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Photolytic release of bioactive carboxylic acids from fused pyran conjugates

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ABSTRACT

New ester cages bearing the coumarin (2*H*-benzopyran-2-one) skeleton with extended π-systems as phototriggers, for glycine and β-alanine, as models of carboxylic acid bifunctional molecules with biological relevance, were evaluated under photolysis conditions at 254, 300, 350 and 419 nm of irradiation in a RPR-100 photochemical reactor. The processes were followed by HPLC-UV detection and ^{1}H NMR with collection of kinetic data. The results showed a correlation between the photolysis efficiency and the increasing extension of the conjugation for both glycine and β-alanine, showing that the 7-aminocoumarin afforded the best results at all wavelengths tested.

From a study of the time-resolved fluorescence behaviour, these compounds were also found to exhibit more complex fluorescence decay kinetics. This was attributed to the presence of conjugated and non-conjugated coumarin species.

1. Introduction

2*H*-Benzopyran-2-one, the IUPAC name of coumarin, is an oxygen heterocycle firstly isolated in 1820 by Voleg from the plant *Dypteryx adorata* and is at the moment known to be present in a huge number of natural products [1–3]. Since ancient Egyptian times, coumarins have been known for their therapeutic properties, namely anticancer, antimicrobial, anesthetic, anti-HIV, anticoagulant and antioxidant activities. Also, coumarins are used in the cosmetic and food industries as fluorescent probes and, more recently, as photocleavable protecting groups (PPGs) [4–9].

PPGs are chemical entities used to temporarily disable the activity of a compound, which is achieved by a covalent bond between the PPG and a functional group essential to its activity. After irradiation, the bond is cleaved and the compound's activity is restored. For a biological application, the employed wavelength needs to be as near as possible to the visible radiation wavelength in order to reduce cellular damage. PPGs can be used to synthesize prodrugs sensitive to light, producing inactive therapeutic agents that regain their biological activity after irradiation. These prodrugs allow the controlled release of drugs, both in time and locally, thus holding the promise of reduced side effects and toxicity [10–15].

In comparison to one of the most common PPGs, *o*-nitrobenzyl, coumarins possess higher molar extinction coefficients at longer wavelengths, higher releasing rates, increased stability and good fluorescence. This makes them excellent options as PPGs for biological applications, besides their interest for organic synthesis, and the study of their fluorescence provides a means by which to assess the photocleavage process [11,16,17]. In addition, some coumarins can be used in two-photon uncaging, a process where two photons are simultaneous absorbed and provide the excitation energy required to initiate the photophysical process. This technique enables photolysis by the absorption of NIR radiation which is less toxic to living systems than UV and presents a higher tissue penetrating capacity [11–18]. Coumarins have been used in the photorelease of several bioactive compounds such as amino acids (glycine, β-alanine, GABA, glutamate), secondary messengers (cGMP, cAMP, diacylglycerol), nucleic acids/oligonucleotides (DNA, mRNA, 8-bromonucleotids, ATP, ADP, AMP), hormones (progesterone), cholesterol and even drugs (anisomycin and paclitaxel) [19–29].

Considering that one of the major drawbacks of PPGs is the fact that their maximum absorption wavelength is at shorter wavelengths, the present work describes the synthesis of a new set of ester conjugates of coumarins possessing an extended π-system or an amino group at position 7 of the system. The intention is to

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bathochromically shift the wavelength of maximum absorption, and consequently, of photolysis, which has been the main objective of our most recent work in this area [30–39]. Glycine and β-alanine were used as models of carboxylic acids, which besides representing bifunctional molecules used in organic synthesis, possess crucial biological roles in humans (as neurotransmitters, for example) [40–42].

For this purpose, the caged amino acids were characterized (using UV-Vis and fluorescence spectroscopy) and irradiated at 254, 300, 350 and 419 nm in a photochemical reactor Rayonet RPR-100. The release of the active compound was monitored by HPLC-UV detection with collection of kinetic data and also by ^{1}H NMR.

