

Design and synthesis of epicocconone analogues with improved fluorescence properties

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I) Experimental Section

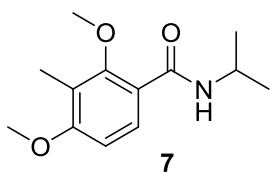
General

All reactions were carried out under a nitrogen or argon atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Dry tetrahydrofuran (THF), diethyl ether (Et₂O), methylene chloride (CH₂Cl₂), and toluene were obtained by distillation (from CaH₂ for CH₂Cl₂, sodium/benzophenone for THF, from sodium for toluene and, from sodium benzophenone for Et₂O). Methanol (MeOH), *N,N*-dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were purchased in anhydrous form and used without further purification. Ethyl acetate (EtOAc), diethyl ether (Et₂O), methylene chloride (CH₂Cl₂), and cyclohexane were purchased at ACS grade quality and used without further purification, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (¹H NMR and ¹³C NMR) homogeneous materials, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on Merck silica gel plates with QF-254 indicator and an ethanolic solution of ammonium molybdate or potassium permanganate and heat as developing agents. E. Merck silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. NMR spectra were recorded on Bruker Advance DMX-300 or -200 instruments and calibrated using residual undeuterated solvent as an internal reference (7.26 ppm and 77.16 ppm for ¹H and ¹³C NMR in CDCl₃; 1.94 ppm and 118.26 ppm for ¹H and ¹³C NMR in CD₃CN). The following abbreviations were used to describe the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, pent = pentet, hex = hexet, br = broad. IR spectra were recorded on a Perkin-Elmer Spectrum One FTIR spectrometer with diamond ATR accessory. High resolution MS data (ESI) was obtained using an Agilent ESI TOF mass spectrometer at 3500 V emitter voltage. Elemental analyses were obtained on a Carlo-Erba 1106 analyzer and Mass Spectra on a HP5890 (electronic impact 70eV) using GC-MS coupling with a Jeol AX500. UV-visible absorption spectra were obtained on a Varian Cary 50 scan spectrophotometer using a rectangular quartz cell (Varian, standard cell, Open Top, 10 × 10 mm, 3.5 mL). Fluorescence spectroscopic studies were performed with a Varian Cary Eclipse spectrophotometer using a semi-micro quartz fluorescence cell (Hellma, 104F-QS, 10 × 4 mm, 1400 μL).

Synthesis of 6,8-bis(methoxymethoxy)-3,3,7-trimethylisochroman-1-one (6):

First strategy:

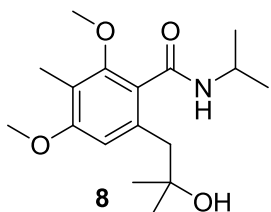
***N*-isopropyl-2,4-dimethoxy-3-methylbenzamide (7):** To a stirred solution of dimethoxytoluene 2



(3.04 g, 20 mmol) in CH₂Cl₂ (200 mL) at room temperature was added aluminum trichloride (AlCl₃, 2.80 g, 21 mmol). The resulting mixture was stirred for 10 min. before isopropylisocyanate (2.36 mL, 25 mmol) was added dropwise. The reaction mixture was stirred for 3h before it was quenched at 0

°C with NH₄Cl (150 mL, sat. aq.). The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (2 x 150 mL). The combined organic layers were washed with HCl (100 mL, 1.0 N aq.), NaHCO₃ (100 mL, sat. aq.), dried (MgSO₄), and concentrated in *vacuo*. Flash column chromatography (silica gel, cyclohexane:EtOAc 4:1) afforded amide 7 (4.5 g, 95%) as a white solid, m.p. 45 °C. IR (neat): $\nu = 3285, 3075, 2969, 2840, 1636 \text{ cm}^{-1}$. ¹H NMR (300 MHz, CDCl₃): $\delta = 7.94$ (d, $J = 8.8$ Hz, 1H), 7.68 (d, $J = 5.6$ Hz, 1H, NH), 6.72 (d, $J = 8.7$ Hz, 1H), 4.26-4.37 (m, 1H), 3.86 (s, 3H), 3.73 (s, 3H), 2.15 (s, 3H), 1.25 (d, $J = 6.5$ Hz, 6H) ppm. ¹³C NMR (75 MHz, CDCl₃): $\delta = 164.6, 161.1, 157.3, 130.0, 119.6, 119.2, 106.5, 61.3, 55.8, 41.2, 23.0, 8.9$ ppm. MS (ESI): $m/z = 238$ [M + H]⁺. Found: C, 65.85; H, 8.04; N, 5.87. C₁₃H₁₉NO₃ requires C, 65.80; H, 8.07; N, 5.90.

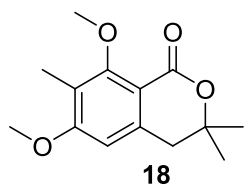
6-(2-hydroxy-2-methylpropyl)-*N*-isopropyl-2,4-dimethoxy-3-methylbenzamide (8): To a stirred



solution of amide 7 (11.85 g, 50 mmol) in THF (200 mL) at room temperature was added TMEDA (15.0 mL, 100 mmol). *t*-BuLi (1.7 M in hexane, 65 mL, 110 mmol) was then slowly added at -78 °C and the reaction mixture was further stirred for 30 min at -15 °C The resulting reaction mixture was then recooled to -78 °C before 2,2-dimethyloxirane (9.9 mL, 110 mmol) was slowly added. The

reaction mixture was finally warmed to room temperature and stirred for 1h30 before it was quenched with H₂O (150 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 150 mL). The combined organic layers were washed with NH₄Cl (2 x 100 mL, sat. aq.), NaHCO₃ (2 x 100 mL, sat. aq.), dried (MgSO₄), and concentrated in *vacuo*. Flash column chromatography (silica gel, cyclohexane:EtOAc 7:3) afforded amido-alcohol 8 (14.3 g, 92%) as a white solid, m.p. 153 °C. IR (neat): $\nu = 3212, 2971, 1619, 1579, 1463, 1402, 1188, 1122 \text{ cm}^{-1}$. ¹H NMR (300 MHz, CDCl₃): $\delta = 6.48$ (s, 1H), 6.26 (d, $J = 7.4$ Hz, 1H, NH), 5.68 (s, 1H, OH), 4.34-4.22 (m, 1H), 3.83 (s, 3H), 3.70 (s, 3H), 2.79 (s, 2H), 2.09 (s, 3H), 1.30 (s, 6H), 1.25 (d, $J = 6.6$ Hz, 6H) ppm. ¹³C NMR (75 MHz, CDCl₃): $\delta = 167.8, 158.9, 156.2, 137.2, 123.1, 118.3, 110.0, 69.3, 62.0, 55.8, 46.7, 42.0, 30.4, 22.6, 8.7$ ppm. MS (ESI): $m/z = 310$ [M + H]⁺. Found: C, 66.19; H, 8.78; N, 4.50. C₁₇H₂₇NO₄ requires C, 65.99; H, 8.80; N, 4.53.

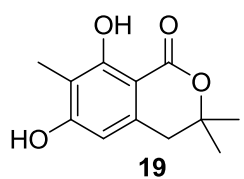
6,8-dimethoxy-3,3,7-trimethyl-3,4-dihydro-1H-isochromen-1-one (18): To a stirred solution of



amido-alcohol **8** (6.36 g, 20.5 mmol) in toluene (200 mL) at room temperature was added camphor sulfonic acid (CSA, 5.86 g, 24.7 mmol). The resulting mixture was heated under reflux for 2h before it was quenched at room temperature with NaHCO₃ (100 mL, sat. aq.). The layers were separated and the

aqueous layer was extracted with EtOAc (2 x 150 mL). The combined organic layers were washed with NaHCO₃ (200 mL, sat. aq.), NH₄Cl (200 mL, sat. aq.), dried (MgSO₄) and concentrated in *vacuo*. Precipitation in cyclohexane (50 mL) afforded lactone **18** (3.91g, 76 %) as a white solid, m.p. 138 °C. IR (neat): $\nu = 2977, 2936, 1710, 1598, 1458, 1300, 1231, 1156, 1127, 1104 \text{ cm}^{-1}$. ¹H NMR (300 MHz, CDCl₃): $\delta = 6.40 \text{ (s, 1H)}, 3.82 \text{ (s, 3H)}, 3.76 \text{ (s, 3H)}, 2.86 \text{ (s, 2H)}, 2.07 \text{ (s, 3H)}, 1.34 \text{ (s, 6H)}$ ppm. ¹³C NMR (75 MHz, CDCl₃): $\delta = 162.4, 162.1, 161.1, 139.1, 120.1, 110.5, 105.3, 79.0, 61.4, 55.7, 40.6, 27.1, 8.5$ ppm. MS (ESI): $m/z = 251 \text{ [M + H]}^+$. Found: C, 67.48; H, 7.54. C₁₄H₁₈O₄ requires C, 67.18; H, 7.25.

