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Title

Light-induced cleavage of model phenylalanine conjugates based on coumarins and quinolones

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Abstract: In order to evaluate the application of quinolone as a new photocleavable protecting group, in comparison with coumarin, a series of model phenylalanine conjugates were prepared by reaction with chloromethylated O and N heterocycles. The photophysical properties of the resulting ester conjugates were evaluated as well as the photosensitivity under irradiation at 250, 300, 350 and 419 nm. The results obtained showed that the quinolone conjugates were readily photolysed, with complete release of the amino acid in short irradiation times and could be considered a new addition to the family of photocleavable protecting groups for the carboxylic acid function of amino acids.

Keywords: Coumarin; Quinolone; Bioconjugates; Amino acids; Photocleavable protecting groups.

Introduction

In organic synthesis, and especially in peptide chemistry, protecting groups are a laborious necessity, as a convenient and efficient synthesis, chemical stability towards different reagents and selective removal are required. Photochemically controllable release is an attractive feature for protecting groups, since typically requires no chemical reagents and provides spatial and temporal resolution in the photorelease process. Additionally, photochemical cleavage is a very mild deprotection strategy that is usually orthogonal to chemical conditions, allowing the removal of protecting groups in sensitive molecules, otherwise incompatible with acidic or basic treatment. Light sensitive groups have become an important tool in organic synthesis, biotechnology and cell biology, for example in molecular caging - a strategy in which a bioactive molecule is rendered inactive by a covalent linkage to a photolabile group. By irradiation of the cage, the caged molecule can be released in its active form. These properties are appealing to both basic and applied research fields, including solution and solid-phase organic synthesis, combinatorial chemistry, photolithography, and biochemical and biophysical research (Guillier et al. 2000; Bochet 2002; Pellicioli and Wirz 2002; Corrie et al. 2005; Mayer and Heckel 2006). The concept of chromatic orthogonality (different photolabile protecting groups that cleave in different wavelengths of irradiation) has been reported in the complete solid-phase synthesis of a peptide using only photodeprotection, with both photolabile linker and protecting groups (Kessler et al. 2003).

Recently reported photolabile protecting groups include coumarin derivatives, which have been successfully studied and applied in the protection of phosphates (Hagen et al. 2003; Furuta et al. 2004; Geissler et al. 2005; Hagen et al. 2005), carboxylates (Hagen et al. 2005; Furuta et al. 1999), sulfates (Geissler et al. 2005), sulfonates (Furuta et al. 2004), diols (Lin and Lawrence 2002) and carbonyl compounds (Lu et al. 2003; Shembekar et al. 2007). Protection of hydroxyl and amino functional groups has also been carried out through carbonate (Suzuki et al. 2003; Gilbert et al. 2007) or carbamate bonds (Hagen et al. 2005; Takaoka et al. 2003; Takaoka et al. 2004). Coumarins have been applied in the preparation of photoresponsive prodrugs that release the parent drug by UV and visible light irradiation (Skwarczynski et al. 2006; Noguchi et al. 2008).

Quinolones and its derivatives constitute a major class of antibacterial chemotherapeutic agents, which have a broad spectrum of activity against bacteria, mycobacteria, parasites, and other diseases (Gootz and Brighty 1998; Ma et al. 2009; Reddy et al. 2009; Senthilkumar et al. 2009). Considering the biological potential, this family of heterocycles is of extreme

importance. Although the structural resemblance between quinolones and coumarins, contrary to the latter, the use of quinolones as photolabile protecting groups has not been reported, while quinolines have been considered for this application with promising results (Fedoryak and Dore 2002; Zhu et al. 2006).

Coumarins and quinolones are fluorophores, which are more convenient than non-fluorescent protecting groups, since they may be useful in the visualisation of processes during synthesis as well as monitoring the course of reaction and thus allow tracing of the location of caged molecules inside living cells by fluorescent techniques. Although fluorescence deactivation may be an inconvenience in some photochemical processes, considering the body of work in this area published in the last years, the direction of improvement on photoreleasable groups has been towards the development and application of polycyclic structures (both benzene and heterocycle derived) which are fluorophores in most cases and have been reported as having improved properties as photolabile protecting groups.

Considering these facts, we decided to evaluate the behaviour of series of quinolones as photocleavable protecting groups of the carboxylic acid function, comparatively to related coumarins. Compounds possessing the carboxylic group are of great interest in organic chemistry and commonly need to be protected in sequential synthesis against various reagents including reactive nucleophiles, reducing and oxidant agents. Among the most appealing carboxylic compounds are amino acids, which play central roles as building blocks of peptides, proteins and as intermediates in metabolism.

Therefore, we synthesised the above mentioned heterocycles as well as their corresponding conjugates with phenylalanine, which was chosen as a model bifunctional molecule given the relevance of amino acids as scaffolds in organic and peptide synthesis. The obtained bioconjugates were studied under different photocleavage conditions, as part of our continuing interest in the investigation of different heteroaromatic fluorophores as fluorescent labels and photocleavable protecting groups, applied to amino acids, including neurotransmitters (Piloto et al. 2005; Piloto et al. 2006a; Piloto et al. 2006b; Fernandes et al. 2007; Fonseca et al. 2007; Fernandes et al. 2008a; Fernandes et al. 2008b).

Experimental Section

General

All melting points were measured on a Stuart SMP3 melting point apparatus and are uncorrected. 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 – 800 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 NMR and 75.4 MHz for ^{13}C NMR or a Bruker Avance III 400 at an operating frequency of 400 MHz for ^{1}H NMR and 100.6 MHz for ^{13}C NMR using the solvent peak as internal reference at 25 ºC. 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 HMBC and HMQC 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 254 (35W), 300 (21W), 350 (24W) and 419 (14W) nm. HPLC analyses were performed using a Licrospher 100 RP18 (5 μm) column in a HPLC system composed by a Jasco PU-980 pump, a UV/vis Shimadzu SPD-GAV detector and a Shimadzu C-RGA Chromatopac register. LC/MS analyses of photolised samples were carried out in a Finnigan LXQ mass detector coupled to a Surveyor Plus LC system, and mass spectra were obtained by ESI technique. All reagents were used as received.

Synthesis of 4-chloromethyl-7-methylcoumarin 1a. 3-Methylphenol (1.034 g, 9.6×10^{-3} mol) was mixed with ethyl 4-chloro-3-oxobutanoate (1.5 eq, 1.94 mL, 1.4×10^{-2} mol) at room temperature and aqueous H_2SO_4 70% (5 mL) was added. The reaction mixture was stirred at room temperature for 17h, poured into ice, the precipitate formed was filtered, washed with water and dried at 50ºC. Compound **1a** was obtained as a white solid (1.785 g, 90%); mp = 209-210 ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.41 (3H, s, CH_3), 5.00 (2H, d, *J* 0.6, CH_2), 6.60 (1H, s, H-3), 7.23 (1H, dd, *J* 8.4 and 1.2 Hz, H-6), 7.27 (1H, s, H-8), 7.72 (1H, d, *J* 8.1 Hz, H-5); ^{13}C NMR (75.4 MHz, DMSO-d_6): δ = 21.08 (CH_3), 41.27 (CH_2), 114.31 (C-3), 114.62 (C-4a), 116.83 (C-8), 125.01 (C-5), 125.56 (C-6), 143.35 (C-7), 150.72 (C-4), 153.43 (C-8a), 159.82 (C-2); IR (KBr 1%, cm^{-1}): ν = 3075, 2850, 1729, 1617, 1555, 1513, 1567, 1445, 1392, 1377, 1276, 1263, 1225, 1193, 1149, 1056, 1037, 1018, 979, 887, 882, 692; UV/Vis (ethanol, nm): λ_{max} (log ε) = 314 (3.83); MS: *m/z* (EI) 210 (M^{+} ^{37}Cl, 8), 208 (M^{+} ^{35}Cl,

25), 173 (28), 146 (25), 145 (100), 115 (26); HRMS: *m/z* (EI) calc. for $C_{11}H_9O_2{}^{35}Cl$ 208.0296, found 208.0291; calc. for $C_{11}H_9O_2{}^{37}Cl$ 210.0271, found 210.0262.

Synthesis of 4-chloromethyl-7-methyl-6-nitrocoumarin 1b. Compound **1a** (0.060 g, 2.9×10^{-4} mol) was dissolved in concentrated H_2SO_4 (0.5 mL), in a ice bath and HNO_3 65% (0.1 mL) was added. The reaction mixture was stirred at room temperature for 30 min and then poured into ice, to give a precipitate which was filtered and dried. Compound **1b** was obtained as an off-white solid (0.067 g, 92%); mp = 133-135 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.62 (3H, s, CH_3), 5.09 (2H, s, CH_2), 6.79 (1H, s, H-3), 7.60 (1H, s, H-8), 8.50 (1H, s, H-5); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 19.86 (CH_3), 40.88 (CH_2), 115.58 (C-4a), 116.60 (C-3), 120.50 (C-8), 122.29 (C-5), 137.89 (C-6), 144.88 (C-7), 149.60 (C-4), 155.26 (C-8a), 158.74 (C-2); IR (KBr 1%, cm^{-1}): ν = 3086, 3066, 2841, 1747, 1625, 1551, 1526, 1494, 1457, 1448, 1420, 1376, 1351, 1324, 1286, 1261, 1214, 1197, 1166, 1086, 1030, 1003, 927, 898, 850, 775; UV/Vis (ethanol, nm): λ_{max} (log ε) = 319 (3.61); MS: *m/z* (EI) 255 (M^{+} ^{37}Cl, 5), 253 (M^{+} ^{35}Cl, 17), 238 (21), 236 (78), 202 (100), 174 (29), 146 (27), 144 (24), 117 (28), 116 (29), 115 (76), 91 (23); HRMS: *m/z* (EI) calc. for $C_{11}H_8NO_4{}^{35}Cl$ 253.0153, found 253.0142; calc. for $C_{11}H_8NO_2{}^{37}Cl$ 255.0112, found 255.0112.