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 using KBr discs. UV/visible 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 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 and excitation − emission matrices (EEM's) were recorded using a FluoroLog 3. Time-resolved fluorescence was measured using a HORIBA Scientific DeltaFlex equipped with a DeltaDiode (DD-350, emitting at 349 nm) excitation source. Fluorescence decays were measured at 5 nm increments over the wavelength range 375 nm–575 nm. The resultant decays were analysed globally to obtain decay associated spectra (using EzTime software) or just made use of the decay obtained at 480 nm in the dataset. During the analysis, an "extra" short-lived component (not plotted in Fig. 3) was required and attributed to scattered excitation. The optical density of the samples used or these fluorescence measurements was kept to ~0.1 to avoid self-absorption effects. Photolysis measurements were carried out using a Rayonet RPR-100 chamber reactor equipped with 10 lamps of 254, 300, 350 and 419 ± 10 nm. HPLC analyses were performed using a Licrospher 100 RP18 (5 μm) column in a JASCO HPLC system composed by a PU-2080 pump and a UV-2070 detector with ChromNav software. All reagents were used as received. The synthesis of chloromethyl precursors **1a-e** is included in the supporting information.

2.2. General procedure for the synthesis of conjugates *4* and *5*

The chloromethyl precursors **1a-e** were dissolved in dry DMF (3 mL), and potassium fluoride (3 equiv) and *N*-(*tert*-butoxycarbonyl)-glycine or *N*-(*tert*-butoxycarbonyl)-β-alanine (1 equiv) were added. The reaction mixture was stirred at room temperature for 24 h. The solvent was removed by evaporation under reduced pressure and the required conjugate was obtained as a solid. The crude residue of compounds **4** and **5** was purified by column chromatography using mixtures of increasing polarity of dichloromethane/methanol (**4a-e**) or light petroleum/ethyl acetate (**5a-e**) as eluent.

2.2.1. *N-(tert-Butyloxycarbonyl)-glycine (2-oxo-2H-benzopyran-4-yl)methyl ester **4a***

Starting from compound **1a** (0.031 g, 1.6×10^{-4} mol) in dry DMF (2 mL), potassium fluoride (0.028 g, 4.8×10^{-4} mol) and *N*-(*tert*-butoxycarbonyl)-glycine **2** (0.028 g, 1.6×10^{-4} mol), the ester conjugate **4a** was obtained as an orange solid (0.031 g, 56%). Mp = 115.1–116.6 °C. R_f = 0.67 (dichloromethane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.47 (s, 9 H, C(CH_3)$_3$), 4.07 (d, *J* 5.6 Hz, 2 H, H-α), 4.98 (br s, 1 H, NH), 5.39 (s, 2 H, CH_2), 6.51 (s, 1 H, H-3), 7.33 (td, *J* 8 and 1.2 Hz, 1 H, H-6), 7.39 (dd, *J* 8 and 0.8 Hz, 1 H, H-8), 7.52 (dd, *J* 8 and 1.6 Hz, 1 H, H-5), 7.58 (td, *J* 8.8 and 1.6 Hz, 1 H, H-7) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 28.27 (C(CH_3)$_3$) 42.41 (C-α), 61.78 (CH_2), 80.45 (*C*(CH_3)$_3$), 113.67 (C-3), 116.99 (C-4a), 117.50 (C-8), 123.41 (C-5), 124.55 (C-6), 132.22 (C-7), 148.25 (C-4), 153.66 (C-8a), 155.70 (C=O Boc), 160.16 (C-2), 169.77 (C=O Gly) ppm. IR (liquid film): ν = 3440, 3058, 2980, 2918, 2850, 1729, 1630, 1608, 1569, 1510, 1452, 1407, 1368, 1266, 1162, 1070, 1006, 937, 862, 738, 703 cm^{-1}. HRMS: *m/z* (ESI): Found [M^++1]: 334.12910; $C_{17}H_{20}NO_6$ requires [M^++1]: 334.12925.

