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Photoactivable heterocyclic cages in a comparative release study of butyric acid as a model drug

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ABSTRACT

Aiming: at the improvement of the photorelease of butyric acid – a model carboxylic acid drug, a set of heteroaromatic compounds based on acridine, naphtho[2,1-*b*]pyran, 3*H*-benzopyran fused julolidine and thioxo-naphtho[2,1-*b*]pyran were evaluated as benzyl-type phototriggers, in comparison with the well-known *o*-nitrobenzyl group. The corresponding ester cages were irradiated in a photochemical reactor at 254, 300, 350 and 419 nm, in two solvent systems (methanol or acetonitrile in 80:20 mixtures with HEPES buffer). Photolysis studies showed that, for some of the cages, the release of the active molecule occurred with short irradiation times using 419 nm. Time-resolved fluorescence was used to elucidate their photophysical properties and determine the decay kinetics. Studies were also carried out to assess the suitability of using two-photon excitation to address these compounds, which is advantageous if their use in biological systems is to be considered.

1. Introduction

The use of several drugs in clinical practice is dependent on the improvement of their therapeutic effects, bioavailability, physico-chemical properties and the minimizing of other undesirable side effects. Prodrugs, pharmacologically latent derivatives of active agents, can be designed to undergo activation through a specific stimulus. In addition to chemical and/or enzymatic triggers, light is an appealing tool to use for conversion of prodrugs to active agents in a spatially and temporally controlled manner [1–8]. Butyric acid, a saturated unbranched monocarboxylic acid, is one of the short-chain fatty acids. The effects of butyric acid include the disruption of cell proliferation and induction of apoptosis; modification of cell morphology and alteration of gene expression [9]. The presence of carboxylic acids or other ionisable polar groups in drugs can result in poor absorption from the gastrointestinal tract owing to lipophilicity/solubility issues.

Light triggered benzyl or heterocyclic benzyl esters represent a large family of photolabile protecting groups. *o*-Nitrobenzylic derivatives have been widely used in various applications, as they combine a satisfactory photosensitivity with a stability for handling and synthesis purposes [10–12]. Nevertheless, *o*-nitrobenzyl cages exhibit some limitations, since the wavelength of excitation required for the uncaging is not the most suitable for bioapplications. The search for protecting groups with improved photochemical properties and even displaying fluorescence has motivated the incorporation of heterocyclic moieties in their design, with coumarinyl methyl groups as relevant examples. It is possible to find a wide range of derivatives possessing different combinations of substituents and/or ring fusions for the release of various active molecules [13–15]. Recently, acridinyl methyl esters have also proved to possess the required photosensitivity for the release of carboxylic acid compounds [16].

Considering the know-how of the authors in the field of fluorescent photoactivable molecules based on aromatic and heteroaromatic skeletons for the release of bioanalytes [17–24], in connection with the interest in the development of alternative light sensitive prodrugs, and following the previous work regarding butyric acid, a new set of oxygen and nitrogen heterocyclic cages were synthesised. The use of these heterocyclic moieties can result in longer maximum wavelengths of absorption, allowing the photo-release of butyric acid at longer wavelengths, not detrimental to bioapplications. It also opens the way to use two-photon excitation (TPE), where if sufficient photon flux is present two longer

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wavelength photons can be used to excite a sample. This is advantageous as it only occurs in a femtolitre volume and the longer wavelength photons are less likely to interact with biological material. Thus, the present work evaluates the behaviour of (acridin-9-yl) methyl, (5-methoxy-3-oxo-3*H*-naphtho[2,1-*b*]pyran-1-yl) methyl, (8-methoxy-3-oxo-3*H*-naphtho[2,1-*b*]pyran-1-yl) methyl, and [11-oxo-2,3,5,6,7,11-hexahydro-1*H*-pyrano[2,3-*f*]pyrido[3,2,1-*ij*]quinolin-9-yl]methyl groups, in comparison with the well-known *o*-nitrobenzyl group in the light-induced release of butyric acid. Additionally, bearing in mind that the replacement of a carbonyl by a thiocarbonyl group results in an improvement in the photolytic release [23,24], thionated groups; namely (5-methoxy-3-thioxo-3*H*-naphtho[2,1-*b*]pyran-1-yl) methyl, (8-methoxy-3-thioxo-3*H*-naphtho[2,1-*b*]pyran-1-yl) methyl, and (9-methoxy-3-thioxo-3*H*-naphtho[2,1-*b*]pyran-1-yl) methyl were tested. The ester cages, in two solvent systems (methanol or acetonitrile in 80:20 mixtures with HEPES buffer), were irradiated at 254, 300, 350 and 419 nm in a photochemical reactor. The fact that the groups exhibit fluorescence enabled time-resolved fluorescence measurements to be employed to elucidate their photophysical properties and determine the decay kinetics, along with their suitability for two-photon excitation. This last point can be important if their use in biological systems is to be considered.