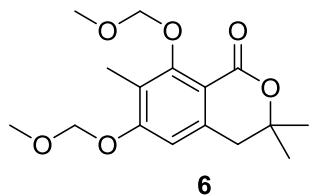
6,8-dihydroxy-3,3,7-trimethyl-3,4-dihydro-1H-isochromen-1-one (19): To a stirred solution of



lactone **18** (5.50 g, 22 mmol) in CH₂Cl₂ (200 mL) at room temperature was added AlCl₃ (30.88 g, 220 mmol). The resulting mixture was heated under reflux for 20 h before it was quenched at room temperature with HCl (200 mL, 1.0 N aq.). The resulting mixture was diluted with EtOAc (400 mL), the layers were separated and

the aqueous layer was extracted with EtOAc (2 x 300 mL). The combined organic layers were washed with brine (400 mL, sat. aq.), dried (MgSO₄) and concentrated in *vacuo*. Precipitation in CH₂Cl₂ (5 mL) afforded diphenol **19** (4.84 g, 99%) as a white solid, m.p. 204 °C. IR (neat): $\nu = 3356, 1622, 1436, 1361, 1104 \text{ cm}^{-1}$. ¹H NMR (300 MHz, CD₃CN): $\delta = 11.60 \text{ (s, 1H, OH)}, 7.76 \text{ (s, 1H, OH)}, 6.26 \text{ (s, 1H)}, 2.91 \text{ (s, 2H)}, 2.01 \text{ (s, 3H)}, 1.40 \text{ (s, 6H)}$ ppm. ¹³C NMR (75 MHz, CD₃CN): $\delta = 170.6, 162.7, 162.3, 138.7, 110.2, 107.1, 101.3, 82.5, 39.1, 27.2, 7.7$ ppm. MS (ESI): $m/z = 221 \text{ [M - H]}^-$. Found: C, 64.80; H, 6.45. C₁₂H₁₄O₄ requires C, 64.85; H, 6.35.

6,8-bis(methoxymethoxy)-3,3,7-trimethylisochroman-1-one (6): To a stirred solution of diphenol **20**



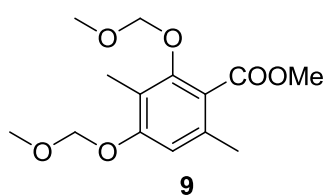
(3.4 g, 15.3 mmol) and NaH (95%, 1.546 g, 31.2 mmol) in THF (250 mL) at room temperature was added MOM-Cl (2.1M in toluene, 36 mL, 76.5 mmol)¹. The resulting mixture was stirred for 2h before it was quenched with NH₄Cl (200 mL, sat. aq.). The layers were separated and the aqueous layer

¹ M. A. Berliner, K. Belecki, *J. Org. Chem.* **2005**, *70*, 9618-9621

was extracted with EtOAc (2 x 200 mL). The combined organic layers were dried (MgSO₄) and concentrated *in vacuo* to afford lactone **6** (4.75 g, quant.) as pale yellow solid, which was used without further purification, m.p. 76 °C. IR (neat): $\nu = 2928, 2110, 1700, 1596, 1474, 1291, 1149, 1108, 1047, 972, 917 \text{ cm}^{-1}$. ¹H NMR (300 MHz, CDCl₃): $\delta = 6.69 \text{ (s, 1H)}, 5.25 \text{ (s, 2H)}, 5.10 \text{ (s, 2H)}, 3.61 \text{ (s, 3H)}, 3.49 \text{ (s, 3H)}, 2.92 \text{ (s, 2H)}, 2.22 \text{ (s, 3H)}, 1.41 \text{ (s, 6H)}$ ppm. ¹³C NMR (75 MHz, CDCl₃): $\delta = 162.6, 160.0, 159.3, 138.9, 121.4, 111.2, 108.7, 101.6, 94.3, 79.4, 57.7, 56.5, 40.7, 27.3, 9.6$ ppm. MS (ESI): $m/z = 311 \text{ [M + H]}^+$.

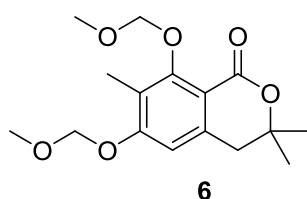
Second strategy:

Methyl 2,4-bis(methoxymethoxy)-3,6-dimethylbenzoate (9): To a stirred solution of methyl atratate



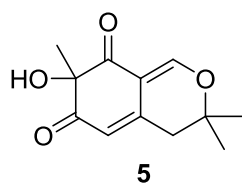
3 (3.92 g, 20 mmol) in THF (200 mL) at 0 °C was added NaH (60%, 2.4 g, 60 mmol). The resulting mixture was stirred at 0 °C for 20 min before MOM-Cl (2.1M in toluene, 28.6 mL, 60 mmol) was slowly added. The resulting mixture was warmed to room temperature and stirred for 3h before it was quenched with NH₄Cl (200 mL, sat. aq.). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 200 mL). The combined organic layers were washed with NaHCO₃ (200 mL, sat. aq.), dried (Na₂SO₄) and concentrated *in vacuo*. Flash column chromatography (silica gel, cyclohexane:EtOAc 9:1) afforded ester **9** (4.95 g, 87%) as colorless oil. IR (neat): $\nu = 2956, 1724, 1605, 1579, 1437, 1273, 1144, 1114, 1042, 978 \text{ cm}^{-1}$. ¹H NMR (300 MHz, CDCl₃): $\delta = 6.72 \text{ (s, 1H)}, 5.19 \text{ (s, 2H)}, 4.96 \text{ (s, 2H)}, 3.89 \text{ (s, 3H)}, 3.54 \text{ (s, 3H)}, 3.47 \text{ (s, 3H)}, 2.28 \text{ (s, 3H)}, 2.15 \text{ (s, 3H)}$ ppm. ¹³C NMR (75 MHz, CDCl₃): $\delta = 168.5, 156.8, 153.9, 134.6, 122.2, 118.3, 111.5, 100.1, 94.2, 57.3, 55.9, 51.8, 19.8, 9.3$ ppm. MS (ESI): $m/z = 285 \text{ [M + H]}^+$.

6,8-bis(methoxymethoxy)-3,3,7-trimethylisochroman-1-one (6): To a stirred solution of DIPA (1.7



mL, 12 mmol) in THF (100 mL) at -78 °C was slowly added *n*-BuLi (2.5 M in hexane, 4.8 mL, 12 mmol). The resulting mixture was warmed to 0 °C and stirred for 30 min before it was cooled to -78 °C and a solution of ester **9** (3.41 g, 12 mmol) in THF (20 mL) was added. The resulting mixture was stirred at -78 °C for 30 min before freshly distilled acetone (0.74 mL, 10 mmol) was added. The resulting mixture was stirred for 5 min before it was quenched at room temperature with HCl (1.0 N, aq.). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 150 mL). The combined organic layers were dried (Na₂SO₄) and concentrated *in vacuo*. Flash column chromatography (silica gel, cyclohexane:EtOAc 8:2) afforded lactone **6** (1.87 g, 60%) as a pale yellow solid.

7-hydroxy-3,3,7-trimethyl-3H-isochromene-6,8(4H,7H)-dione (5): To a stirred solution of lactone **6**



(4.75 g, 15.3 mmol) in toluene (150 mL) at -78 °C was added DIBAL-H (1.2 M in toluene, 16.6 mL, 19.9 mmol). The resulting mixture was stirred at -78 °C for 20 min before it was quenched at -78 °C with H₂O (150 mL). The resulting mixture was diluted with EtOAc (100 mL), the layers were separated and the aqueous

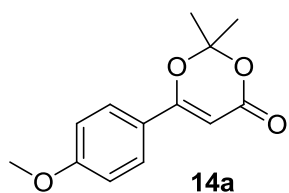
layer was extracted with EtOAc (2 x 200 mL). The combined organic layers were washed with HCl (1.0 N aq., 2 x 200 mL), brine (2 x 200 mL, sat. aq.), dried (MgSO₄) and concentrated in *vacuo* to afford the corresponding reduced lactol **10** (4.78 g, 100 %), as a light yellow oil, which was immediately used without further purification.

To a stirred solution of lactol **10** (312 mg, 1 mmol) in CH₂Cl₂ (10 mL) at room temperature were added TFA (1.50 mL, 7 mmol), water (0.36 mL, 20 mmol) and IBX (560 mg, 2 mmol). The resulting mixture was stirred for 3h before it was filtered to separate the IBX residue and the filtrate was concentrated in *vacuo*. Flash column chromatography (silica gel, cyclohexane:EtOAc 4:1 to 2:3) afforded alcohol **5** (152.6 mg, 68%) as a brown oil. IR (neat): $\nu = 3433, 2979, 2932, 1706, 1660, 1615, 1568, 1287, 1269, 1179, 1091 \text{ cm}^{-1}$. ¹H NMR (300 MHz, CDCl₃): $\delta = 7.75$ (s, 1H), 5.78 (s, 1H), 4.01 (brs, 1H, OH), 2.66 (s, 2H), 1.49 (s, 3H), 1.37 (s, 6H) ppm. ¹³C NMR (75 MHz, CDCl₃): $\delta = 197.6, 195.1, 159.1, 146.9, 116.6, 109.6, 83.1, 81.0, 39.6, 28.8, 26.5, 25.9$ ppm. MS (ESI): $m/z = 223$ [M + H]⁺. HRMS (ESI): calcd. for [C₁₂H₁₄O₄ + H]⁺ 223.0970; found 223.0968.