Synthesis of 1,4-dimethylquinolin-2(1*H*)-one 3a. *N*-Methylaniline **2a** (2 mL, 1.9×10^{-2} mol) and ethyl 3-oxobutanoate (4.67 mL, 3.8×10^{-2} mol) were heated at reflux in acetic acid (10 mL) for 48h. The solvent was evaporated under reduced pressure and the residue was heated in H_2SO_4 96% (8 mL) at 100ºC for 1h. After cooling to room temperature, the mixture was neutralised with aqueous NaOH 6M and a precipitate formed, which was filtered, washed with water and dried. Compound **3a** was obtained as a white solid (0.212 g, 66%); mp = 126-127 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.48 (3H, d, *J* 0.9 Hz, CH_3), 3.72 (3H, s, NCH_3), 6.62 (1H, d, *J* 0.9 Hz, H-3), 7.27 (1H, dt, *J* 8.7 and 1.2 Hz, H-6), 7.39 (1H, d, *J* 8.7 Hz, H-8), 7.59 (1H, dt, *J* 8.7 and 1.2 Hz, H-7), 7.72 (1H, dd, *J* 7.8 and 1.2 Hz, H-5); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 18.95 (CH_3), 29.21 (NCH_3), 114.39 (C-8), 121.13 (C-3), 121.43 (C-4a), 121.87 (C-6), 125.19 (C-5), 130.42 (C-7), 139.81 (C-8a), 146.34 (C-4), 162.11 (C-2); IR (KBr 1%, cm^{-1}): ν = 2919, 2886, 2850, 1652, 1615, 1592, 1562, 1503, 1455, 1409, 1387, 1371, 1322, 1268, 1162, 1139, 1113, 1066, 1041, 924, 874, 863, 843, 769, 753; UV/Vis (ethanol, nm): λ_{max} (log ε) = 329 (3.62); MS: *m/z* (EI) 174 (M^{+}+1, 21), 173 (M^{+}, 100), 145 (21), 144 (89), 130 (52); HRMS: *m/z* (EI) calc. for $C_{11}H_{11}NO$ 173.0847, found 173.0841.

General procedure for the synthesis of 4-methylquinolin-2(1*H*)-ones 3b,c. Compounds **3b**,**c** were prepared by reaction of the corresponding *N*-methylated aniline **2b**,**c** (1 equiv) with ethyl 3-oxobutanoate (2 equiv), by stirring at 180 ºC for 45 min. After evaporation of the excess ethyl 3-oxobutanoate under vacuum, the residue was stirred with aqueous H_2SO_4 70% (5 mL) at 95 ºC 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 $MgSO_4$, filtered and evaporated under reduced pressure. The resulting solid was purified by silica gel column chromatography using chloroform/methanol (50:1) as eluent. Fractions containing the product were combined and evaporated, yielding compounds **3b**,**c** as solids.

1,4,6-Trimethylquinolin-2(1*H*)-one 3b. Starting from *N*-methyl-4-methylaniline **2b** (0.31 mL, 2.5 × 10^{-3} mol) and ethyl 3-oxobutanoate (0.63 mL, 5.0 × 10^{-3} mol), compound **3b** was obtained as an off-white solid (0.346 g, 75%); mp = 105-107 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.45 (6H, s, 2 × CH_3), 3.69 (3H, s, NCH_3), 6.58 (1H, d, *J* 1.2 Hz, H-3), 7.26 (1H, d, *J* 8.7 Hz, H-8), 7.39 (1H, dd, *J* 8.7 and 1.8 Hz, H-7), 7.48 (1H, br s, H-5); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 18.93 (CH_3), 20.78 (CH_3), 29.18 (NCH_3), 114.28 (C-8), 121.02 (C-3), 121.31 (C-4a), 125.07 (C-5), 131.33 (C-6), 131.54 (C-7), 137.73 (C-8a), 146.13 (C-4), 162.01 (C-2); IR (KBr 1%, cm^{-1}): ν = 2850, 1671, 1640, 1572, 1490, 1460, 1375, 1200, 879, 817, 722; UV/Vis (ethanol, nm): λ_{max} (log ε) = 337 (3.49); MS: *m/z* (EI) 187 (M^+, 100), 158 (54), 144 (49); HRMS: *m/z* (EI) calc. for $C_{12}H_{13}NO$ 187.0997, found 187.0990.

1,4-Dimethyl-6-methoxyquinolin-2(1*H*)-one 3c. Starting from *N*-methyl-4-methoxyaniline **2c** (0.25 mL, 1.8 × 10^{-3} mol) and ethyl 3-oxobutanoate (0.46 mL, 3.6 × 10^{-3} mol), compound **3c** was obtained as an off-white solid (0.263 g, 71%); mp = 122-124 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.43 (3H, d, *J* 0.9 Hz, CH_3), 3.68 (3H, s, NCH_3), 3.88 (3H, s, OCH_3), 6.60 (1H, d, *J* 0.9 Hz, H-3), 7.11 (1H, d, *J* 2.7 Hz, H-5), 7.18 (1H, dd, *J* 9.3 and 2.7 Hz, H-7), 7.30 (1H, d, *J* 9.3 Hz, H-8); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 18.99 (CH_3), 29.27 (NCH_3), 55.66 (OCH_3), 107.91 (C-5), 115.55 (C-8), 118.12 (C-7), 121.62 (C-3), 122.17 (C-4a), 134.27 (C-8a), 145.61 (C-4), 154.49 (C-6), 161.61 (C-2); IR (KBr 1%, cm^{-1}): ν = 3003, 2950, 2831, 1650, 1624, 1591, 1570, 1505, 1465, 1429, 1417, 1371, 1314, 1276, 1244, 1201, 1163, 1092, 1033, 910, 886, 844, 806; UV/Vis (ethanol, nm): λ_{max} (log ε) = 351 (3.90); MS: *m/z* (EI) 203 (M^+, 75), 188 (100), 160 (24); HRMS: *m/z* (EI) calc. for $C_{12}H_{13}NO_2$ 203.0949, found 203.0946.

General procedure for the synthesis of quinolin-2(1*H*)-one-4-carbaldehydes 4a-c. Compounds **3a-c** (1 equiv) were reacted with selenium dioxide (4 equiv) in chlorobenzene (30 mL), by heating at reflux for 2-4 days. The mixture was filtered hot and the solvent was removed by rotary evaporation. The crude residue was used in the next reaction without further purification.

1-Methylquinolin-2(1*H*)-one-4-carbaldehyde 4a. Starting from compound **3a** (0.100 g, 5.8 × 10^{-4} mol) and selenium dioxide (0.257 g, 2.3 × 10^{-3} mol), compound **4a** was obtained as a yellow solid (0.080 g, 74%); mp = 166-168 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.78 (3H, s, NCH_3), 7.17 (1H, s, H-3), 7.35 (1H, dt, *J* 8.1 and 1.5 Hz, H-6), 7.44 (1H, d, *J* 8.1 Hz, H-8), 7.66 (1H, dt, *J* 7.5 and 1.5 Hz, H-7), 8.84 (1H, dd, *J* 8.1 and 1.5 Hz, H-5), 10.15 (1H, s, CHO); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 29.94 (CH_3), 114.43 (C-8), 116.46 (C-4a), 123.35 (C-6), 126.61 (C-5), 131.61 (C-7), 131.66 (C-3), 140.04 (C-4), 140.48 (C-8a), 161.58 (C-2), 192.78 (CHO). IR (KBr 1%, cm^{-1}): ν = 3023, 2919, 1670, 1664, 1588, 1561, 1454, 1430, 1413, 1391, 1325, 1212, 1160, 1054, 936, 898, 751; UV/Vis (ethanol, nm): λ_{max} (log ε) = 330 (3.78); MS: *m/z* (EI) 187 (M^+, 100), 158 (53), 130 (58); HRMS: *m/z* (EI) calc. for $C_{11}H_9NO_2$ 187.0636, found 187.0633.