2. Experimental

2.1. Synthesis 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 60 F_{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 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 made by comparison of chemical shifts, peak multiplicities and *J* values and 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 spectrofluorometer. All reagents were used as received. Compounds **5**, **6** and **11** were synthesised as previously reported [20,25,26].

2.2. Synthesis of 1-chloromethyl-5-methoxy-3-oxo-3H-naphtho[2,1-b]pyran **3**

To a solution of 3-methoxy-2-naphthol (0.104 g, 5.97×10^{-4} mol) in 70% aqueous sulphuric acid (5 mL), ethyl 4-chloro-3-oxobutanoate (0.089 mL, 6.57×10^{-4} mol) was added. The reaction was followed by TLC (ethyl acetate/*n*-hexane, 1:4), and stirred at room temperature for 96 h. The mixture was poured into ice water and stirred for 2 h to give a fine pale precipitate. The solid was collected by filtration, washed with cold water, dried and purified by column chromatography, using ethyl acetate/light petroleum as eluent, with mixtures of increasing polarity. Compound **3** was obtained as pale brown solid (0.012 g, 7%). Mp 169.5–171.8 °C. R_f = 0.50 (ethyl acetate/*n*-hexane, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 4.03 (s, 3H, OCH_3), 5.05 (s, 2H, CH_2), 6.75 (s, 1H, H-2), 7.36 (s, 1H, H-6), 7.54–7.57 (m, 2H, H-8 and H-9), 7.82–7.84 (m, 1H, H-7), 8.30–8.32 (m, 1H, H-10). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 45.79 (CH_2), 56.16 (OCH_3), 111.17 (C-6), 113.51 (C-4b), 117.74 (C-2), 123.69 (C-6b), 124.71 (C-10), 125.95 (C-9), 126.17 (C-8), 128.46 (C-7), 131.25 (C-6a), 146.75 (C-5), 147.21 (C-4a), 151.33 (C-1), 159.22 (C-3). IR (KBr 1%): ν = 1728, 1627, 1599, 1556, 1513, 1486, 1462, 1439, 1422, 1378, 1348, 1325, 1264, 1217, 1168, 1140, 1122, 1046, 1024, 1001, 938, 896, 866, 844, 778, 737, 704 cm^{-1}. HRMS (ESI) for $C_{15}H_{12}{}^{37}ClO_3$ [M^+ + H]: calculated 277.04456, found 277.04478; for $C_{15}H_{12}{}^{35}ClO_3$ [M^+ + H]: calculated 275.04756, found 275.04770.