General procedure for the synthesis of dioxin-4-ones 14a-d (Procedure A)²: To a stirred solution of *tert*-butyl ester (1.0 equiv, prepared by addition lithium enolate of *tert*-butyl acetate on the corresponding Weinreb amide) in acetone (10.0 equiv) at 0 °C were added acetic anhydride (15.0 equiv) and sulfuric acid (1.0 equiv). The resulting mixture was warmed to room temperature and stirred for 45 min before it was quenched with Na₂CO₃ (30.0 equiv, aq.). The resulting mixture was diluted with EtOAc (100 mL) and stirred for 40 min (hydrolysis of the remaining acetic anhydride). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 200 mL). The combined organic layers were washed with NH₄Cl (2 x 200 mL, sat. aq.), dried (MgSO₄) and concentrated in *vacuo*. Flash column chromatography (silica gel, cyclohexane:EtOAc 8:2) afforded the desired dioxinone **14a-d**.

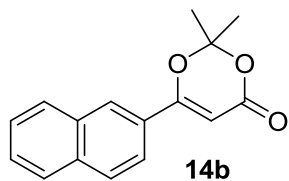
² P. A. Peixoto, A. Boulangé, S. Leleu, X. Franck, *Eur J. Org. Chem.* **2013**, 3316-3327.

R=pOMePh: 2,2-dimethyl-6-(4-methoxyphenyl)-4H-1,3-dioxin-4-one (14a): Following procedure



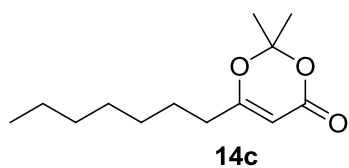
A, *tert*-butyl 3-(4-methoxyphenyl)-3-oxopropanoate (4.66 g, 18.6 mmol) was used to afford dioxinone **14a** (3.57 g, 82%) as a yellow solid, m.p. 68 °C. IR (neat): $\nu = 2999, 2940, 2842, 1721, 1607, 1513, 1363, 1252, 1179, 1027, 804$ cm^{-1} . ^1H NMR (300 MHz, CDCl_3): $\delta = 7.68$ (d, $J = 9.0$ Hz, 2H), 6.92 (d, $J = 9.0$ Hz, 2H), 5.77 (s, 1H), 3.84 (s, 3H), 1.77 (s, 6H) ppm. ^{13}C NMR (75 MHz, CDCl_3): $\delta = 165.0, 162.9, 162.3, 128.3, 123.4, 114.3, 106.4, 89.4, 55.6, 25.1$ ppm. MS (ESI): $m/z = 235$ $[\text{M} + \text{H}]^+$. HRMS (ESI): calcd. for $[\text{C}_{13}\text{H}_{14}\text{O}_4 + \text{H}]^+$ 235.0970; found 235.0976.

R=2-naphtyl: 2,2-dimethyl-6-(naphthalen-2-yl)-4H-1,3-dioxin-4-one (14b): Following procedure A,



tert-butyl 3-(naphthalen-2-yl)-3-oxopropanoate (4.85 g, 17.9 mmol) was used to afford dioxinone **14b** (3.56 g, 78%) as a yellow solid, m.p. 129 °C. IR (neat): $\nu = 1721, 1615, 1374, 1336, 1278, 1253, 1204, 1040, 996, 903, 809$ cm^{-1} . ^1H NMR (300 MHz, CDCl_3): $\delta = 8.21$ (s, 1H), 7.92-7.84 (m, 3H), 7.67 (dd, $J = 1.5, 8.7$ Hz, 1H), 7.60-7.51 (m, 2H), 6.02 (s, 1H), 1.85 (s, 6H) ppm. ^{13}C NMR (75 MHz, CDCl_3): $\delta = 164.9, 162.0, 134.9, 132.7, 129.1, 128.7, 128.3, 128.2, 127.8, 127.2, 127.1, 122.4, 106.8, 91.7, 25.1$ ppm. MS (ESI): $m/z = 255$ $[\text{M} + \text{H}]^+$. HRMS (ESI): calcd for $[\text{C}_{16}\text{H}_{14}\text{O}_3 + \text{H}]^+$ 255.1021; found 255.1014.

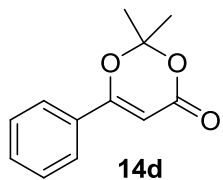
R=*n*-heptyl: 6-heptyl-2,2-dimethyl-4H-1,3-dioxin-4-one (14c): Following procedure A, *tert*-butyl 3-



oxodecanoate (3.79 g, 15.7 mmol) was used to afford dioxinone **14c** (2.52 g, 71%) as a yellow oil. IR (neat): $\nu = 2999, 2929, 2858, 1732, 1634, 1463, 1392, 1463, 1392, 1271, 1205, 1013, 901, 806$ cm^{-1} . ^1H NMR (300

MHz, CDCl_3): $\delta = 5.16$ (s, 1H), 2.15 (t, $J = 7.5$ Hz, 2H), 1.62 (s, 6H), 1.48-1.45 (m, 2H), 1.23-1.22 (m, 8H), 0.84-0.80 (m, 3H) ppm. ^{13}C NMR (75 MHz, CDCl_3): $\delta = 172.2, 161.4, 106.2, 93.1, 33.6, 31.6, 28.9$ (2C), 25.7, 25.0, 22.6, 14.0 ppm. MS (ESI): $m/z = 227$ $[\text{M} + \text{H}]^+$.

R=Ph: 2,2-dimethyl-6-phenyl-4H-1,3-dioxin-4-one (14d): Following procedure A, *tert*-butyl 3-oxo-

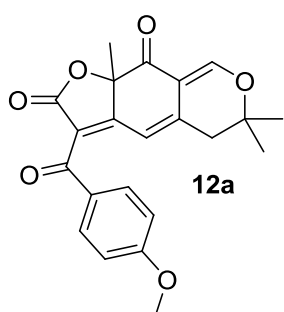


3-phenylpropanoate (4.27 g, 19.4 mmol) was used to afford dioxinone **14d** (2.89 g, 73%) as a white solid, m.p. 66 °C. IR (neat): $\nu = 2998, 1727, 1450, 1389, 1363, 1279, 1225, 1204, 990, 770, 691$ cm^{-1} . ^1H NMR (300 MHz, CDCl_3): $\delta = 7.67$ (d, $J = 7.4$ Hz, 2H), 7.52-7.40 (m, 3H), 5.87 (s, 1H), 1.78 (s, 6H) ppm. ^{13}C NMR (75 MHz,

CDCl₃): δ = 165.0, 161.9, 132.1, 131.0, 128.8, 126.4, 106.7, 91.2, 25.0 ppm. HRMS (ESI): calcd. for [C₁₂H₁₂O₃ + H]⁺ 205.0865; found 205.0869.

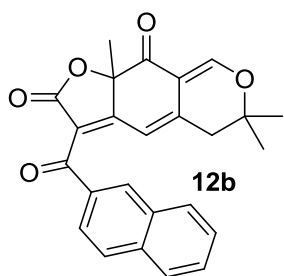
General method for the synthesis of acylfuranones 12a-d (method B): To a stirred solution of alcohol **5** (1.0 equiv), dioxinone **14a-d** (1.5 equiv) and molecular sieves (4 Å, 200 mg per mmol) in toluene (0.1 mol.L⁻¹) at room temperature was added Et₃N (2.0 equiv). The resulting mixture was heated at 110 °C for 3 h before it was quenched at room temperature with HCl (1.0 N, aq.). The layers were separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were dried (MgSO₄) and concentrated in *vacuo*. Flash column chromatography (silica gel, cyclohexane:EtOAc 8:2) afforded the desired acylfuranone **12a-d**.

R=pOMePh: 3-(4-methoxybenzoyl)-6,6,9a-trimethyl-5,6-dihydro-2H-furo[3,2-g]isochromene-2,9(9aH)-dione (12a): Following method B, alcohol **5** (109 mg, 0.49 mmol) and dioxinone **14a** (172



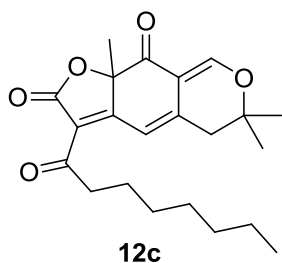
mg, 0.74 mmol) were used to afford acylfuranone **12a** (161 mg, 86%) as a yellow solid. ¹H NMR (300 MHz, CDCl₃): δ = 7.83 (d, *J* = 8.9 Hz, 2H), 7.74 (s, 1H), 6.93 (d, *J* = 9.1 Hz, 2H), 6.47 (s, 1H), 5.29 (s, CH₂Cl₂ residual), 3.86 (s, 3H), 2.68 (d, *J* = 16.6 Hz, 1H), 2.61 (d, *J* = 16.6 Hz, 1H), 1.76 (s, 3H), 1.42 (s, 3H), 1.36 (s, 3H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 189.8, 187.2, 169.3, 168.5, 164.4, 158.8, 141.4, 132.3, 129.4, 119.9, 113.8, 111.4, 110.6, 87.0, 81.2, 55.7, 39.8, 27.8, 26.4, 26.2 ppm. MS (IC): *m/z* = 381 [M + H]⁺. HRMS (ESI): calcd. for [C₂₂H₂₀O₆ + H]⁺ 381.1338; found 381.1343.

