Supporting Information

Self-immolative Versatile Fluorogenic Probes for Screening of Hydrolytic Enzymes Activity

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Materials and methods

¹H- and ¹³C-NMR spectra were recorded in CDCl₃ solution with Bruker 400 MHz spectrometer. Chemical shifts are expressed in parts per million using TMS as an internal standard. TLC was done on Kieselgel 60 F₂₅₄ aluminum sheets. Fluorescence spectra and fluorescence time profiles were recorded at room temperature in quartz cuvettes with an F-7000 spectrofluorometer (Shimadzu, Kyoto, Japan). Commercial enzymes were purchased from Sigma–Aldrich. All chemicals were commercial products of analytical grade.

Fluorogenic Enzyme Assays Procedure

Substrates were conditioned as 5mM stock solution in acetonitrile, and tested with series of lipases, esterases and proteases. Assays were initiated by addition of 40 μ L of substrate solution to a solution of enzyme (50 μ g) in a phosphate buffer saline (2 mL, 100 mM, pH 7.4). Fluorescence time profiles were recorded in cuvettes by SHIMAZU F7000 Spectrofluorometer. The fluorescence data were acquired over one hour in each experiment, which was sufficient for completion of the reaction with more reactive enzymes. Fluorescence data were converted to umbelliferone concentration by means of a calibration curve. The linear portion of each curve was used to generate the reaction rates.

Standard procedure

CAUTION: Phosgene (b.p. 88°C) is highly toxic and corrosive, and reacts violently with many nucleophiles. Excess phosgene (formed by the amine-mediated decomposition of triphosgene) was caught in the primary liquid nitrogen trap and destroyed when still cold in CH₂Cl₂ by dropwise addition to a vigorously stirred, cold mixture of 2-aminoethanol or piperidine (5 mL) and ethanol (30 mL) in dichloromethane (100 mL) in a well-ventilated hood.

Ethyl-(2-oxo-4-methyl-2H-chromen-7-yl) carbonate (5)



Compound **5** was obtained according to a literature procedure.[1] ¹HNMR (CDCl₃, 400 MHz, ppm): δ 7.60 (d, *J*=8.8 Hz, 1H), 7.21–7.14 (m, 2H), 6.26 (d, *J*=1.2 Hz, 1H), 4.35 (q, *J*=7.2 Hz, 2H), 2.42 (d, *J*=1.2 Hz,

3H), 1.40 (t, *J*=7.2 Hz, 3H); ¹³CNMR (CDCl₃, 100 MHz, ppm): δ 160.4, 154.1, 153.2, 152.7, 151.8, 125.4, 117.9, 117.4, 114.6, 109.9, 65.3, 18.7, 14.1; Element. Anal. calc. for C₁₃H₁₂O₅: C 62.90, H 4.87; found: C 62.76, H 4.79.





General procedure for synthesis of 2{[(benzyloxy)carbonyl]amino}ethyl 4-methyl-2-oxo-2*H*chromen-7-yl carbonate (6)



Benzyl chloroformate (0.42 mL, 3 mmol) and then Et_3N (0.63 mL, 4.5 mmol) were added dropwise to a stirred solution of aminoethanol (0.2 mL, 3.3 mmol) in CH_2Cl_2 (8 mL) at 0 °C. Then the mixture

was stirred at room temperature for 2 h, the solvent was evaporated and the crude product was purified by column chromatography on silica gel using Hexane/Ethyl acetate (1:1, v/v) as eluent to afford benzyl 2-hydroxyethylcarbamate as a white solid with 50% yield (293 mg).

Then benzyl 2-hydroxyethylcarbamate (195 mg, 1 mmol) was dissolved in anhydrous THF (20 mL) and cooled to 0 °C. Et₃N (0.42 mL, 3 mmol) and phosgene (3,6 mL, 15% solution in toluene) were slowly added to the solution, which was stirred for 3 hours at room temperature under argon atmosphere. The excess of phosgene was evaporated and the resulted mixture was cooled to 0 °C. A mixture of DMAP (19.5 mg, 20% by weight), Et₃N (0.42 mL, 3 mmol), and 7-hydroxy-4-methyl- 2H-chromen-2-one (352 mg, 2 mmol) in anhydrous THF (4 mL) was slowly added to the solution. The mixture was stirred for 12 h under argon atmosphere. Then the solvent was evaporated and crude product was purified by column chromatography on silica gel (CH₂Cl₂/MeOH, 98:2, v/v) to afford 6 as a colorless oil: yield 61% (141.4 mg).