1,6-Dimethylquinolin-2(1*H*)-one-4-carbaldehyde 4b. Starting from compound **3b** (0.450 g, 2.4 × 10^{-3} mol) and selenium dioxide (1.067 g, 9.6 × 10^{-3} mol), compound **4b** was obtained as a yellow solid (0.429 g, 89%); mp = 179-180 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.47 (3H, s, CH_3), 3.76 (3H, s, NCH_3), 7.16 (1H, s, H-3), 7.34 (1H, d, *J* 8.4 Hz, H-7), 7.48 (1H, dd, *J* 8.7 and 1.8 Hz, H-8), 8.64 (1H, d, *J* 0.9 Hz, H-5), 10.15 (1H, s, CHO); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 20.88 (CH_3), 29.41 (NCH_3), 114.28 (C-8), 116.41 (C-4a), 126.28 (C-5), 131.70 (C-3), 132.80 (C-7), 133.12 (C-6), 138.55 (C-8a), 139.86 (C-4), 161.46 (C-2), 192.96 (CHO). IR (KBr 1%, cm^{-1}): ν = 3039, 2925, 2859, 1702, 1673, 1656, 1585, 1567, 1451, 1413, 1378, 1322, 1303, 1189, 1108, 1055, 939, 902, 813, 758; UV/Vis (ethanol, nm): λ_{max} (log ε) = 338 (3.72); MS: *m/z* (EI) 201 (M^+, 100), 172 (48), 144 (49); HRMS: *m/z* (EI) calc. for $C_{12}H_{11}NO_2$ 201.0790, found 201.0790.

6-Methoxy-1-methylquinolin-2(1*H*)-one-4-carbaldehyde 4c. Starting from compound **3c** (0.170 g, 8.4 × 10^{-4} mol) and selenium dioxide (0.376 g, 3.4 × 10^{-3} mol), compound **4c** was obtained as a yellow solid (0.113 g, 62%); mp = 202-204 ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ

= 3.77 (3H, s, NCH_3), 3.91 (3H, s, OCH_3), 7.19 (1H, s, H-3), 7.27 (1H, dd, *J* 6.9 and 2.1 Hz, H-7), 7.39 (1H, d, *J* 6.9 Hz, H-8), 8.41 (1H, d, *J* 2.1 Hz, H-5), 10.12 (1H, s, CHO); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 30.07 (NCH_3), 55.70 (OCH_3), 108.06 (C-5), 115.58 (C-8), 117.13 (C-4a), 120.66 (C-7), 132.83 (C-3), 135.19 (C-8a), 139.32 (C-4), 155.66 (C-6), 161.95 (C-2), 193.17 (CHO). IR (KBr 1%, cm^{-1}): ν = 3020, 2929, 1671, 1659, 1580, 1454, 1433, 1415, 1385, 1331, 1213, 1161, 1055, 936, 898; UV/Vis (ethanol, nm): λ_{max} (log ε) = 341 (3.69); MS: *m/z* (EI) 217 (M^+, 100), 188 (43); HRMS: *m/z* (EI) calc. for $C_{12}H_{11}NO_3$ 217.0739, found 217.0744.

General procedure for the synthesis of 4-hydroxymethylquinolin-2(1*H*)-ones 5a-c. Compounds **4a-c** (1 equiv) were reacted with sodium borohydride (0.7 equiv) in ethanol (10 mL) for 1 day at room temperature. The solvent was removed under reduced pressure and after purification by column chromatography (for **5a** and **5c**), compounds **5a-c** were obtained as solids.

4-Hydroxymethyl-1-methylquinolin-2(1*H*)-one 5a. Starting from compound **4a** (0.807 g, 4.3×10^{-3} mol) and sodium borohydride (0.114 g, 3.0×10^{-3} mol), and purification by silica gel column chromatography using dichloromethane/methanol (25:1) as eluent, compound **5a** was obtained as a light pink solid (0.549 g, 67%); mp = 182-183 ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.60 (3H, s, NCH_3), 4.77 (2H, d, *J* 1.2 Hz, CH_2), 5.51 (1H, s, OH), 6.66 (1H, s, H-3), 7.25 (1H, dt, *J* 8.0 and 1.2 Hz, H-6), 7.55 (1H, dd, *J* 8.4 and 0.8 Hz, H-8), 7.61 (1H, dt, *J* 8.4 and 1.2 Hz, H-7), 7.72 (1H, dd, *J* 8.0 and 1.2 Hz, H-5); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 28.83 (NCH_3), 59.40 (CH_2), 114.96 (C-8), 116.80 (C-3), 118.31 (C-4a), 121.73 (C-6), 124.19 (C-5), 130.56 (C-7), 139.46 (C-8a), 149.74 (C-4), 161.04 (C-2); IR (KBr 1%, cm^{-1}): ν = 3324, 2954, 2924, 2854, 1643, 1575, 1509, 1458, 1442, 1421, 1401, 1377, 1351, 1333, 1302, 1231, 1198, 1140, 1096, 1080, 1050, 1017, 980, 753; UV/Vis (ethanol, nm): λ_{max} (log ε) = 328 (3.72); MS: *m/z* (EI) 189 (M^+, 100), 160 (26), 144 (39), 130 (22), 118 (36), 117 (20); HRMS: *m/z* (EI) calc. for $C_{11}H_{11}NO_2$ 189.0793, found 189.0790.

1,6-Dimethyl-4-hydroxymethylquinolin-2(1*H*)-one 5b. Starting from compound **4b** (0.221 g, 1.1×10^{-3} mol) and sodium borohydride (0.029 g, 7.7×10^{-4} mol), compound **5b** was obtained as a beige solid (0.188 g, 84%); mp = 148-150 ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.42 (3H, s, CH_3), 3.49 (3H, s, NCH_3), 4.25 (1H, s, OH), 4.88 (2H, s, CH_2), 6.83 (1H, s, H-3),

7.14 (1H, d, *J* 8.8 Hz, H-8), 7.34 (1H, d, *J* 8.4 Hz, H-7), 7.48 (1H, s, H-5); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 20.76 (CH_3), 29.16 (NCH_3), 61.32 (CH_2), 114.43 (C-8), 118.00 (C-3), 119.06 (C-4a), 124.18 (C-5), 131.63 (C-7), 131.75 (C-6), 137.51 (C-8a), 149.01 (C-4), 162.38 (C-2); IR (KBr 1%, cm^{-1}): ν = 3356, 2912, 2857, 1660, 1644, 1599, 1573, 1557, 1464, 1417, 1350, 1296, 1279, 1184, 1131, 1095, 1076, 1002, 946, 868, 807; UV/Vis (ethanol, nm): λ_{max} (log ε) = 335 (3.51); MS: *m/z* (EI) 203 (M^+, 100), 174 (22), 158 (42), 144 (29), 132 (38); HRMS: *m/z* (EI) calc. for $C_{12}H_{13}NO_2$ 203.0955, found 203.0946.

4-Hydroxymethyl-6-methoxy-1-methylquinolin-2(1*H*)-one 5c. Starting from compound **4c** (0.113 g, 5.2×10^{-4} mol) and sodium borohydride (0.134 g, 3.6×10^{-4} mol), and purification by silica gel column chromatography using dichloromethane/methanol (100:1) as eluent, compound **5c** was obtained as a white solid (0.111 g, 97%); mp = 177-179 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.60 (3H, s, NCH_3), 3.90 (3H, s, OCH_3), 3.17 (1H, br s, OH), 4.91 (2H, s, CH_2), 6.87 (1H, s, H-3), 7.13-7.28 (3H, m, H-5, H-7 and H-8); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 29.68 (NCH_3), 55.73 (OCH_3), 61.78 (CH_2), 106.94 (C-5), 115.81 (C-8), 118.69 (C-7), 119.04 (C-3), 119.86 (C-4a), 134.33 (C-8a), 147.92 (C-4), 154.68 (C-6), 161.90 (C-2). IR (KBr 1%, cm^{-1}): ν = 3338, 2918, 2849, 1648, 1620, 1573, 1507, 1465, 1430, 1372, 1279, 1241, 1185, 1079, 1034, 864, 809; UV/Vis (ethanol, nm): λ_{max} (log ε) = 352 (3.60); MS: *m/z* (EI) 219 (M^+, 100), 204 (81), 203 (25), 190 (21), 188 (35), 86 (28), 84 (45); HRMS: *m/z* (EI) calc. for $C_{12}H_{13}NO_3$ 219.0901, found 219.0895.

General procedure for the synthesis of 4-chloromethylquinolin-2(1*H*)-ones 6a-c. Compounds **5a-c** (1 equiv) were stirred with thionyl chloride (20 equiv) in dichloromethane (10 mL) for 1 day at room temperature. After removal of the solvent under reduced pressure, compounds **6a-c** were obtained as solids.

4-Chloromethyl-1-methylquinolin-2(1*H*)-one 6a. Starting from compound **5a** (0.068 g, 3.6×10^{-4} mol) and thionyl chloride (0.5 mL, 7.2×10^{-3} mol), compound **6a** was obtained as a white solid (0.078 g, 79%); mp = 170-171 ºC; ^{1}H NMR (300 MHz, $CDCl_3$/DMSO-d_6, 1:1): δ = 3.56 (3H, s, NCH_3), 4.61 (2H, d, *J* 0.6 Hz, CH_2), 6.67 (1H, s, H-3), 7.16 (1H, dt, *J* 8.1 and 1.2 Hz, H-6), 7.28 (1H, d, *J* 6.9 Hz, H-8), 7.45 (1H, dt, *J* 8.7 and 1.5 Hz, H-7), 7.66 (1H, dd, *J* 8.4 and 1.5 Hz, H-5); ^{13}C NMR (75.4 MHz, $CDCl_3$/DMSO-d_6, 1:1): δ = 29.03 (CH_3), 41.84 (NCH_3), 114.44 (C-8), 118.12 (C-4a), 121.29 (C-3), 121.92 (C-6), 124.46 (C-5), 130.67 (C-7), 139.74 (C-8a), 143.91 (C-4), 161.12 (C-2); IR (KBr 1%, cm^{-1}): ν = 3073, 3021, 1674,

1662, 1646, 1593, 1563, 1505, 1453, 1415, 1400, 1325, 1266, 1222, 1169, 1081, 1052, 949, 915, 896, 793, 774; UV/Vis (ethanol, nm): λ_{max} (log ε) = 333 (3.78); MS: *m/z* (EI) 209 (M^+ ^{37}Cl, 15), 207 (M^+ ^{35}Cl, 50), 173 (36), 172 (62), 144 (100), 130 (20); HRMS: *m/z* (EI) calc. for $C_{11}H_{10}NO^{35}Cl$ 207.0457, found 207.0451; calc. for $C_{11}H_{10}NO^{37}Cl$ 209.0431, found 209.0421.