2.3. Synthesis of 1-chloromethyl-8-methoxy-3-oxo-3H-naphtho[2,1-b]pyran **4**

To a solution of 6-methoxy-2-naphthol (0.248 g, 1.42×10^{-3} mol) in 70% aqueous sulphuric acid (5 mL), ethyl 4-chloro-3-oxobutanoate (0.288 mL, 2.13×10^{-3} mol) was added. The reaction was followed by TLC (ethyl acetate/*n*-hexane, 1:4), and stirred at room temperature for 72 h. The mixture was poured into ice water and stirred for 2 h to give a fine pale precipitate. The solid was collected by filtration, washed with cold water, dried and purified by column chromatography, using ethyl acetate/light petroleum as eluent, with mixtures of increasing polarity. Compound **4** was obtained as pale yellow solid (0.145 g, 37%). Mp 207.3–209.9 °C. R_f = 0.50 (ethyl acetate/*n*-hexane, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 3.97 (s, 3H, OCH_3), 5.03 (s, 2H, CH_2), 6.72 (s, 1H, H-2), 7.26 (d, *J* = 2.8 Hz, 1H, H-7), 7.36 (dd, *J* = 9.6 and 2.8 Hz, 1H, H-9), 7.46 (d, *J* = 8.8 Hz, 1H, H-5), 7.92 (d, *J* = 8.8 Hz, 1H, H-6), 8.33 (d, *J* = 9.2 Hz, 1H, H-10). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 45.84 (CH_2), 55.41 (OCH_3), 108.61 (C-7), 112.70 (C-4b), 117.51 (C-2), 118.18 (C-5), 120.09 (C-9), 123.44 (C-6b), 126.36 (C-10), 132.97 (C-6a), 133.16 (C-6), 150.98 (C-1), 153.76 (C-4a), 157.13 (C-8), 160.09 (C-3). IR (KBr 1%): ν = 1722, 1611, 1553, 1514, 1468, 1422, 1362, 1318, 1264, 1213, 1182, 1154, 1118, 1034, 1015, 998, 912, 897, 878, 858, 816, 737, 704 cm^{-1}. HRMS (ESI) for $C_{15}H_{12}{}^{37}ClO_3$ [M^+ + H]: calculated 277.04456, found 277.04449; for $C_{15}H_{12}{}^{35}ClO_3$ [M^+ + H]: calculated 275.04756, found 275.04751.

2.4. Synthesis of 2-nitrobenzyl butyrate, **7**

To a solution of butyric acid (0.263 mL, 2.88×10^{-3} mol) in dry DMF (4 mL) at 0 °C, 1-hydroxybenzotriazole (HOBt) (0.072 g, 5.33×10^{-4} mol) was added. After stirring for 10 min, *N*,*N*-dicyclohexylcarbodiimide (DCC) (0.114 g, 5.52×10^{-4} mol) was added, followed by (2-nitrophenyl) methanol **1** (0.402 g, 2.62×10^{-3} mol). The reaction mixture was stirred at room temperature for 72 h and followed by TLC (ethyl acetate/light petroleum, 1:4). The solid was filtered and the residue was evaporated under vacuum. Cold acetone was added and the dicyclohexylurea precipitate was filtered. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using ethyl acetate/light petroleum, with mixtures of increasing polarity as eluent. Compound **7** was obtained as an orange oily solid (0.169 g, 29%). R_f = 0.88 (ethyl acetate/light petroleum, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 0.92 (t, *J* = 7.6 Hz, 3H, CH_3—CH_2—CH_2), 1.65 (sext, *J* = 7.2 Hz, 2H, CH_3—CH_2—CH_2), 2.36 (t, *J* = 7.2 Hz, 2H, CH_3—CH_2—CH_2), 5.47 (s, 2H, CH_2), 7.44 (dt, *J* = 7.4 and 1.6 Hz, 1H, H-4), 7.55 (dd, *J* = 7.6 and 0.8 Hz, 1H, H-6), 7.62 (dt, *J* = 7.4 and 0.8 Hz, 1H, H-5), 8.02 (dd, *J* = 8.0 and 0.8 Hz, 1H, H-3). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 13.43 (CH_3-CH_2-CH_2), 18.19 (CH_3—CH_2—CH_2), 35.80 (CH_3—CH_2—CH_2), 62.54 (CH_2), 124.79 (C-3), 128.56 (C-4), 128.84 (C-6), 132.06 (C-1), 133.54 (C-5), 147.39 (C-2), 172.80 (C=O). IR (KBr 1%): ν = 2964, 2931, 2851, 1711, 1613, 1574, 1525, 1476, 1445, 1434, 1366, 1338, 1305, 1251, 1187, 1144, 1085, 1037, 989, 858, 792, 726 cm^{-1}. HRMS (ESI) for $C_{11}H_{14}NO_4$ [M^+ + H]: calculated 224.09232, found: 224.09240.