R=2-naphthyl: 3-(2-naphthoyl)-6,6,9a-trimethyl-5,6-dihydro-2H-furo[3,2-g]isochromene-2,9(9aH)-dione (12b): Following method B, alcohol **5** (60 mg, 0.27 mmol) and dioxinone **14b** (104 mg, 0.41



mmol) were used to afford acylfuranone **12b** (69 mg, 64%) as a yellow solid. ¹H NMR (300 MHz, CDCl₃): δ = 8.34 (s, 1H), 7.95-7.76 (m, 4H), 7.61 (s, 1H), 7.59-7.49 (m, 2H), 6.54 (s, 1H), 2.65 (q, *J* = 16.6 Hz, 2H), 1.82 (s, 3H), 1.43 (s, 3H), 1.37 (s, 3H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 189.9, 188.9, 170.1, 168.4, 159.2, 142.0, 136.1, 133.9, 132.4 (2C), 130.0, 129.1, 128.5, 128.0, 127.0, 124.7, 119.6, 111.5, 110.7, 87.1, 81.3, 39.9, 28.0, 26.4, 26.3 ppm. MS (ESI): *m/z* = 401 [M + H]⁺. HRMS (ESI): calcd. for [C₂₅H₂₀O₅ + H]⁺ 401.1389; found 401.1384.

R=n-heptyl: 6,6,9a-trimethyl-3-octanoyl-5,6-dihydro-2H-furo[3,2-g]isochromene-2,9(9aH)-dione

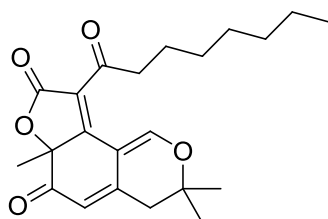


12c

(**12c**): Following method B, alcohol **5** (67 mg, 0.30 mmol) and dioxinone **14c** (102 mg, 0.45 mmol) were used to afford acylfuranone **12c** (22 mg, 20%) as a yellow oil. IR (neat): $\nu = 2927, 2856, 1756, 1714, 1681, 1593, 1556, 1455, 1415, 1374, 1265, 1178, 1090, 1017 \text{ cm}^{-1}$. $^1\text{H NMR}$ (300 MHz, CDCl_3): $\delta = 7.75$ (s, 1H), 7.04 (s, 1H), 3.00 (ddd, $J = 17.5, 14.7, 7.3 \text{ Hz}$, 1H), 2.92 (ddd, $J = 17.7, 14.7, 7.2 \text{ Hz}$, 1H), 2.75 (d, $J = 16.6 \text{ Hz}$, 1H), 2.67 (d, $J = 16.6 \text{ Hz}$, 1H),

1.69 (s, 3H), 1.63 - 1.57 (m, 2H), 1.45 (s, 3H), 1.38 (s, 3H), 1.31 - 1.25 (m, 8H), 0.87 (t, $J = 6.7 \text{ Hz}$, 3H) ppm. $^{13}\text{C NMR}$ (75 MHz, CDCl_3): $\delta = 197.4, 189.8, 171.4, 169.0, 159.2, 143.4, 117.4, 112.9, 110.7, 86.4, 81.4, 42.1, 40.0, 31.8, 29.8, 29.3, 28.1, 26.4, 26.3, 23.6, 22.8, 14.3 \text{ ppm}$. MS (ESI): $m/z = 373$ [$\text{M} + \text{H}$] $^+$. HRMS (ESI): calcd. for $[\text{C}_{22}\text{H}_{28}\text{O}_5 + \text{H}]^+$ 373.2015; found 373.2018.

R=n-heptyl: 3,3,6a-trimethyl-9-octanoyl-3H-furo[2,3-h]isochromene-6,8(4H,6aH)-dione (13c):

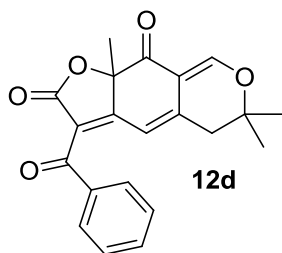


13c

Isolated along with compound **12c** to give acylfuranone **13c** (34 mg, 30%) as a yellow solid. IR (neat): $\nu = 2931, 2857, 1760, 1694, 1606, 1557, 1456, 1375, 1302, 1253, 1175, 1100, 1026 \text{ cm}^{-1}$. $^1\text{H NMR}$ (300 MHz, CDCl_3): $\delta = 8.72$ (s, 1H), 5.58 (s, 1H), 3.09 (ddd, $J = 17.9, 8.7, 6.4 \text{ Hz}$, 1H), 2.84 (ddd, $J = 17.9, 8.5, 6.0 \text{ Hz}$, 1H), 2.75 (d, $J = 17.0 \text{ Hz}$, 1H), 2.64 (d, $J = 17.0 \text{ Hz}$,

1H), 1.66 (s, 3H), 1.60 - 1.53 (m, 2H), 1.46 (s, 3H), 1.40 (s, 3H), 1.26 - 1.23 (m, 8H), 0.84 (t, $J = 7.0 \text{ Hz}$, 3H) ppm. $^{13}\text{C NMR}$ (75 MHz, CDCl_3): $\delta = 197.4, 192.4, 168.9, 167.8, 160.0, 147.6, 119.7, 116.4, 105.2, 87.3, 79.5, 42.1, 40.1, 31.8, 29.2, 29.1, 27.2, 27.1, 25.2, 23.7, 22.7, 14.2 \text{ ppm}$. MS (ESI): $m/z = 373$ [$\text{M} + \text{H}$] $^+$. HRMS (ESI): calcd. for $[\text{C}_{22}\text{H}_{28}\text{O}_5 + \text{H}]^+$ 373.2015; found 373.2012.

R=Ph: 3-benzoyl-6,6,9a-trimethyl-5,6-dihydro-2H-furo[3,2-g]isochromene-2,9(9aH)-dione (12d):



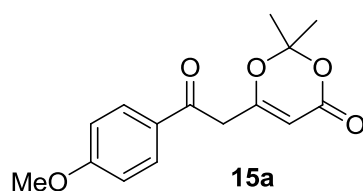
12d

Following method B, alcohol **5** (60 mg, 0.27 mmol) and dioxinone **14d** (82 mg, 0.40 mmol) were used to afford acylfuranone **12d** (56 mg, 60%) as a yellow. $^1\text{H NMR}$ (300 MHz, CDCl_3): $\delta = 7.84$ (d, $J = 7.4 \text{ Hz}$, 2H), 7.77 (s, 1H), 7.60 (t, $J = 7.4 \text{ Hz}$, 1H), 7.46 (t, $J = 7.4 \text{ Hz}$, 2H), 6.54 (s, 1H), 2.71 (d, $J = 16.4 \text{ Hz}$, 1H), 2.63 (d, $J = 16.4 \text{ Hz}$, 1H), 1.78 (s, 3H), 1.44 (s, 3H), 1.38 (s, 3H) ppm. $^{13}\text{C NMR}$ (75 MHz, CDCl_3): $\delta = 189.8, 189.1, 170.4, 168.3, 159.2, 142.1, 136.6, 134.0, 129.8, 128.6,$

$119.3, 111.4, 110.6, 87.0, 81.3, 39.9, 28.0, 26.4, 26.3 \text{ ppm}$. MS (ESI): $m/z = 351$ [$\text{M} + \text{H}$] $^+$. HRMS (ESI): calcd. for $[\text{C}_{21}\text{H}_{18}\text{O}_5 + \text{H}]^+$ 351.1232; found 351.1227.

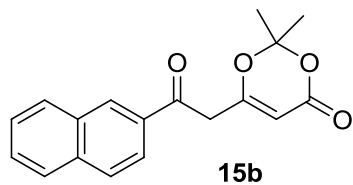
General Procedure for the synthesis of dioxinones 15a-d (Procedure B): To a stirred solution of DIPA (2.0 equiv) in THF (0.1 mol.L⁻¹) at -78 °C was added *n*-BuLi (1.2 M in hexane, 2.0 equiv). The resulting mixture was to 0 °C and stirred for 30 min before it was cooled to -78 °C and 2,2,6-trimethyl-2,4-dihydro-1,3-dioxin-4-one (2.0 equiv) was added. The resulting mixture was stirred at -78 °C for 30 min before the appropriate acid chloride (1.0 equiv) was added. The resulting mixture was warmed to -40 °C and stirred for 2 h before it was quenched at 0 °C with HCl (1.0 N, aq.). The layers were separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were dried (MgSO₄) and concentrated in *vacuo*. Precipitation in a mixture of Et₂O/pentane 1:10 (first Et₂O and then pentane) afforded the desired dioxinone **15a-d**.