4-Chloromethyl-1,6-dimethylquinolin-2(1*H*)-one 6b. Starting from compound **5b** (0.136 g, 6.7×10^{-4} mol) and thionyl chloride (1.0 mL, 1.3×10^{-2} mol), compound **6b** was obtained as a beige solid (0.122 g, 82%); mp = 153-155 ºC; ^1H NMR (400 MHz, $CDCl_3$): δ = 2.48 (3H, s, CH_3), 3.72 (3H, s NCH_3), 4.73 (2H, s, CH_2), 6.82 (1H, s, H-3), 7.32 (1H, d, *J* 8.4 Hz, H-8), 7.44 (1H, dd, *J* 8.8 and 1.6 Hz, H-7), 7.59 (1H, s, H-5); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 20.88 (CH_3), 29.49 (NCH_3), 42.34 (CH_2), 114.73 (C-8), 118.58 (C-4a), 122.04 (C-3), 124.67 (C-5), 131.89 (C-6), 132.20 (C-7), 138.33 (C-8a), 143.89 (C-4), 161.61 (C-2); IR (KBr 1%, cm^{-1}): ν = 3033, 2964, 1656, 1590, 1568, 1505, 1461, 1438, 1418, 1381, 1326, 1262, 1165, 1097, 1052, 1022, 945, 911, 876, 805; UV/Vis (ethanol, nm): λ_{max} (log ε) = 343 (3.48); MS: *m/z* (EI) 223 (M^+ ^{37}Cl, 21), 221 (M^+ ^{35}Cl, 70), 187 (30), 186 (68), 158 (100), 157 (23), 115 (22); HRMS: *m/z* (EI) calc. for $C_{12}H_{12}NO^{35}Cl$ 221.0598, found 221.0607; calc. for $C_{12}H_{12}NO^{37}Cl$ 223.0583, found 223.0578.

4-Chloromethyl-6-methoxy-1-methylquinolin-2(1*H*)-one 6c. Starting from compound **5c** (0.100 g, 4.6×10^{-4} mol) and thionyl chloride (0.7 mL, 9.2×10^{-3} mol), compound **6c** was obtained as a yellowish solid (0.056 g, 52%); mp = 171-172 ºC; ^1H NMR (300 MHz, $CDCl_3$): δ = 3.72 (3H, s, NCH_3), 3.91 (3H, s, OCH_3), 4.71 (2H, s, CH_2), 6.84 (1H, s, H-3), 7.23 (1H, dd, *J* 8.7 and 2.7 Hz, H-7), 7.25 (1H, s, H-5), 7.36 (1H, d, *J* 8.7 Hz, H-8); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 29.61 (NCH_3), 42.49 (CH_2), 55.76 (OCH_3), 107.56 (C-5), 116.05 (C-8), 119.06 (C-7), 119.44 (C-4a), 122.67 (C-3), 134.83 (C-8a), 143.46 (C-4), 154.73 (C-6), 161.22 (C-2). IR (KBr 1%, cm^{-1}): ν = 3081, 2995, 2966, 2942, 2839, 1652, 1622, 1592, 1573, 1508, 1458, 1428, 1415, 1344, 1272, 1246, 1199, 1084, 1043, 929, 865, 808; UV/Vis (ethanol, nm): λ_{max} (log ε) = 358 (3.38); MS: *m/z* (EI) 239 (M^+ ^{37}Cl, 33), 237 (M^+ ^{35}Cl, 100), 222 (55), 203 (62), 202 (25), 188 (70), 174 (34), 160 (22), 159 (33), 130 (19); HRMS: *m/z* (EI) calc. for $C_{12}H_{12}NO_2{}^{35}Cl$ 237.0561, found 237.0557; calc. for $C_{12}H_{12}NO_2{}^{37}Cl$ 239.0517, found 239.0527.

Synthesis of 4-chloromethyl-1,6-dimethyl-5-nitroquinolin-2(1*H*)-one 6d. Compound **6b** (0.060g, 2.7×10^{-4} mol) was dissolved in concentrated H_2SO_4 (0.5 mL), in an ice bath, and HNO_3 65% (0.05 mL) was added. The mixture was stirred for 30 min at room temperature and then poured over ice. A precipitate was collected by filtration, washed with water and dried to yield compound **6d** as a yellow solid (0.042 g, 58%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.35 (3H, s, CH_3), 3.76 (3H, s NCH_3), 4.65 (2H, d, *J* 0.8 Hz, CH_2), 7.17 (1H, t, *J* 0.8 Hz, H-3), 7.51 (2H, m, H-7 and H-8); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 17.05 (CH_3), 30.25 (NCH_3), 41.26 (CH_2), 110.68 (C-4a), 116.82 (C-8), 124.23 (C-6), 125.64 (C-3), 133.00 (C-7), 139.57 (C-8a), 140.94 (C-4), 147.90 (C-5), 160.46 (C-2). IR (KBr 1%, cm^{-1}): ν = 2927, 2855, 1663, 1591, 1557, 1530, 1459, 1419, 1376, 1314, 1274, 1107, 980, 869, 817; UV/Vis (ethanol, nm): λ_{max} (log ε) = 343 (3.61); MS: *m/z* (EI) 268 (M^+ ^{37}Cl, 34), 266 (M^+ ^{35}Cl, 100), 188 (52); HRMS: *m/z* (EI) calc. for $C_{12}H_{11}N_2O_3{}^{35}Cl$ 266.0464, found 266.0461; $C_{12}H_{11}N_2O_3{}^{37}Cl$ 268.0438, found 268.0440;

General procedure for the synthesis of coumarin conjugates 7a,b and quinolin-2(1*H*)-one conjugates 8a-d. The corresponding chloromethylcoumarin **1a**,**b** or chloromethylquinolin-2(1*H*)-one **6a-d** (1 equiv) was stirred with *N*-benzyloxycarbonyl-L-phenylalanine (1 equiv) and KF (3 equiv) in DMF (5 mL), at room temperature for 1-3 days. The solvent was removed in a rotary evaporator and a crude solid was obtained.

***N*-Benzyloxycarbonyl-L-phenylalanine (7-methyl-coumarin-4-yl)methyl ester 7a**. Starting from compound **1a** (0.100 g, 4.79×10^{-4} mol), Z-Phe-OH (0.143 g, 4.79×10^{-4} mol) and KF (0.083 g, 1.44×10^{-3} mol), the crude solid was recrystalised from ethyl acetate and petroleum ether 40-60, yielding conjugate **7a** as a white solid (0.190 g, 84%); mp = 151-152 ºC; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.46 (3H, s, CH_3), 3.15 (2H, d, *J* 6.3 Hz, β-CH_2 Phe), 4.72-4.79 (1H, m, α-H Phe), 5.12 (2H, s, CH_2 Z), 5.24-5.26 (3H, m, CH_2 and NH), 6.27 (1H, s, H-3), 7.09-7.35 (13H, m, H-5, H-6, H-8 and 10 × Ph-H); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 21.63 (CH_3), 38.19 (β-CH_2 Phe), 55.06 (α-C Phe), 62.01 (CH_2), 67.19 (CH_2 Z), 112.76 (C-3), 114.52 (C-4a), 117.52 (C-8), 123.14 (C-5), 125.64 (C-6), 127.43 (C-4' Phe), 128.16 (C-3'' and C-5'' Z), 128.25 (C-4'' Z), 128.51 (C-2'' and C-6'' Z), 128.77 (C-3' and C-5' Phe), 129.06 (C-2' and C-6' Phe), 135.14 (C-1' Phe), 135.96 (C-1'' Z), 143.46 (C-7), 147.85 (C-4), 153.72 (C-8a), 155.64 (C=O urethane), 160.39 (C-2), 171.06 (C=O ester); IR (KBr 1%, cm^{-1}): ν = 3311, 3062, 3027, 1742, 1693, 1620, 1544, 1494, 1456, 1409, 1377, 1272, 1212, 1181, 1149, 1069, 1052, 1016, 962, 863; UV/Vis (ethanol, nm): λ_{max} (log ε) = 317 (3.92); MS: *m/z*

(ESI) 472 (M^++1, 58), 324 (20), 143 (51), 139 (83); HRMS: *m/z* (ESI) calc. for $C_{28}H_{26}NO_6$ 472.17546, found 472.17537.

