodologies to circumvent these limitations. Butyric acid can be taken as an example, as it is a pleotropic anticancer agent that has a specific effect on the inhibition of nuclear histone deacetylase enzymes, leading to an increase in the acetylation level of H3 and H4 histones. However, *in vivo* it displays low potency because of rapid metabolisation [10–12]. In order to bypass this problem, butyric acid prodrugs, including those that are photoactivatable, have been described in the literature [13,14].

Our research has been involved in studies related with the design, synthesis and evaluation of light-sensitive moieties for the release of bioactive molecules, including butyric acid [1,2,15–20]. Recently, we have reported on coumarin, benzocoumarin thio(-benzo)coumarin, coumarin fused with julolidine and amino-substituted benzocoumarin cages [19–21]. Also, our studies with photoactive prodrugs of butyric acid were initiated with the use of naphthoxazoles and coumarin fused oxazoles; namely naphtho[2,3-*d*]oxazole, naphtho[1,2-*d*]oxazole and 6-oxo-6*H*-benzopyrano[6,7-*d*]oxazole (with a linkage between the heterocycle and the active molecule through oxopyran or oxazole moieties) [22]. In this regard, and considering that the more promising results were

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found with benzopyranoxazole with a linkage to butyric acid through the pyranone ring, we describe here the synthesis of a new set of benzopyranoxazoles, with an improved capability for the photorelease of butyric acid using longer wavelength light to initiate the release. Longer wavelengths are advantageous as they help to avoid the absorption of light by intrinsic species (biological material including amino acids, proteins and nucleic acids), and enable the resulting conjugates to be addressed using two-photon excitation.

In this work, novel 6-oxo-6*H*-benzopyrano[6,7-*d*]oxazol-8-yl) methyl groups were synthesised and used in the caging of butyric acid. The resulting ester cages were irradiated at 254, 300 and 350 nm in a photochemical reactor in a solution of methanol/HEPES buffer (80:20). Because the caging groups exhibit fluorescence, it is possible to make use of this fact and employ fluorescence techniques, principally time-resolved methods to characterise their photophysical properties. Since the photocleavage proceeds via intermediate species (eg ion pairs) it is helpful to ascertain if the substituent groups have any marked effect on processes that can be elucidated using changes in their fluorescence behaviour. The determination of decay associated spectra enables both spectral (energetic) and decay kinetics to be compared.

2. Experimental section

2.1. Material and instruments

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/visible absorption spectra (200–700 nm) were obtained using a Shimadzu UV/2501 PC spectrophotometer. NMR spectra were obtained on 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 ppm using δ_H Me_4Si = 0 ppm as reference and *J* values are given in Hz. Assignments were supported by spin decoupling-double resonance and bidimensional heteronuclear correlation techniques. 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 fluorometer. Time-resolved fluorescence measurements were performed on a HORIBA Scientific DeltaFlex with a DeltaDiode excitation source emitting at 336 nm (DD-340). All reagents were used as received.

2.2. General procedure for the synthesis of oxo-benzopyranoxazoles ***3a-g***

To a solution of 6-amino-4-(chloromethyl)-7-hydroxy-2-oxo-2*H*-benzopyran **1** (1 equiv.) in polyphosphoric acid (0.500 g), the acid derivative **2** (2 equiv.) 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 precipitate. The solid was collected by filtration, washed with cold water and dried in a vacuum oven.

2.2.1. 8-(Chloromethyl)-2-phenyl-6-oxo-6H-benzopyrano[6,7-d] oxazole, ***3a***

Starting from benzopyran **1** (0.095 g, 0.42 mmol) in polyphosphoric acid (0.500 g) and benzoic acid **2a** (0.101 g, 0.83 mmol), compound **3a** was obtained as a grey solid (0.080 g, 62%). mp = 251.1–251.9 °C. λ_{max} (MeOH-HEPES 80/20)/nm 341 (log ε 3.98). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 5.11 (s, 2 H, CH_2), 6.69 (s, 1 H, H-7), 7.61-7-67 (m, 3 H, H-3′, H-4′ and H-5′), 7.92 (s, 1 H, H-4), 8.21 (dd, *J* = 7.6 and 1.6 Hz, 2 H, H-2′ and H-6′), 8.28 (s, 1 H, H-9) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ = 41.47 (CH_2), 99.58 (C-4), 114.13 (C-7), 114.69 (C-8a), 115.46 (C-9), 125.65 (C-1′), 127.28 (C-2′ and C-6′), 129.20 (C-3′ and C-5′), 132.27 (C-4′), 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. IR (KBr 1%): ν_{max} 3377, 2922, 2315, 1714 (br), 1632, 1595, 1554, 1489, 1438, 1406, 1353, 1267, 1137, 1043, 1018, 961, 886, 843, 778, 729, 700, 664 cm^{-1}. HRMS: *m*/*z* (EI): Found [M$^+$]: 311.03585; $C_{17}H_{10}NO_3Cl$ requires [M$^+$]: 311.03492.