Ar=pOMePh: 6-[2-(4-methoxyphenyl)-2-oxoethyl]-2,2-dimethyl-4H-1,3-dioxin-4-one (15a):



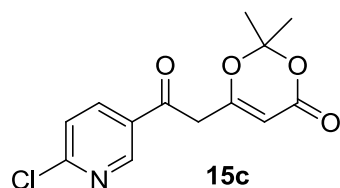
Following procedure B, *p*-methoxybenzoyl chloride (1.86 g, 10.9 mmol) was used to afford dioxinone **15a** (1.24 g, 41%) as a yellow solid, m.p. 82 °C. IR (neat): $\nu = 3000, 2842, 1728, 1682, 1639, 1601, 1575, 1376, 1273, 1173, 1018, 838 \text{ cm}^{-1}$. ¹H NMR (300 MHz, CDCl₃): $\delta = 7.90$ (d, $J = 8.9$ Hz, 2H), 6.94 (d, $J = 8.9$ Hz, 2H), 5.39 (s, 1H), 3.87 (s, 3H), 3.84 (s, 2H), 1.69 (s, 6H) ppm. ¹³C NMR (75 MHz, CDCl₃): $\delta = 191.6, 165.7, 164.3, 160.9, 130.8, 129.0, 114.2, 107.4, 96.9, 55.7, 43.2, 25.1$ ppm. MS (ESI): $m/z = 277$ [M + H]⁺.

Ar=2-naphtyl: 2,2-dimethyl-6-(2-(naphthalen-2-yl)-2-oxoethyl)-4H-1,3-dioxin-4-one (15b):



Following procedure B, 2-naphthoyl chloride (3.85 g, 20.2 mmol) was used to afford dioxinone **15b** (2.57 g, 43 %) as a yellow solid, m.p. 119 °C. IR (neat): $\nu = 2999, 1726, 1677, 1641, 1371, 1315, 1271, 1199, 1179, 1015, 861, 811, 758 \text{ cm}^{-1}$. ¹H NMR (300 MHz, CDCl₃): $\delta = 8.45$ (s, 1H), 8.09-7.89 (m, 4H), 7.68-7.57 (m, 2H), 5.48 (s, 1H), 4.04 (s, 2H), 1.72 (s, 6H) ppm. ¹³C NMR (75 MHz, CDCl₃): $\delta = 193.1, 165.4, 160.9, 136.0, 133.3, 132.5, 130.5, 129.7, 129.2, 129.0, 128.0, 127.3, 123.6, 107.5, 97.2, 43.5, 25.1$ ppm. MS (ESI): $m/z = 295$ [M - H]⁻.

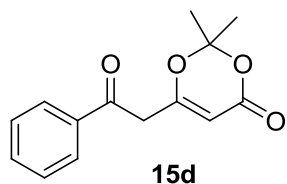
Ar=6-chloropyridin-3-yl: 6-(2-(6-chloropyridin-3-yl)-2-oxoethyl)-2,2-dimethyl-4H-1,3-dioxin-4-one (15c): Following procedure B, 6-chloropyridin-3-oyl chloride (1.06 g, 6.0 mmol) was used to



afford dioxinone **15c** (1.05 g, 62%) as a yellow solid, m.p. 117 °C. IR (neat): $\nu = 3103, 2998, 1723, 1687, 1635, 1574, 1374, 1357, 1268, 1200, 1101, 1015, 989, 812 \text{ cm}^{-1}$. ¹H NMR (300 MHz, CDCl₃): $\delta = 8.93$ (d, $J = 2.4$ Hz, 1H), 8.19 (dd, $J = 2.4, 8.5$ Hz, 1H), 7.50 (d, $J = 8.5$ Hz, 1H), 5.44

(s, 1H), 3.89 (s, 2H), 1.71 (s, 6H) ppm. ^{13}C NMR (75 MHz, CDCl_3): $\delta = 190.9, 163.9, 160.5, 156.6, 150.1, 138.3, 130.2, 125.0, 107.6, 97.5, 43.5, 25.1$ ppm. MS (ESI): $m/z = 280$ $[\text{M} - \text{H}]^-$.

Ar=Ph: 2,2-dimethyl-6-(2-oxo-2-phenylethyl)-4H-1,3-dioxin-4-one (15d): Following procedure B,



benzoyl chloride (2.3 mL, 20 mmol) was used to afford dioxinone **15d** (2.28 g, 47%) as a white solid, m.p. 114 °C. IR (neat): $\nu = 3098, 2999, 2962, 2930, 1716, 1687, 1644, 1392, 1377, 1222, 1019, 822$ cm^{-1} . ^1H NMR (300 MHz, CDCl_3): $\delta = 7.93$ (d, $J = 7.2$ Hz, 2H), 7.62 (t, $J = 7.4$ Hz, 1H), 7.49 (t, $J = 7.7$

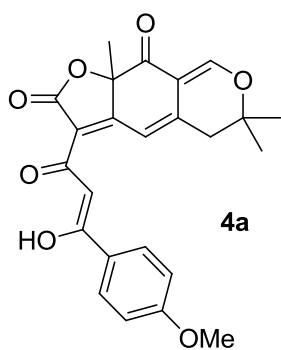
Hz, 2H), 5.41 (s, 1H), 3.90 (s, 2H), 1.69 (s, 6H) ppm. ^{13}C NMR (75 MHz, CDCl_3): $\delta = 193.2, 165.3, 160.9, 136.0, 134.2, 129.0, 128.4, 107.4, 97.1, 43.4, 25.1$ ppm. MS (ESI): $m/z = 247$ $[\text{M} + \text{H}]^+$.

General procedure for the synthesis of methylated dioxinones 16a and 16d (Procedure C): To a stirred solution of dioxinone **15a** or **15d** (1.0 equiv) and K_2CO_3 (5.0 equiv) in acetone (0.2 mol.L^{-1}) at room temperature was added MeI (2.0 equiv). The resulting mixture was heated under reflux and stirred for 2 h before it was quenched at room temperature with H_2O . The layers were separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with NH_4Cl (sat. aq.), dried (MgSO_4) and concentrated in *vacuo* to afford methylated dioxinones (100% conversion) which were used without further purification.

To a stirred solution of methylated dioxinones (1.0 equiv) in CH_2Cl_2 (0.1 mol.L^{-1}) at room temperature were added DIEA (3.0 equiv) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf; 1.5 equiv). The resulting mixture was stirred for 2 h before it was quenched with NaHCO_3 (sat. aq.). The layers were separated and the aqueous layer was extracted twice with CH_2Cl_2 . The combined organic layers were washed with NH_4Cl (sat. aq.), dried (MgSO_4) and concentrated in *vacuo*. Flash column chromatography (silica gel, cyclohexane:EtOAc 9:1) afforded the silylated dioxinones **16a** and **16d** which were directly engaged in the next step due to their instability.

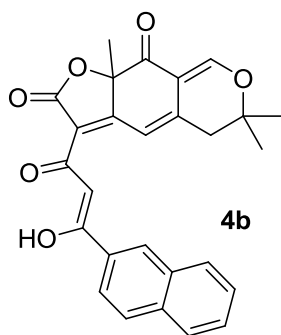
General method for the synthesis of epicocconone analogues 4a-d (Method A): To a stirred solution of alcohol **5** (1.0 equiv) and molecular sieves (4 Å, 200 mg per mmol) in toluene (0.1 mol.L^{-1}) at room temperature was added dioxinone **15a-d** (1.5 equiv). The resulting mixture was heated to 110 °C and stirred for 20 min before Et_3N (2.0 equiv) was added. The reaction mixture was stirred for 1 h before it was quenched at room temperature with HCl (1.0 N, aq.). The layers were separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated in *vacuo*. Flash column chromatography (silica gel, cyclohexane:EtOAc 9:1) afforded the desired epicocconone analogues **4a-d**.