***N*-Benzyloxycarbonyl-L-phenylalanine (7-methyl-6-nitrocoumarin-4-yl)methyl ester 7b**. Starting from compound **1b** (0.085 g, 3.35×10^{-4} mol), Z-Phe-OH (0.100 g, 3.35×10^{-4} mol) and KF (0.058 g, 1.00×10^{-3} mol), the crude solid was recrystalised from ethyl acetate and petroleum ether 40-60, yielding conjugate **7b** as a white solid (0.122 g, 70%); mp = 140-142 ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.73 (3H, s, CH_3), 3.13-3.16 (2H, m, β-CH_2 Phe), 4.18-4.20 (1H, m, α-H Phe), 5.11 (2H, s, CH_2 Z), 5.25-5.27 (2H, m, CH_2), 6.37 (1H, s, H-3), 7.00-7.12 (5H, m, 5 × Ph-H), 7.21-7.35 (6H, m, H-8 and 5 × Ph-H), 8.19 (1H, s, H-5); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 21.19 (CH_3), 38.21 (β-CH_2 Phe), 55.13 (α-C Phe), 61.44 (CH_2), 67.30 (CH_2 Z), 115.19 (C-3), 115.37 (C-4a), 121.01 (C-5), 121.19 (C-8), 127.50 (C-4' Phe), 127.81 (C-4'' Z), 128.23 (C-3'' and C-5'' Z), 128.55 (C-2'' and C-6'' Z), 128.81 (C-3' and C-5' Phe), 129.02 (C-2' and C-6' Phe), 135.05 (C-1' Phe), 135.94 (C-1'' Z), 138.84 (C-6), 145.09 (C-7), 146.95 (C-4), 155.52 (C-8a), 155.66 (C=O urethane), 158.54 (C-2), 171.07 (C=O ester); IR (KBr 1%, cm^{-1}): ν = 3325, 2925, 1736, 1698, 1629, 1594, 1530, 1496, 1454, 1402, 1382, 1344, 1314, 1260, 1123, 1045, 868, 841, 751; UV/Vis (ethanol, nm): λ_{max} (log ε) = 316 (3.58); MS: *m/z* (ESI) 539 (M^+ + 1 + Na, 83), 474 (53), 374 (27), 338 (38), 323 (20), 322 (100); HRMS: *m/z* (ESI) calc. for $C_{28}H_{24}N_2O_8Na$ 539.14135, found 539.14249.

***N*-Benzyloxycarbonyl-L-phenylalanine (1-methylquinolin-2(1*H*)-one-4-yl) methyl ester 8a**. Starting from compound **6a** (0.080 g, 3.9×10^{-4} mol), Z-Phe-OH (0.115 g, 3.9×10^{-4} mol) and KF (0.067 g, 1.2×10^{-3} mol), the crude solid was recrystalised from dichloromethane yielding conjugate **8a** as a beige oily solid (0.165 g, 91%); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.94-3.11 (2H, m, β-CH_2 Phe), 3.62 (3H, s, NCH_3), 4.36-4.42 (1H, m, α-H Phe), 4.90-5.00 (2H, m, CH_2 Z), 5.36-5.45 (2H, m, CH_2), 6.64 (1H, s, H-3), 7.10-7.33 (10H, m, 10 × Ph-H), 7.56 (1H, d, *J* 8.4 Hz, H-8), 7.63-7.69 (2H, m, H-5 and H-7), 7.95 (1H, d, *J* 8.0 Hz, NH); ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 29.03 (CH_3), 36.31 (β-CH_2 Phe), 55.67 (α-C Phe), 62.48 (CH_2), 65.51 (CH_2 Z), 115.12 (C-8), 117.88 (C-4a), 119.01 (C-3), 122.03 (C-6), 124.62 (C-5), 126.53 (C-4' Phe), 127.64 (C-2'' and C-6'' Z), 127.78 (C-4'' Z), 128.24 (C-3'' and C-5'' Z), 128.28 (C-3' and C-5' Phe), 129.08 (C-2' and C-6' Phe), 130.99 (C-7), 136.77 (C-1'' Z), 137.19 (C-1' Phe), 139.62 (C-8a), 143.33 (C-4), 156.01 (C=O urethane), 160.54 (C-2), 171.40 (C=O ester); IR (KBr 1%, cm^{-1}): ν = 3031, 2925, 1755, 1720, 1657, 1592, 1498, 1455, 1399, 1325, 1258, 1179, 1082, 1063, 875, 848, 751; UV/Vis (ethanol, nm): λ_{max} (log ε)

= 330 (3.68); MS: *m/z* (ESI) 471 (M^++1, 100); HRMS: *m/z* (ESI) calc. for $C_{28}H_{27}N_2O_5$ 471.19184, found 471.19145.

***N*-Benzyloxycarbonyl-L-phenylalanine (1,6-dimethylquinolin-2(1*H*)-one-4-yl) methyl ester 8b**. Starting from compound **6b** (0.050 g, 2.3×10^{-4} mol), Z-Phe-OH (0.068 g, 2.3×10^{-4} mol) and KF (0.039 g, 6.8×10^{-4} mol), the crude solid was purified by silica gel column chromatography using ethyl acetate/*n*-hexane (1:2) as eluent, and conjugate **8b** was obtained as a light yellow oily solid (0.061 g, 56%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.44 (3H, s, CH_3), 3.14-3.17 (2H, m, β-CH_2 Phe), 3.72 (3H, s, NCH_3), 4.74-4.80 (1H, m, α-H Phe), 5.05-5.15 (2H, m, CH_2 Z), 5.26 (1H, d, *J* 8.1 Hz, NH), 5.34-5.39 (2H, m, CH_2), 6.72 (1H, s, H-3), 7.07-7.10 (2H, m, H-2' and H-6' Phe), 7.21-7.45 (12H, m, H-5, H-8 and 10 × Ph-H), 7.43 (1H, dd, *J* 8.7 and 2.1 Hz, H-7); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 20.80 (CH_3), 29.42 (NCH_3), 38.13 (β-CH_2 Phe), 54.96 (α-C Phe), 63.05 (CH_2), 67.08 (CH_2 Z), 114.64 (C-8), 118.48 (C-4a), 120.28 (C-3), 123.99 (C-5), 127.26 (C-4'' Z), 128.11 (C-2'' and C-6'' Z), 128.19 (C-4' Phe), 128.50 (C3'' and C-5'' Z), 128.67 (C-3' and C-5' Phe), 129.12 (C-2' and C-6' Phe), 131.89 (C-6), 132.11 (C-7), 135.26 (C-1' Phe), 136.08 (C-1'' Z), 130.03 (C-8a), 141.98 (C-4), 155.63 (C=O urethane), 161.51 (C-2), 171.17 (C=O ester); IR (KBr 1%, cm^{-1}): ν = 3285, 3062, 3031, 2940, 1751, 1720, 1652, 1592, 1570, 1531, 1498, 1455, 1418, 1327, 1260, 1178, 1082, 1062, 880, 809, 749; UV/Vis (ethanol, nm): λ_{max} (log ε) = 339 (3.71); MS: *m/z* (ESI) 485 (M^++1, 100), 486 (M^++2, 50); HRMS: *m/z* (ESI) calc. for $C_{29}H_{29}N_2O_5$ 485.20918, found 485.20710.

***N*-Benzyloxycarbonyl-L-phenylalanine (6-methoxy-1-methylquinolin-2(1*H*)-one-4-yl) methyl ester 8c**. Starting from compound **6c** (0.050 g, 2.1×10^{-4} mol), Z-Phe-OH (0.063 g, 2.1×10^{-4} mol) and KF (0.037 g, 6.3×10^{-4} mol), the crude solid was purified by silica gel column chromatography using dichloromethane/methanol (100:1) as eluent, and conjugate **8c** was obtained as a light yellow oily solid (0.065 g, 62%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.13-3.16 (2H, m, β-CH_2 Phe), 3.72 (3H, s, NCH_3), 3.90 (3H, s, OCH_3), 4.72-4.78 (1H, m, α-H Phe), 5.05-5.12 (2H, m, CH_2 Z), 5.27 (1H, d, *J* 8.0 Hz, NH), 5.33 (2H, s, CH_2), 6.75 (1H, s, H-3), 7.02 (1H, d, *J* 2.4 Hz, H-5), 7.07-7.09 (2H, m, H-2' and H-6' Phe), 7.21-7.36 (10H, m, H-7, H-8 and 8 × Ph-H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 29.55 (NCH_3), 38.10 (β-CH_2 Phe), 54.99 (α-C Phe), 55.73 (OCH_3), 63.12 (CH_2), 67.08 (CH_2 Z), 106.70 (C-5), 115.97 (C-8), 119.13 (C-7), 119.36 (C-4a), 121.28 (C-3), 127.26 (C-4' Phe), 128.09 (C-2'' and C-6'' Z), 128.18 (C-4'' Z), 128.49 (C-3'' and C-5'' Z), 128.65 (C-3' and C-5' Phe), 129.10 (C-2' and

C-6’ Phe), 134.57 (C-8a), 135.23 (C-1’ Phe), 136.07 (C-1’’ Z), 141.61 (C-4), 154.80 (C-6), 155.62 (C=O urethane), 161.15 (C-2), 171.18 (C=O ester); IR (KBr 1%, cm^{-1}): ν = 3291, 3029, 3006, 2940, 1736, 1652, 1621, 1587, 1571, 1509, 1464, 1456, 1431, 1385, 1313, 1281, 1247, 1205, 1181, 1083, 1041, 876; UV/Vis (ethanol, nm): λ_{max} (log ε) = 354 (3.70); MS: *m/z* (ESI) 501 (M^++1, 63), 407 (21), 204 (100); HRMS: *m/z* (ESI) calc. for $C_{29}H_{29}N_2O_6$ 501.20181, found 501.20201.