2.2.2. 2-(3′-Aminophenyl)-8-(chloromethyl)-6-oxo-6H-benzopyrano[6,7-d]oxazole, ***3b***

Starting from benzopyran **1** (0.050 g, 0.22 mmol) in polyphosphoric acid (0.500 g) and 3-aminobenzoic acid **2b** (0.060 g, 0.22 mmol), compound **3b** was obtained as a grey solid (0.065 g, 89%). mp = 242.5–243.5 °C. λ_{max} (MeOH-HEPES 80/20)/nm 340 (log ε 3.90). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 5.13 (s, 2 H, CH_2), 6.70 (s, 1 H, H-7), 7.02 (d, *J* = 7.6 Hz, 1 H, H-4′), 7.36 (t, *J* = 7.6 Hz, 1 H, H-5′), 7.53 (dd, *J* = 7.6 and 1.6 Hz, 1 H, H-6′), 7.60 (s, 1 H, H-2′), 7.96 (s, 1 H, H-4), 8.26 (s, 1 H, H-9) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ = 41.61 (CH_2), 99.70 (C-4), 114.36 (C-7), 114.65 (C-2′), 114.83 (C-8a), 115.66 (C-9), 117.92 (C-6′), 120.22 (C-4′), 126.48 (C-1′), 130.29 (C-5′), 138.56 (C-9a), 145.52 (C-3′), 151.10 (C-8), 151.80 (C-4a), 152.06 (C-3a), 159.70 (C-6), 164.36 (C-2) ppm. IR (KBr 1%): ν_{max} 3385, 2965, 2320, 1720 (br), 1635, 1593, 1557, 1487, 1442, 1410, 1328, 1143, 1094, 995, 877, 744, 666 cm^{-1}. HRMS: *m*/*z* (EI): Found [M$^+$]: 326.04501; $C_{17}H_{11}N_2O_3Cl$ requires [M$^+$]: 326.04582.

2.2.3. 2-(3′-Amino-4′-methylphenyl)-8-(chloromethyl)-6-oxo-6H-benzopyrano[6,7-d]oxazole, ***3c***

Starting from benzopyran **1** (0.100 g, 0.44 mmol) in polyphosphoric acid (0.500 g) and 4-methyl-3-aminobenzoic acid **2c** (0.066 g, 0.44 mmol), compound **3c** was obtained as a grey solid (0.080 g, 54%). mp = 201.3–202.0 °C. λ_{max} (MeOH-HEPES 80/20)/nm 342 (log ε 3.46). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 5.12 (s, 2 H, CH_2), 6.69 (s, 1 H, H-7), 7.20 (d, *J* = 7.6 Hz, 1 H, H-5′), 7.41 (d, *J* = 7.6 Hz, 1 H, H-6′), 7.58 (s, 1 H, H-2′), 7.92 (s, 1 H, H-4), 8.22 (s, 1 H, H-9) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ = 17.57 (CH_3), 41.67 (CH_2), 99.60 (C-4), 113.74 (C-2′), 114.11 (C-7), 114.72 (C-8a), 115.24 (C-9), 117.14 (C-6′), 123.90 (C-4′), 128.01 (C-1′), 131.00 (C-5′), 138.57 (C-9a), 144.88 (C-3′), 150.98 (C-8), 151.53 (C-4a), 151.88 (C-3a), 159.57 (C-6), 164.55 (C-2) ppm. IR (KBr 1%): ν_{max} 3377, 2970, 2316, 1719 (br), 1635, 1585, 1558, 1489, 1438, 1406, 1328, 1141, 1094, 993, 876, 743, 663 cm^{-1}. HRMS: *m*/*z* (EI): Found [M$^+$]: 340.06272; $C_{18}H_{13}N_2O_3Cl$ requires [M$^+$]: 340.06147.

2.2.4. 2-(3′-Amino-4′-chlorophenyl)-8-(chloromethyl)-6-oxo-6H-benzopyrano[6,7-d]oxazole, ***3d***

Starting from benzopyran **1** (0.100 g, 0.44 mmol) in polyphosphoric acid (0.500 g) and 4-chloro-3-aminobenzoic acid **2d** (0.075 g, 0.44 mmol), compound **3d** was obtained as a grey solid (0.110 g, 70%). mp = 323.4–324.2 °C. λ_{max} (MeOH-HEPES 80/20)/nm 341 (log ε 3.85). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 5.13 (s, 2 H, CH_2), 6.70 (s, 1 H, H-7), 7.32 (dd, *J* = 7.6 and 2.0 Hz, 1 H, H-6′), 7.41 (d, *J* = 7.6 Hz, 1 H, H-5′), 7.64 (d, *J* = 2.0 Hz, 1 H, H-2′), 7.95 (s, 1 H, H-4), 8.25 (s, 1 H, H-9) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ = 41.70 (CH_2), 99.76 (C-4), 113.58 (C-2′), 114.27 (C-7), 114.76 (C-8a), 115.54 (C-6′), 115.56 (C-9), 121.04 (C-4′), 125.03 (C-1′), 130.08 (C-5′), 138.46 (C-9a), 145.40 (C-3′), 150.98 (C-8), 151.73 (C-4a), 151.96 (C-3a), 159.59 (C-6), 163.83 (C-2) ppm. IR (KBr 1%): ν_{max} 3380, 2970, 2316, 1722 (br), 1638, 1600, 1567, 1498, 1440, 1406, 1335, 1151, 1084, 997, 882, 745, 666 cm^{-1}. HRMS: *m*/*z* (EI): Found [M$^+$]: 360.00710; $C_{17}H_{10}N_2O_3Cl_2$ requires [M$^+$]: 360.00685.