Ar=pOMePh: (Z)-3-(3-hydroxy-3-(4-methoxyphenyl) acryloyl)-6,6,9a-trimethyl-5,6-dihydro-2H-furo[3,2-g]isochromene-2,9(9aH)-dione (4a): Following method A, alcohol **5** (222 mg, 1 mmol) and



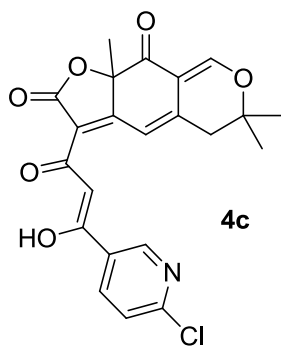
dioxinone **15a** (414 mg, 1.5 mmol) were used to afford analogue **4a** (126 mg, 30%) as a yellow solid. ^1H NMR (300 MHz, CDCl_3): δ = 8.00 (d, J = 8.9 Hz, 2H), 7.75 (s, 1H), 7.45 (s, 1H), 7.10 (s, 1H), 6.95 (d, J = 9.0 Hz, 2H), 3.88 (s, 3H), 2.74 (d, J = 6.8 Hz, 2H), 1.72 (s, 3H), 1.46 (s, 3H), 1.40 (s, 3H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 190.4, 189.9, 172.6, 168.5, 167.3, 163.9, 158.8, 140.9, 130.2, 128.6, 114.7, 114.1, 113.6, 110.7, 96.9, 86.5, 81.3, 55.7, 40.1, 28.0, 26.6, 26.2 ppm. MS (ESI): m/z = 423 $[\text{M} + \text{H}]^+$. HRMS (ESI): calcd. for $[\text{C}_{24}\text{H}_{22}\text{O}_7 + \text{H}]^+$ 423.1444; found 423.1440.

Ar=2-naphtyl: (Z)-3-(3-hydroxy-3-(naphthalen-2-yl)acryloyl)-6,6,9a-trimethyl-5,6-dihydro-2H-furo[3,2-g]isochromene-2,9(9aH)-dione (4b): Following method A, alcohol **5** (222 mg, 1 mmol) and



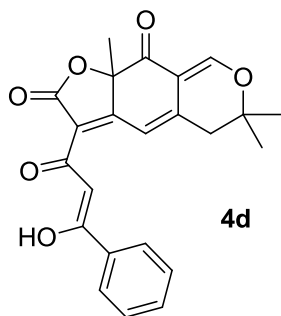
dioxinone **15b** (429 mg, 1.5 mmol) were used to afford analogue **4b** (146 mg, 33%) as a yellow solid. ^1H NMR (300 MHz, CDCl_3): δ = 8.56 (s, 1H), 8.07-7.98 (m, 2H), 7.92-7.86 (m, 2H), 7.77 (s, 1H), 7.65 (s, 1H), 7.62-7.53 (m, 2H), 7.14 (s, 1H), 2.79 (d, J = 17.1 Hz, 1H), 2.71 (d, J = 17.1 Hz, 1H), 1.75 (s, 3H), 1.47 (s, 3H), 1.40 (s, 3H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 190.3, 189.8, 174.4, 168.5, 168.1, 159.0, 141.4, 135.8, 133.2, 132.8, 129.8, 129.4, 128.7, 128.6, 127.9, 127.0, 123.7, 114.7, 113.5, 110.7, 97.6, 86.6, 81.4, 40.1, 28.1, 26.5, 26.3 ppm. MS (ESI): m/z = 443 $[\text{M} + \text{H}]^+$. HRMS (ESI): calcd. for $[\text{C}_{27}\text{H}_{22}\text{O}_6 + \text{H}]^+$ 443.1495; found 443.1500.

Ar=6-chloropyridin-3-yl: 3-[(1Z)-1-hydroxy-3-oxo-3-(6-chloro-pyridin-3-yl)prop-1-en-1-yl]-6,6,9a-trimethyl-5,6-dihydro-2H-furo[3,2-g]isochromene-2,9(9aH)-dione (4c): Following method



A, alcohol **5** (222 mg, 1 mmol) and dioxinone **15c** (422 mg, 1.5 mmol) were used to afford analogue **4c** (115 mg, 27%) as a yellow solid. ^1H NMR (300 MHz, CDCl_3): δ = 8.99 (d, J = 2.4 Hz, 1H), 8.19 (dd, J = 2.4, 8.4 Hz, 1H), 7.78 (s, 1H), 7.45 (s, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.09 (s, 1H), 2.80 (d, J = 17.3 Hz, 1H), 2.72 (d, J = 17.3 Hz, 1H), 1.74 (s, 3H), 1.47 (s, 3H), 1.41 (s, 3H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 189.5, 186.9, 175.7, 169.3, 168.2, 159.4, 155.6, 149.5, 142.4, 137.6, 130.4, 124.5, 114.0, 113.3, 110.7, 97.3, 86.7, 81.5, 40.1, 28.2, 26.5, 26.3 ppm. HRMS (ESI): calcd. for $[\text{C}_{27}\text{H}_{18}\text{ClNO}_6 - \text{H}]^-$ 426.0744; found 426.0754.

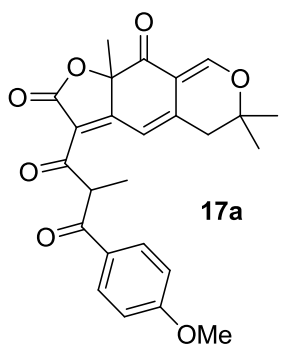
Ar=Ph: **3-[(1Z)-1-hydroxy-3-oxo-3-phenylprop-1-en-1-yl]-6,6,9a-trimethyl-5,6-dihydro-2H-furo[3,2-g]isochromene-2,9(9aH)-dione (4d):** Following method A, alcohol **5** (222 mg, 1 mmol) and



dioxinone **15d** (369 mg, 1.5 mmol) were used to afford analogue **4d** (137 mg, 35%) as a yellow solid. ^1H NMR (300 MHz, CDCl_3): δ = 8.00 (d, J = 7.4 Hz, 2H), 7.76 (s, 1H), 7.55 (t, J = 7.2 Hz, 1H), 7.51 (s, 1H), 7.46 (t, J = 7.2 Hz, 2H), 7.11 (s, 1H), 2.78 (d, J = 16.6 Hz, 1H), 2.71 (d, J = 16.6 Hz, 1H), 1.73 (s, 3H), 1.47 (s, 3H), 1.40 (s, 3H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 190.5, 189.8, 174.4, 168.4, 168.1, 159.0, 141.4, 135.9, 133.2, 128.8, 127.9, 114.7, 113.5, 110.7, 97.3, 86.5, 81.4, 40.1, 28.0, 26.5, 26.3 ppm. MS (ESI): m/z = 393 $[\text{M} + \text{H}]^+$. HRMS (ESI): calcd. for $[\text{C}_{23}\text{H}_{20}\text{O}_6 + \text{H}]^+$ 393.1338; found 393.1327.

General method for the synthesis of epicocconone analogues 17a and 17d (Method B): To a stirred solution of alcohol **5** (1.0 equiv), silylated dioxinone **16a** or **16d** (1.5 equiv) and molecular sieves (4 Å, 200 mg per mmol) in toluene ($0.1 \text{ mol}\cdot\text{L}^{-1}$) at room temperature was added Et_3N (2.0 equiv). The resulting mixture was heated to 110 °C and stirred for 3 h before it was quenched at room temperature with HCl (1.0 N, aq.). The layers were separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated in *vacuo*. Flash column chromatography (silica gel, cyclohexane:EtOAc) afforded the desired epicocconone analogues **17a** and **17d**.

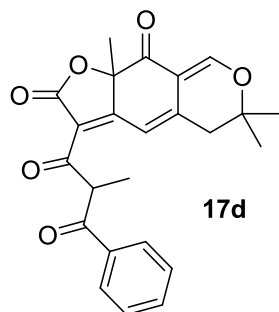
Ar=pOMePh: **3-[3-(4-methoxyphenyl)-2-methyl-3-oxopropanoyl]-6,6,9a-trimethyl-5,6-dihydro-2H-furo[3,2-g]isochromene-2,9(9aH)-dione (17a):** Following method B, alcohol **5** (84 mg, 0.38



mmol) and dioxinone **16a** (165 mg, 0.57 mmol) were used to afford analogue **17a** (41 mg, 25%), obtained in a mixture of diastereoisomers (45:55), as a yellow solid after purification by flash chromatography on silica gel (cyclohexane:EtOAc, 60:40). ^1H NMR (300 MHz, CDCl_3): δ = 7.99 (d, J = 8.9 Hz, 1.1H), 7.95 (d, J = 8.9 Hz, 0.9H), 7.75 (s, 1H), 7.08 (s, 1H), 6.95 (d, J = 8.9 Hz, 1.1H), 6.93 (d, J = 8.9 Hz, 0.9H), 5.51 (q, J = 7.1, 14.2 Hz, 0.45H), 5.45 (q, J = 7.1, 14.2 Hz, 0.55H), 3.87 (s, 1.65H), 3.86 (s, 1.35H), 2.78-2.64 (m, 2H), 1.69 (s, 1.35H), 1.68 (s, 1.65H), 1.44-1.38 (m, 9H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 197.6, 197.1, 193.7 (2C), 189.7, 189.5, 172.9, 172.4, 169.2, 169.1, 163.9, 163.8, 159.6, 159.4, 144.4, 143.8, 131.3, 131.2, 128.8 (2C), 116.1, 116.0, 114.1 (2C), 113.1, 112.8, 110.8, 110.7, 86.8, 86.6, 81.6, 81.5, 55.6

(2C), 52.4, 52.3, 40.1 (2C), 28.4, 28.2, 26.6, 26.4, 26.3, 13.5, 13.3 ppm. MS (ESI): $m/z = 454$ $[M + H_2O]^+$. HRMS (ESI): calcd. for $[C_{25}H_{24}O_7 + H]^+$ 437.1600; found 437.1596.