***N*-Benzyloxycarbonyl-L-phenylalanine (1,6-dimethyl-5-nitroquinolin-2(1*H*)-one-4-yl) methyl ester 8d**. Starting from compound **6d** (0.022 g, 8.3×10^{-5} mol), Z-Phe-OH (0.025 g, 8.3×10^{-5} mol) and KF (0.015 g, 2.5×10^{-4} mol), the crude solid was purified by silica gel column chromatography using dichloromethane/methanol (50:1) as eluent, and conjugate **8d** was obtained as a yellow oily solid (0.032 g, 72%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.35 (3H, s, CH_3), 3.06-3.22 (2H, m, β-CH_2 Phe), 3.75 (3H, s, NCH_3), 4.70-4.73 (1H, m, α-H Phe), 5.08-5.10 (2H, m, CH_2 Z), 5.13 (1H, d, *J* 8.1 Hz, NH), 5.21-5.26 (2H, m, CH_2), 6.83 (1H, s, H-3), 7.09-7.12 (2H, m, H-2’ and H-6’ Phe), 7.21-7.45 (8H, m, 8 × Ph-H), 7.46-7.51 (2H, m, H-7 and H-8); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 17.13 (CH_3), 30.20 (NCH_3), 38.04 (β-CH_2 Phe), 54.93 (α-C Phe), 62.01 (CH_2), 67.07 (CH_2 Z), 110.57 (C-4a), 116.78 (C-8), 123.90 (C-3), 124.25 (C-6), 127.19 (C-4’ Phe), 128.11 (C-2’’ and C-6’’ Z), 128.14 (C-4’’ Z), 128.48 (C-3’’ and C-5’’ Z), 128.64 (C-3’ and C-5’ Phe), 129.12 (C-2’ and C-6’ Phe), 133.00 (C-7), 135.40 (C-1’ Phe), 136.08 (C-1’’ Z), 139.38 (C-4), 139.61 (C-8a), 149.00 (C-5), 156.00 (C=O urethane), 160.20 (C-2), 170.57 (C=O ester); IR (KBr 1%, cm^{-1}): ν = 3303, 3062, 3031, 2921, 2850, 1752, 1720, 1652, 1589, 1558, 1496, 1455, 1418, 1372, 1258, 1210, 1174, 1107, 1083, 1052, 869, 817, 744; UV/Vis (ethanol, nm): λ_{max} (log ε) = 341 (3.86); MS: *m/z* (ESI) 530 (M^++1, 100), 474 (25); HRMS: *m/z* (ESI) calc. for $C_{29}H_{28}N_3O_7$ 530.19109, found 530.19218.

General photolysis procedure. A 1×10^{-4} M methanol/HEPES buffer (80:20) solution of conjugates **7** and **8** (5 mL) were 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, 350 and 419 ± 10 nm. HEPES buffer solution was prepared in distilled water with HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) (10 mM), NaCl (120 mM), KCl (3 mM), $CaCl_2$ (1 mM) and $MgCl_2$ (1mM) 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, except for compound

8c (0.6 mL/min), 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 for each conjugate (retention time: **7a**, 6.5; **7b**, 6.3; **8a**, 4.8; **8b**, 5.7; **8c**, 6.4; **8d**, 5.6 min).

Results and Discussion

Synthesis

4-Chloromethyl-7-methylcoumarin **1a** was synthesised through a Pechmann reaction, between 3-methylphenol and ethyl 4-chloro-3-oxobutanoate, catalysed by aqueous sulphuric acid at room temperature, through a method reported earlier (Fonseca et al. 2007). This compound was nitrated in standard conditions with nitric acid and sulphuric acid, yielding 4-chloromethyl-7-methyl-6-nitrocoumarin **1b** (Scheme 1).

1,4-Dimethylquinolin-2(1*H*)-one **3a**, 1,4,6-trimethylquinolin-2(1*H*)-one **3b** and 1,4-dimethyl-6-methoxyquinolin-2(1*H*)-one **3c** were prepared by a modified Knorr synthesis (Uray et al. 1999) between ethyl 3-oxobutanoate and *N*-methylaniline **2a**, *N*,4-dimethylaniline **2b** and *N*-methyl-4-methoxyaniline **2c**, respectively. The methyl group at position 4 was oxidised to the aldehyde, by reaction with selenium dioxide, affording 1-methylquinolin-2(1*H*)-one-4-carbaldehyde **4a**, 1,6-dimethylquinolin-2(1*H*)-one-4-carbaldehyde **4b** and 6-methoxy-1-methylquinolin-2(1*H*)-one-4-carbaldehyde **4c**, which were then reacted with sodium borohydride, giving 4-hydroxymethyl-1-methylquinolin-2(1*H*)-one **5a**, 1,6-dimethyl-4-hydroxymethylquinolin-2(1*H*)-one **5b** and 4-hydroxymethyl-6-methoxy-1-methylquinolin-2(1*H*)-one **5c**. These hydroxymethylated derivatives were converted through treatment with thionyl chloride to the chloromethylated counterparts, namely 4-chloromethyl-1-methylquinolin-2(1*H*)-one **6a**, 4-chloromethyl-1,6-dimethylquinolin-2(1*H*)-one **6b** and 4-chloromethyl-6-methoxy-1-methylquinolin-2(1*H*)-one **6c**. This strategy was followed due to difficulties encountered when the reaction was carried out directly between the *N*-methylated amines **2a-c** and ethyl 4-chloro-3-oxobutanoate, with no expected product being obtained. 4-Chloromethyl-1,6-dimethyl-5-nitroquinolin-2(1*H*)-one **6d** was obtained by nitration of compound **6b** (Scheme 1). The introduction of the nitro group will later on allow the design of an alternative bifunctional heterocyclic analogue of the well known *o*-nitrobenzyl photocleavable protecting group, by subsequent transformation of the adjacent methyl group. The chloromethyl derivatives were obtained in good yields (Table 1) and characterised by IR, ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry.

< SCHEME 1>

Given the need for protecting groups in synthetic strategies involving bifunctional molecules, such as amino acids, as well as the interest in photocleavable protecting groups, we evaluated the behaviour of the above heterocycles as photocleavable protecting groups for the carboxylic function. Thus, chloromethyl coumarins **1a**,**b** and quinolones **6a-d** were used in the preparation of several fluorescent bioconjugates using phenylalanine as a model, via coupling of *N*-benzyloxycarbonyl-phenylalanine (Z-Phe-OH) in DMF, at room temperature, in the presence of potassium fluoride (Tjoeng and Heavner 1981). The corresponding phenylalanine conjugates **7a**,**b** and **8a-d** (Scheme 2, Table 1) were obtained in good to excellent yields (56-91%) and fully characterised by the usual analytical techniques.

< SCHEME 2>

< TABLE 1>

The IR spectra of conjugates **7a**,**b** and **8a-d** showed bands due to stretching vibrations of the ester carbonyl group of the fluorophore-amino acid linkage from 1736 to 1755 cm^{-1}. ^{1}H NMR spectra showed signals of the phenylalanine α-CH (δ 4.18 to 4.80 ppm), as well as the characteristic protons of the fluorophore methylene group (δ 5.21 to 5.45 ppm). The confirmation of the presence of the newly formed ester bond was also supported by ^{13}C NMR spectra signals of the carbonyl group, which were found between δ 170.57 and 171.40 ppm.

Considering that the present work involves the evaluation of new quinolone derivatives as photocleavable protecting groups, we carried out the photophysical characterisation to study the properties of the conjugates in comparison with the starting heterocycles, for the determination of the parameters needed for monitorisation during photolysis. As they are novel compounds and for the sake of comprehensiveness, the UV-vis and fluorescence data of the synthesized compounds was collected and is presented in Table 1.

The UV-vis absorption and emission spectra of degassed 1×10^{-5} to 2×10^{-5} M solutions in absolute ethanol of all tags and precursors (**1a**,**b**, **3a-c**, **4a-c**, **5a-c** and **6a-d**) and conjugates **7a,b** and **8a-d** were measured, absorption and emission maxima, molar absorptivities and fluorescence quantum yields (Φ_F) are reported (Table 1). Fluorescence quantum yields were calculated using 9,10-diphenylanthracene as standard (Φ_F = 0.95 in ethanol) (Morris et al. 1976). 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 comparison of the data of coumarin conjugates **7a**,**b** with the corresponding precursors **1a**,**b**, it was found that the fluorescence quantum yield did not vary after covalent linkage to the amino acid. Regarding the quinolone precursors, the fluorescence quantum yields of the methylated compounds **3a-c** are similar to those of hydroxymethyl derivatives **5a-c**, decreasing for the chloromethylated derivatives **6a-d**. The fluorescence quantum yields for quinolone conjugates **8a-c** revealed an increase in their values, upon linkage of the quinolone precursor to the amino acid. Considering the Stokes' shifts of all compounds, it was found that the data for fluorophores in their free form and their conjugates are comparable, varying from 50 to 85 nm.