¹HNMR (CDCl₃, 400 MHz, ppm) δ 7.60 (d, 1H, *J*= 8.56 Hz), 7.37-7.28 (m, 5H), 7.21-7.14 (m, 2H), 6.27 (d, 1H, *J*=1.22 Hz), 5.30 (m, 1H), 5.13 (s, 1H), 4.37-4.34 (m, 2H), 3.59 (q, *J*=5.22 Hz), 2.42 (d, 3H, *J*=1.22

Hz); ¹³CNMR (CDCl₃, 100 MHz, ppm) δ 160.3, 154.2, 153.1, 152.7, 151.8, 136.2, 128.6, 128.5, 128.2, 128.1, 125.5, 118.1, 117.3, 114.7, 109.9, 68.0, 67.0, 40.0, 18.7; Element. Anal. calc. for C₂₁H₁₉NO₇: C 63.47, H 4.82, N 3.53; found: C 63.17, H 4.37, N 3.49.



2[(tert-butoxycarbonyl)amino]ethyl 4-methyl-2-oxo-2H-chromen-7-yl carbonate (7)



The general procedure was used starting with aminoethanol (0.2 mL, 3.0 mmol) and di-*tert*-butyl-dicarbonate (720 mg, 3.3 mmol) afforded 2-(N-tert-butoxycarbonylamino)ethanol as a white solid with 98%

yield (473 mg). The next reaction step with 7-hydroxy-4-methyl-2H-chromen-2-one (352 mg, 2 mmol) afforded 7 as a colorless oil with 48% yield (173.6 mg).

¹HNMR (CDCl₃, 400 MHz, ppm) δ 7.26 (d, 1H, *J*=8.56 Hz), 7.23 (d, 1H, *J*=2.20 Hz), 7.18 (dd, 1H, *J*₁=8.68 Hz, *J*₂=2.32 Hz), 6.28 (d, 1H, *J*=1.22 Hz), 4.79-5.03 (m, 1H), 4.34 (t, 3H, *J*=5.26 Hz), 3.51 (d, 2H, *J*=5.14 Hz), 2.44 (d, 4H, *J*=1.22), 1.47 (s, 9H); ¹³CNMR (CDCl₃, 100 MHz, ppm) δ 160.3, 154.2, 153.2, 152.7, 151.7, 125.5, 118.1, 117.3, 114.7, 109.9, 68.3, 61.3, 28.4, 18.7; Element. Anal. calc. for C₁₈H₂₁NO₇: C 59.50, H 5.83, N 3.85; found: C 59.36, H 5.94, N 3.77.



4-methyl-2-oxo-2H-chromen-7-yl 2{[(prop-2en-1-yloxy)carbonyl]amino}ethyl carbonate (8)



The general procedure was used starting with aminoethanol (0.2 mL, 3.0 mmol) and allil chloroformate (0.32 mL, 3.3 mmol) afforded N-Allocethanolamine as a white solid with 86% yield (374

mg). The next reaction step with 7-hydroxy-4-methyl-2H-chromen-2-one (352 mg, 2 mmol) afforded **8** as a colorless oil with 39% yield (134.8 mg).

¹HNMR (CDCl₃, 400 MHz, ppm) δ 7.62 (d, 1H, *J*=8.56 Hz), 7.22 (d, 1H, *J*=2.45 Hz), 7.17 (dd, 1H, *J*₁=8.68 Hz, *J*₂=2.32 Hz), 6.28 (d, 1H, *J*=1.22 Hz), 5.86-6.07 (m, 1H), 5.27-5.40 (m, 1H), 5.23 (dd, 1H, *J*₁=10.51 Hz, *J*₂=1.22 Hz), 5.16 (br. s., 1H), 4.60 (d, 2H, *J*=5.38 Hz), 4.37 (t, 2H, *J*=5.26 Hz), 3.58 (q, 2H, *J*=5.54 Hz), 2.44 (d, 3H, *J*=1.22 Hz); ¹³CNMR (CDCl₃, 100 MHz, ppm) δ 160.3, 156.2, 154.2, 153.1, 152.7, 151.7, 132.6, 125.5, 118.1, 117.9, 117.3, 114.7, 109.9, 68.0, 65.8, 39.9, 18.7; Element. Anal. calc. for C₁₇H₁₇NO₇: C 58.79, H 4.93, N 4.03; found: C 58.94, H 5.05, N 4.04.





References

[1] A. Zadlo, D. Koszelewski, F. Borys, R. Ostaszewski, ChemBioChem, 2015, 16, 677-682.