Ar=Ph: 6,6,9a-trimethyl-3-(2-methyl-3-oxo-3-phenylpropanoyl)-5,6-dihydro-2H-furo[3,2-g]isochromene-2,9(9aH)-dione (17d): Following method B, alcohol **5** (84 mg, 0.38 mmol) and



dioxinone **16d** (148 mg, 0.57 mmol) were used to afford analogue **17d** (45 mg, 29%), obtained in a mixture of diastereoisomers (42:58), as a yellow solid after purification by flash chromatography on silica gel (cyclohexane:EtOAc, 70:30).

1H NMR (300 MHz, $CDCl_3$): $\delta = 8.01-7.95$ (m, 2H), 7.76 (s, 1H), 7.59-7.43 (m, 3H), 7.09 (s, 1H), 5.57-5.45 (m, 1H), 2.79-2.65 (m, 2H), 1.70 (s, 1.25H), 1.69 (s, 1.75H), 1.45-1.39 (m, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 199.1,$

198.6, 193.5 (2C), 189.6, 189.5, 173.1, 172.7, 169.2, 169.1, 159.7, 159.5, 144.6, 144.0, 135.9, 135.8, 133.4, 131.9, 128.90 (2C), 128.85 (2C), 115.9, 115.7, 113.0 (2C), 112.8, 110.8, 110.7, 110.5, 86.8, 86.7, 81.6, 81.5, 52.7, 52.6, 40.1, 40.0, 28.4, 28.2, 26.5, 26.3, 13.3, 13.0 ppm. MS (ESI): $m/z = 424$ $[M + H_2O]^+$. HRMS (ESI): calcd. for $[C_{24}H_{22}O_6 + H]^+$ 407.1495; found 407.1514.

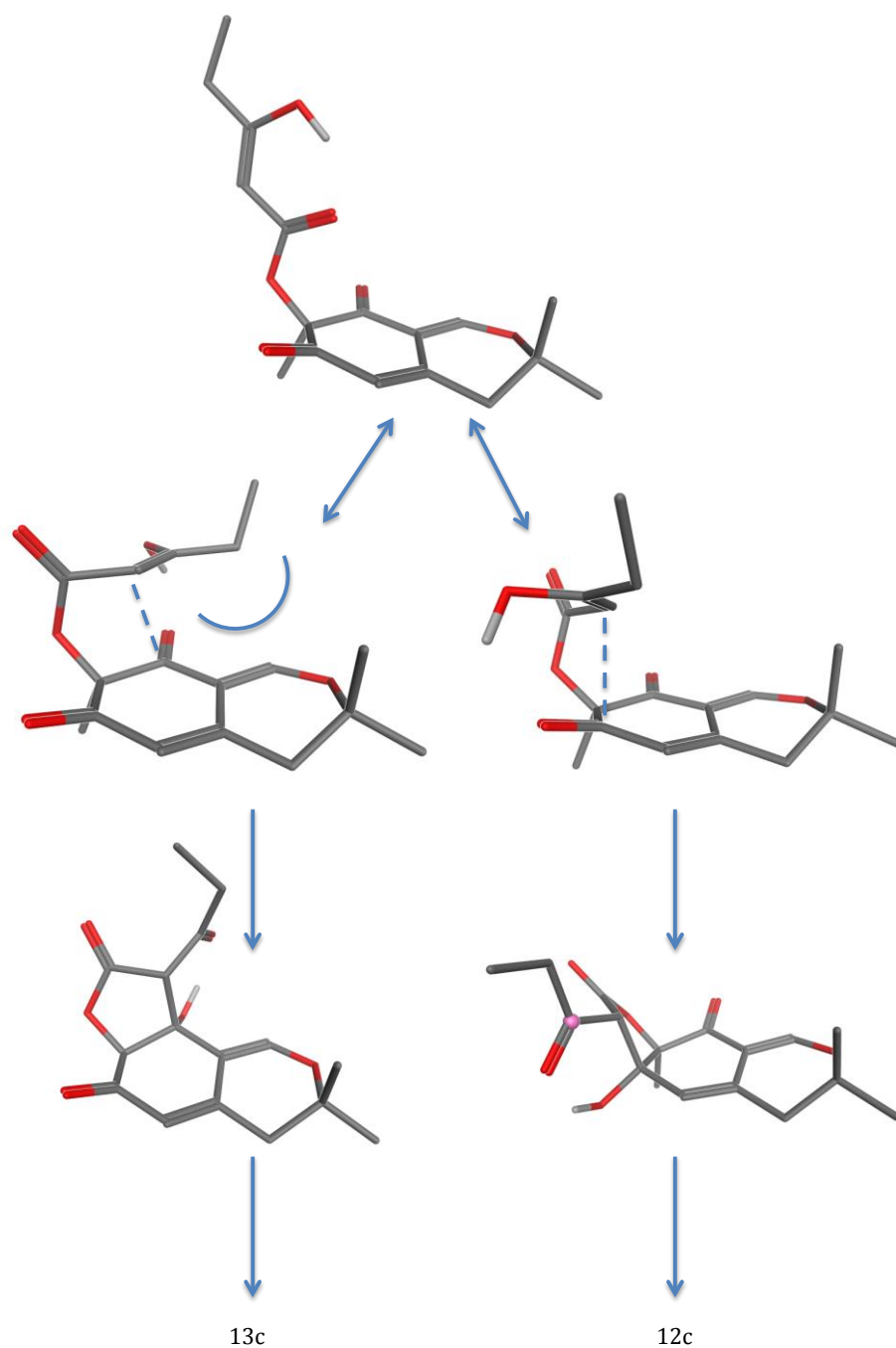


Figure S1. Molecular modeling of the cyclization of the β -ketoester formed from **5** and **14c**. The MOE package was used incorporating the MMFF94x forcefield. All calculations were performed *in vacuo* but with a distance dependent dielectric ($\epsilon = 2r$) to simulate solvent. Note that for formation of **11'** (Scheme 6) there is a steric interaction between the acyl side-chain and the β -methyl, which is less of an issue for R= alkyl than R = aryl.

II) PHOTOPHYSICAL PROPERTIES

Absorption and Fluorescence Spectra in Water (pH = 2.5) with SDS (0.1 % w/v)

Stock solutions: epicocconone **1** and epicocconone analogues (**12a-d**, **4a-d**, **17a-d**) were dissolved in DMSO (2.26 mM).

Mixture *a*: pH = 2.5 buffer (prepared from an universal buffer solution*) containing SDS (0.1% w/v)

Mixture *b*: pH = 10.5 buffer (prepared from universal buffer solution*) containing BSA (1 mg/mL).

Analogues 12a-d:

Without BSA: To the 3.5 mL rectangular quartz cell, 40 μL of stock dye solution **12a-d** and 2960 μL of mixture *a* were sequentially added. The resulting mixture was homogenised by inversion for 30 s at 25 °C and the absorption spectra recorded from 250 to 800 nm (Figure S2_A1, E1).

Emission spectra were recorded under the same conditions on the same sample after excitation at 420 nm and normalized to λ_{max} using the equation:

$$I_n = \frac{I \times A_{\text{max}}}{A_{\text{ex}}}$$

where *I* is the fluorescence intensity at a given wavelength and A_{ex} is the absorbance at the excitation wavelength and A_{max} is the absorbance at the absorption maximum.

Absorbance and fluorescence intensities of analogues **12c** and **13c** are too weak to be correctly measured.

With BSA: To the 3.5 mL rectangular quartz cell, 160 μL of dye stock solution **12a-d** and 120 μL of mixture *b* were added. The resulting reaction mixture was homogenised by inversion. After 15 min, 2720 μL of mixture *a* was added. The resulting reaction mixture was homogenized by inversion for 30 s, at 25 °C, the absorption spectra were recorded from 250 to 800 nm (Figure S2_A2, E2).

Emission spectra were recorded under the same conditions on the same sample after excitation at 488 nm and normalized (as above) relative to the absorption maximum (λ_{max}).

* Citric acid (10.505 g), sodium tetraborate (9.535 g), tris(hydroxymethyl)aminomethane (6.055 g), sodium dihydrogenphosphate (6.000 g) and lithium chloride (2.100 g) were dissolved in water (500 mL) and adjusted to pH 2.5 with hydrochloric acid (2M) or to pH 10.5 with sodium hydroxide (2M).