Photolysis studies of phenylalanine conjugates 7a,b and 8a-d

The evaluation of heterocycles **1a**,**b** and **6a-d** as photocleavable protecting groups was carried out by photolysis studies of the corresponding ester conjugates **7a**,**b** and **8a-d** under irradiation at different wavelengths. Solutions of the mentioned compounds (1×10^{-4} M) in methanol/HEPES buffer (80:20) solution were irradiated in a Rayonet RPR-100 reactor, at 254, 300, 350 and 419 nm, in order to determine the most favourable cleavage conditions. The solvent system was chosen based on previous studies regarding different organic solvents with varying aqueous content, which revealed that photocleavage was faster in a mixture of methanol and HEPES buffer (Fernandes et al. 2007). The course of the photocleavage reaction was followed by reverse phase HPLC with UV detection.

The plots of peak area (*A*) of the starting material *versus* irradiation time were obtained for each compound, at the considered wavelengths. Peak areas were determined by HPLC, which revealed a gradual decrease with time, and were the average of 3 runs. 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).

For each compound and 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 correlation coefficients varying from 0.9874 to 0.9987 (Figure 1). The corresponding rate constants were calculated and are presented in Table 2.

<TABLE 2>

< FIGURE 1>

In the present work, it is intended to assess, in a preliminary approach, the potential for the applicability of quinolone as a photocleavable protecting group, when compared to the well-established coumarin. As such, considering the chromophores presented, definitive conclusions about the influence of the substituents or their position on the photocleavage properties cannot be drawn. Taking into consideration the influence of the structure of the heterocycle on the photocleavage rates, it was found that the quinolone conjugates **8a-d** displayed shorter irradiation times, in all wavelengths of irradiation studied, when compared to the coumarin conjugates **7a**,**b**. The presence of a nitro group increased the irradiation times, for compound **8d** in all wavelengths. From this fact and considering the results in Table 2 for compounds **8b** or **8c** and **8d** (with a nitro group), one might suggest the feasibility of selective photodeprotection at 300 and 350 nm, if both the quinolone precursors were used to protect a bifunctional molecule through ester bonds. At 300 and 350 nm, the irradiation times for the nitro conjugate **8d** were *ca* 40 times larger when compared to the irradiation time for conjugate **8b**. The same suggestion could be made for the combination of a coumarin and a quinolone as protecting groups, as can be seen by comparison of the behaviour of coumarin conjugate **7a** and quinolone conjugate **8b** at 300 and 350 nm. Under irradiation at 350 nm, there was a striking difference between the irradiation times (the quinolone conjugate cleaved more than 800 times faster than the coumarin conjugate), which could allow a selective photolysis.

Regarding irradiation times in the different wavelengths studied, and accordingly to the lamp power, it was found for the coumarin conjugates that shorter values were obtained at 254 nm. For the quinolone conjugates, irradiation at 254 and 300 nm resulted in similar irradiation times for compounds **8a-c** (0.8 to 9 min), with compounds **8b** and **8c** showing short irradiation times at 350 nm (0.6 and 4 min, respectively). Conjugate **8d** showed the largest irradiation times in all wavelengths of irradiation studied.

Considering the short irradiation time obtained for compounds **8b** and **8c** at the studied wavelengths, photocleavage at 419 nm was also tested. For compound **8b**, a large increase in the irradiation time was observed (193 min, *ca* 3.5 h) and for compound **8c**, the increase in the irradiation time was moderate (34 min, *ca* 0.5h). Once again considering the possibility of selective photodeprotection, these interesting results suggest that the corresponding methylquinoline **6b** and methoxyquinoline **6c** precursors can be used as photoremovable

protecting groups to be used at 419 nm. These heterocycles, when linked through ester bonds, could be selectively removed in the presence of other protecting groups which cleave with much slower rates or that are not affected by radiation of that wavelength. Photolysis at 419 nm can be considered the most suitable for certain practical bioapplications involving caging strategies, as it avoids cell damage due to short-wavelength light.

As reported before (Piloto et al. 2006b), the *N*-benzyloxycarbonyl urethane-type blocking group was stable in the tested conditions, no cleavage being detected at the various wavelengths of irradiation. Even at 254 nm, where all the chromophores present at the molecule absorb, in the reported conditions (using a Rayonet reactor) no tendency to form side products has been detected, for example by cleavage of the *N*-protecting group or any other rearrangement.

Additionally to monitoring of the photolysis process through HPLC/UV detection in the case of the quinolone conjugates, the samples collected at different irradiation times were also analysed by LC/MS. The obtained mass spectra contained peaks that could be attributed to the released Z-protected phenylalanine, the hydroxymethylated quinolone precursor, as well as the methoxymethylated quinolone. These data are in agreement with a proposed mechanism (Singh and Khade 2005; Yamaji 2009), where either by homolytical or heterolytical cleavage of the ester bond, the observed cleavage products are obtained: once the methylene carbocation is formed, it can be attacked by a proper nucleophile (water or methanol) yielding the above mentioned products (Scheme 3).

< SCHEME 3>

Furthermore, the release of Z-protected phenylalanine, as the expected photolysis product, was also followed by ^{1}H NMR in a methanol-d_4/D_2O (80:20) solution of conjugates **7** and **8** in a concentration from 5.4×10^{-3} to 9.1×10^{-3} M (*ca*. 54 to 91 times larger than the concentration used of the above described experiments), which led to the expected increase in the photolysis time for the complete release of the amino acid. Upon irradiation at the usual wavelengths, a new set of signals appeared, related to phenylalanine α-CH and β-CH_2, at δ 4.44 and 2.95 ppm, respectively, and a decrease of the benzylic-type CH_2 of the fluorophore was visible at δ 5.44 ppm (see Figure 2 for conjugate **8b**). After a variable irradiation period (depending on the wavelength of irradiation and structure of the conjugate) the signals due to the amino acid residue in the conjugate form disappeared completely and were replaced by

the corresponding set of signals of Z-Phe-OH, thus confirming the quantitative release of the amino acid. Moreover, signals due to by-products related to the fluorophore were also detected in the ^{1}H NMR spectra.

< FIGURE 2>

Conclusions

Phenylalanine ester bioconjugates **7** and **8** were prepared in good to excellent yields by using a simple synthetic method involving chloromethylated coumarin **1** and quinolone **6** precursors and the carboxylic acid group of *N*-benzyloxycarbonyl-protected phenylalanine as model of bifunctional compounds.

Photocleavage studies of the fluorescent conjugates, in methanol/ HEPES buffer (80:20) solution at 254, 300, 350 and 419 nm, confirmed their photosensitivity, which was influenced by the structure of the heterocycle. The quinolone conjugates **8a-d** displayed shorter irradiation times, that can be considered useful for practical applications, when compared to the coumarin conjugates **7a**,**b**, at all wavelengths of irradiation.

In summary, the synthesised fluorescent conjugates required short irradiation times for photocleavage to occur, with quantitative release of the amino acid, making them appropriate for using as photolabile protecting groups for organic molecules, including amino acids. These results suggest that quinolones could be an alternative and further exploited as photoremovable protecting groups, when considering the promising results obtained in the photocleavage under all considered wavelengths of irradiation. Although the synthesis of quinolones is longer (four sequential steps are necessary), this fact is largely compensated by the short irradiation times necessary to release the model amino acid from the conjugates.

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CAPTIONS

Scheme 1. Synthesis of coumarins **1a**,**b** and quinolin-2(1*H*)-ones **3a-c**, **4a-c**, **5a-c** and **6a-d**.

Scheme 2. Synthesis of phenylalanine ester conjugates **7a**,**b** and **8a-d**.

Scheme 3. Proposed mechanism for the formation of photolysis products from quinolin-2(1*H*)-one conjugates **8a-d**.

Table 1. Yields, UV-vis and fluorescence data for coumarins **1a**,**b**, quinolin-2(1*H*)-ones **6a-d** and fluorescent bioconjugates **7a**,**b** and **8a-d** in absolute ethanol.