2.2.2. *N-(tert-Butyloxycarbonyl)-glycine (7-amino-2-oxo-2H-benzopyran-4-yl)methyl ester **4b***

Starting from compound **1b** (0.031 g, 1.6×10^{-4} mol) in dry DMF (2 mL), potassium fluoride (0.016 g, 8.0×10^{-5} mol) and *N*-(*tert*-butoxycarbonyl)-glycine **2** (0.028 g, 1.6×10^{-4} mol), the ester conjugate **4b** was obtained as an yellow solid (0.038 g, 70%). Mp = 179.6–180.2 °C. R_f = 0.6 (dichloromethane/methanol 20:1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.38 (s, 9 H, C(CH_3)$_3$), 3.83 (d, *J* 6 Hz, 2 H, H-α), 5.30 (s, 2 H, CH_2), 6.00 (s, 1 H, H-3), 6.72 (s, 2 H, NH_2), 6.42 (d, *J* 2 Hz, H-8), 6.55 (dd, *J* 8.8 and 2 Hz, 1 H, H-6), 7.31–7.36 (m, 2 H, H-5 and NH) ppm. ^{13}C NMR (DMSO-*d6*, 100.6 MHz): δ = 28.12 (C(CH_3)$_3$), 42.05 (C-α), 61.43 (CH_2), 78.40 (*C*(CH_3)$_3$), 98.58 (C-8), 104.65 (C-3), 105.70 (C-4a), 111.31 (C-6), 125.41 (C-5), 150.56 (C-4), 153.25 (C-8a), 155.57 (C-7), 155.94 (C=O Boc), 160.54 (C-2), 170.03 (C=O Gly) ppm. IR (KBr 1%): ν = 3383, 3231, 2976, 2363, 1743, 1702, 1616, 1526, 1404, 1279, 1169, 1056, 869, 850, 819, 747 cm^{-1}. HRMS: *m/z* (ESI): Found [M^+ +1]: 349.14003; $C_{17}H_{21}N_2O_6$ requires [M^++1]: 349.14010.

2.2.3. *N-(tert-Butyloxycarbonyl)-glycine (7-phenyl-2-oxo-2H-benzopyran-4-yl)methyl ester **4c***

Starting from compound **1c** (0.037 g, 1.4×10^{-4} mol) in dry DMF (2 mL), potassium fluoride (0.025 g, 4.3×10^{-3} mol) and *N*-(*tert*-butoxycarbonyl)-glycine **2** (0.025 g, 1.4×10^{-5} mol), the ester conjugate **4c** was obtained as an yellow solid (0.009 g, 16%). Mp = 129.5–131.6 °C. R_f = 0.55 (dichloromethane/methanol 100:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.46 (s, 9 H, C(CH_3)$_3$), 4.08 (d, *J* 5 Hz, 2 H, H-α), 5.07 (t, *J* 5 Hz 1 H, NH), 5.41 (s, 2 H, CH_2), 6.51 (s, 1 H, H-3), 7.45 (dt, *J* 7.2 and 1.2 Hz, 1 H, H-4′), 7.51 (dt, *J* 7.2 and 1.2 Hz, 2 H, H-3'and H-5′), 7.57–7.60 (m, 3 H, H-5, H-6 and H-8), 7.64 (dt, *J* 7.2 and 1.2 Hz, 2 H, H-2′ and H-6′) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 28.27 (C(CH_3)$_3$), 42.41 (C-α), 61.81 (CH_2), 80.46 (*C*(CH_3)$_3$), 113.27 (C-3), 115.51 (C-6), 115.86 (C-4a), 123.38 (C-8), 123.79 (C-5), 127.19 (C-2′ and C-6′), 128.71 (C-4′), 129.14 (C-3′ and C-5′), 138.81 (C-1′), 145.41 (C-7), 148.13 (C-4), 154.10 (C-8a), 155.71 (C=O Boc), 160.31 (C-2), 169.78 (C=O Gly) ppm. IR (liquid film): ν = 3371, 3061, 2978, 2932, 1722, 1617, 1583, 1514, 1452, 1406, 1368, 1337, 1279, 1252, 1163, 1074, 1055, 1004, 952, 866, 766, 750, 737, 699 cm^{-1}. HRMS: *m/z* (ESI): Found [M^+ +1]: 410.16042; $C_{23}H_{24}NO_6$ requires [M^++1]: 410.16037.