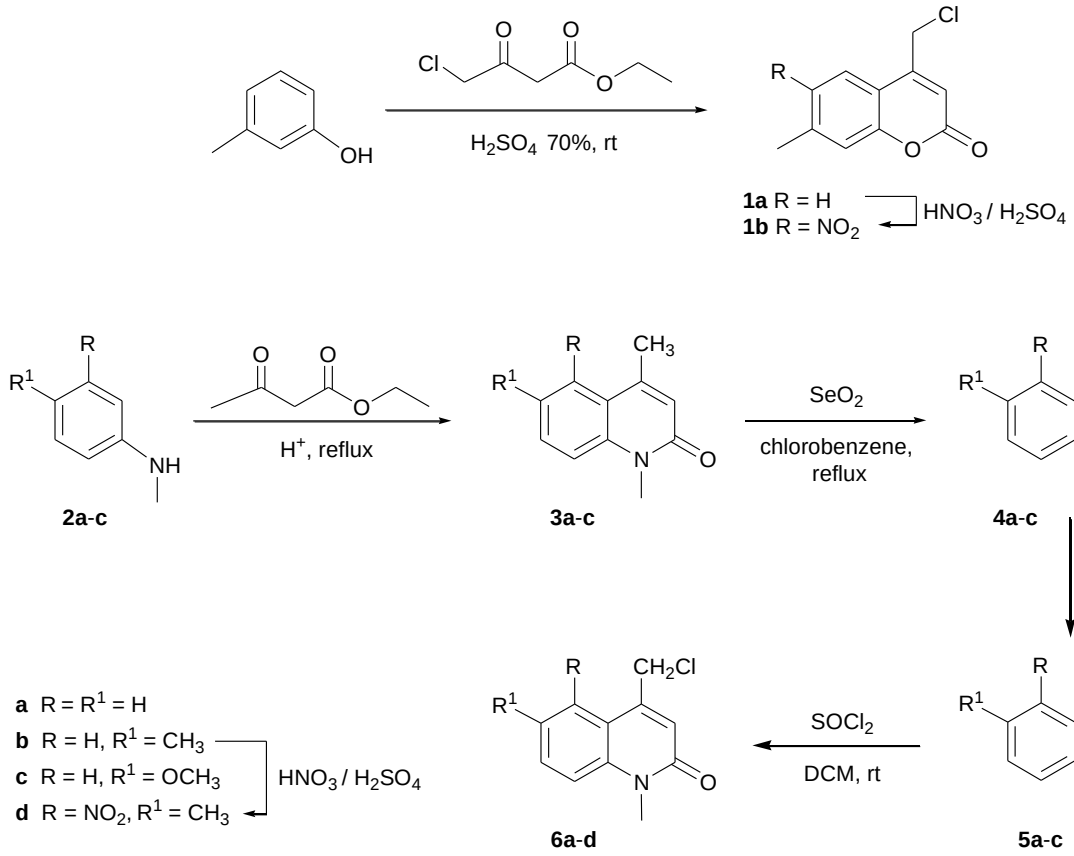
Table 2. Irradiation times (in min) and rate constants (k, in min^{-1}) for the photolysis of bioconjugates **7a**,**b** and **8a-d** at different wavelengths in methanol/HEPES buffer (80:20) solution.

Figure 1. Plot of ln A *vs* irradiation time for the photolysis of bioconjugates **7a** (+),**7b** (□), **8a** (Δ), **8b** (×), **8c** (◊) and **8d** (○) at 254 nm in methanol/HEPES buffer (80:20) solution.

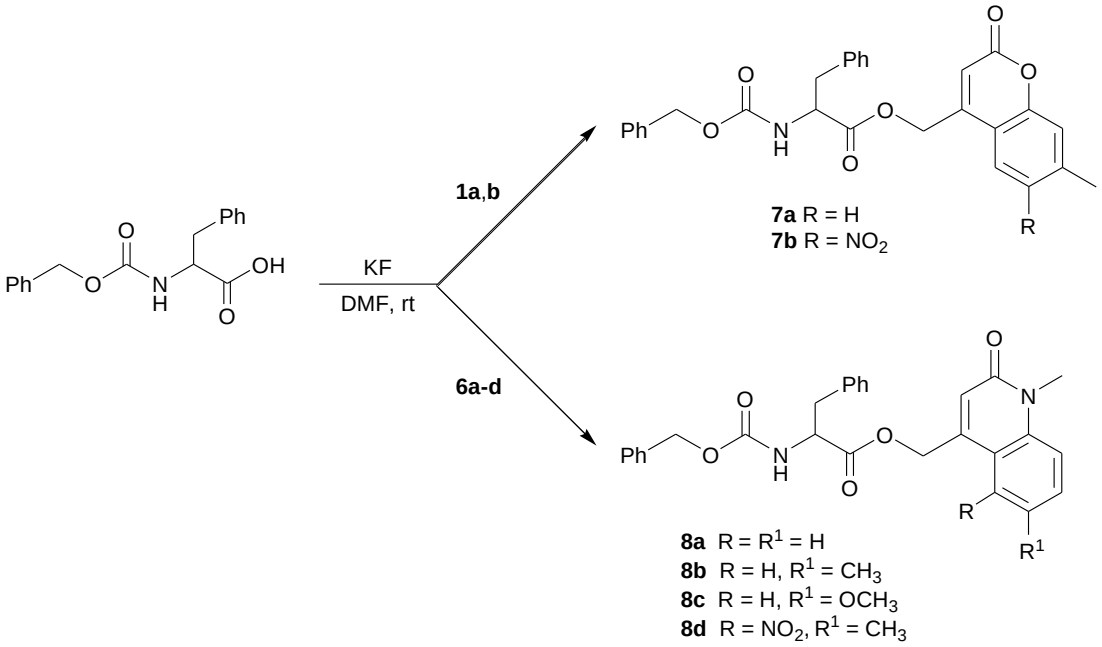
Figure 2. ^{1}H NMR spectra in methanol-d_4/D_2O (80:20) of the photolysis of conjugate **8b** (C = 8.8×10^{-3} M) at 350 nm: (a) before irradiation; (b) after irradiation for 15 min; (c) after irradiation for 95 min; (d) Z-Phe-OH.

SCHEMES

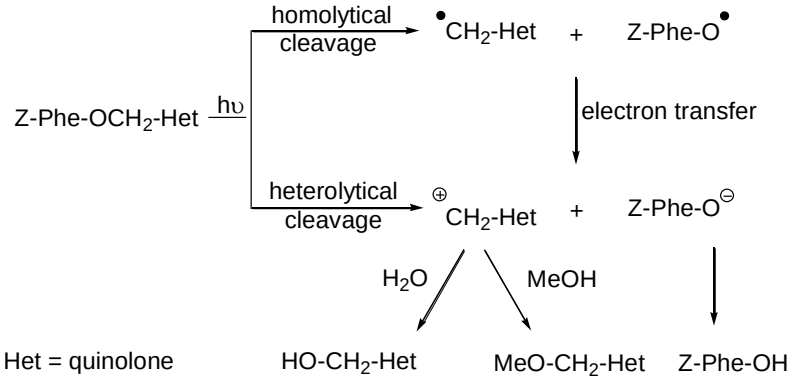
Scheme 1



Scheme 2



Scheme 3



TABLES

Table 1

		UV-vis		Fluorescence		
Compound	Yield (%)	λ_{max} (nm)	log ε	λ_{em} (nm)	Φ_F	Stokes' shift (nm)
1a	90	314	3.83	397	0.03	83
1b	92	320	3.61	384	0.02	64
3a	66	329	3.62	383	0.13	54
3b	77	337	3.49	377	0.12	40
3c	71	351	3.90	400	0.05	49
4a	74	330	3.78	404	0.11	74
4b	89	338	3.72	389	0.06	51
4c	62	341	3.69	395	0.02	54
5a	67	328	3.72	384	0.14	56
5b	84	335	3.51	388	0.13	53
5c	97	352	3.60	410	0.06	58
6a	79	333	3.78	386	0.05	53
6b	82	343	3.48	396	0.05	53
6c	52	358	3.38	408	0.01	50
6d	58	343	3.61	423	0.02	80
7a	84	317	3.92	397	0.03	80
7b	70	316	3.58	401	0.02	85
8a	91	330	3.68	385	0.10	55
8b	56	339	3.71	396	0.07	57
8c	62	354	3.70	418	0.05	64
8d	72	341	3.86	414	0.01	73

Table 2

Compound	254 nm		300 nm		350 nm		419 nm	
	Irr time	*k*	Irr time	*k*	Irr time	*k*	Irr time	*k*
7a	34	0.082	129	0.023	3293	0.001	---	---
7b	18	0.166	176	0.016	1317	0.002	---	---
8a	9	0.321	8	0.384	69	0.043	---	---
8b	2	1.279	2	1.665	4	0.754	193	0.015
8c	0.8	3.227	1.2	2.274	0.6	9.347	34	0.086
8d	25	0.122	85	0.035	144	0.021	---	---

FIGURES

Figure 1

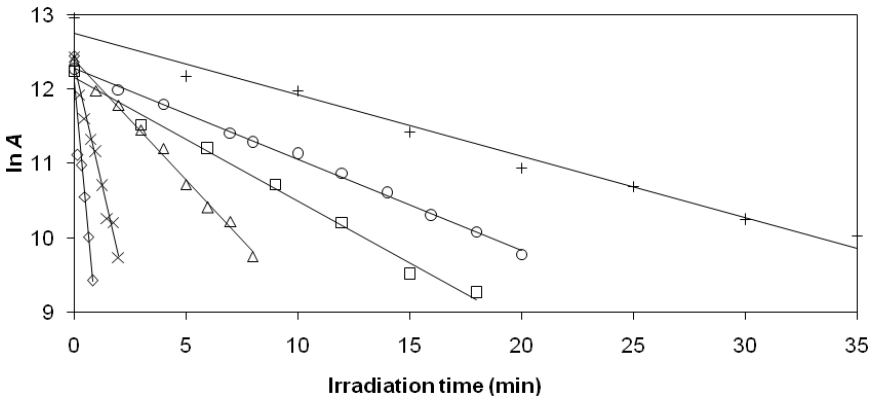


Figure 2