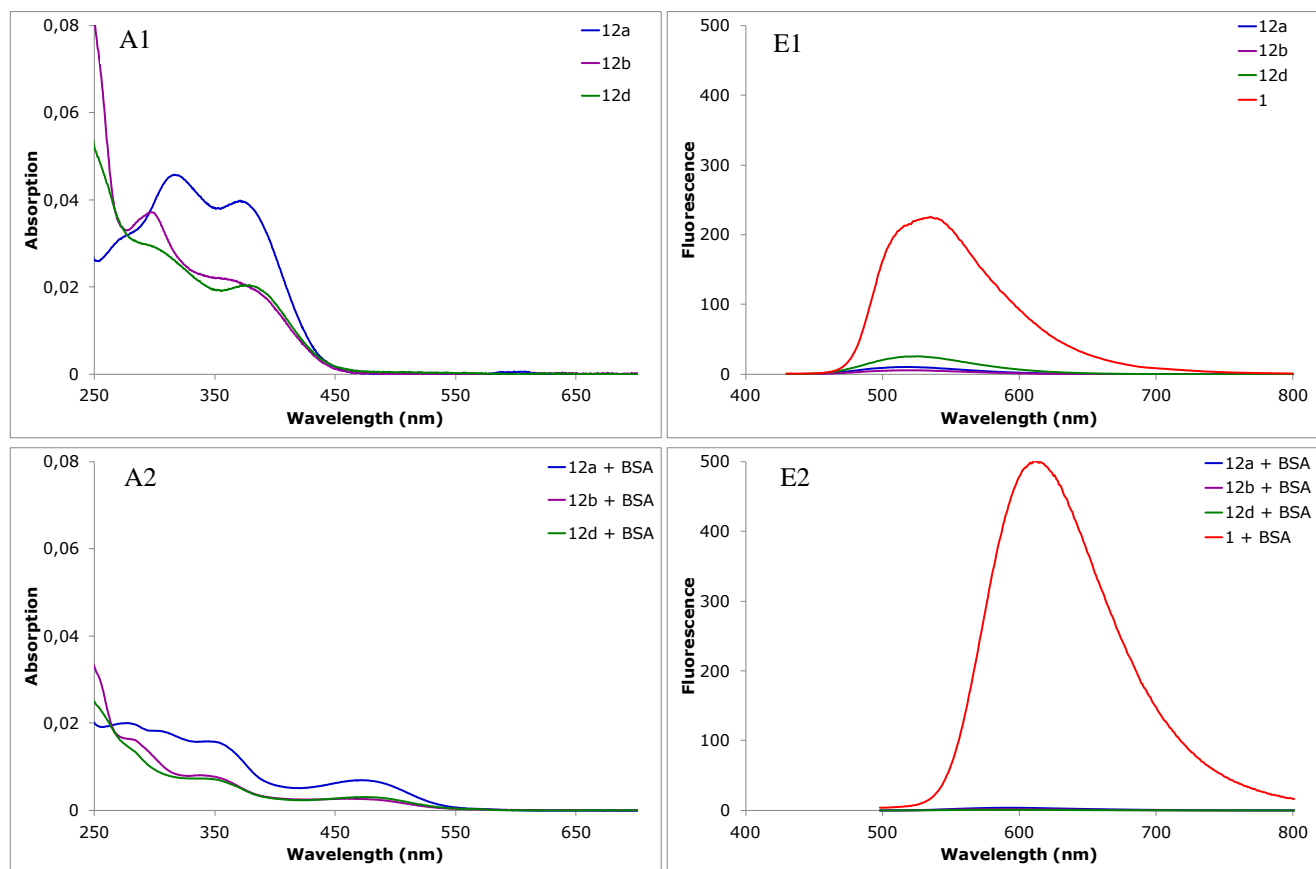


Figure S2. Absorption (A1 and A2) and emission (E1 and E2) spectra of epicocconone (**1**) and analogues **12a-d** in H₂O (pH=2.5) with 0.1% SDS and in the presence of excess of BSA, with 0.1% SDS. All spectra are normalized on identical concentrations of fluorophores (10 μM) and the emissions normalized to the excitation maximum.

Analogues 4a-d, 17a-d and 1:

Without BSA: To the 3.5 mL rectangular quartz cell, 10 μL of stock dye solution **4a-d**, **17a-d** and **1** and 2990 μL of mixture *a* were sequentially added. The resulting mixture was homogenised by inversion for 30 s at 25 °C and the absorption spectra recorded from 250 to 800 nm (Figure S3_A3, E3).

Emission spectra were recorded under the same conditions on the same sample after excitation at 420 nm and normalized to λ_{\max} using the equation:

$$I_n = \frac{I \times A_{\max}}{A_{ex}}$$

where I is the fluorescence intensity at a given wavelength and A_{ex} is the absorbance at the excitation wavelength and A_{\max} is the absorbance at the absorption maximum.

With BSA: To the 3.5 mL rectangular quartz cell, 10 μL for **4a-d** or **1** or 20 μL for **17a-d** of dye stock solution were added. Then, 40 μL of mixture *b* were added. The resulting reaction mixture was homogenised by inversion. After 15 min, the solution was completed to 3 mL with mixture *a*. The resulting reaction mixture was homogenized by inversion for 30 s, at 25 °C, the absorption spectra were recorded from 250 to 800 nm (Figure S3_A4, E4).

Emission spectra were recorded under the same conditions on the same sample after excitation at 488 nm and normalized (as above) relative to the absorption maximum (λ_{\max}).

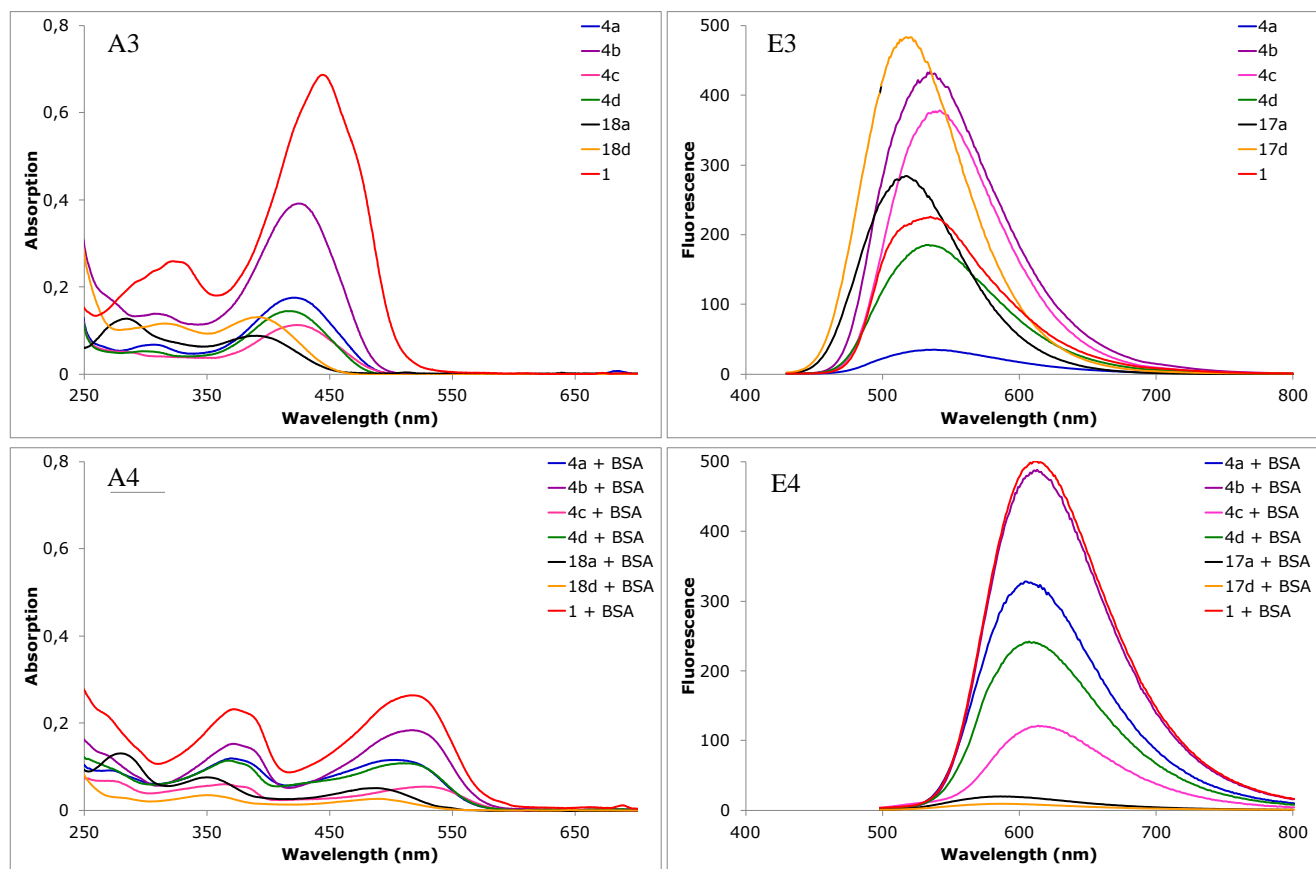


Figure S3. Absorption (A3 and A4) and emission (E3 and E4) spectra of epicocconone (**1**) and analogues **4a-d**, **17a-d** in H₂O (pH=2.5) with 0.1% SDS and in the presence of excess of BSA, with 0.1% SDS. All spectra are normalized on identical concentrations of fluorophores (10 μM) and the emissions normalized to the excitation maximum.

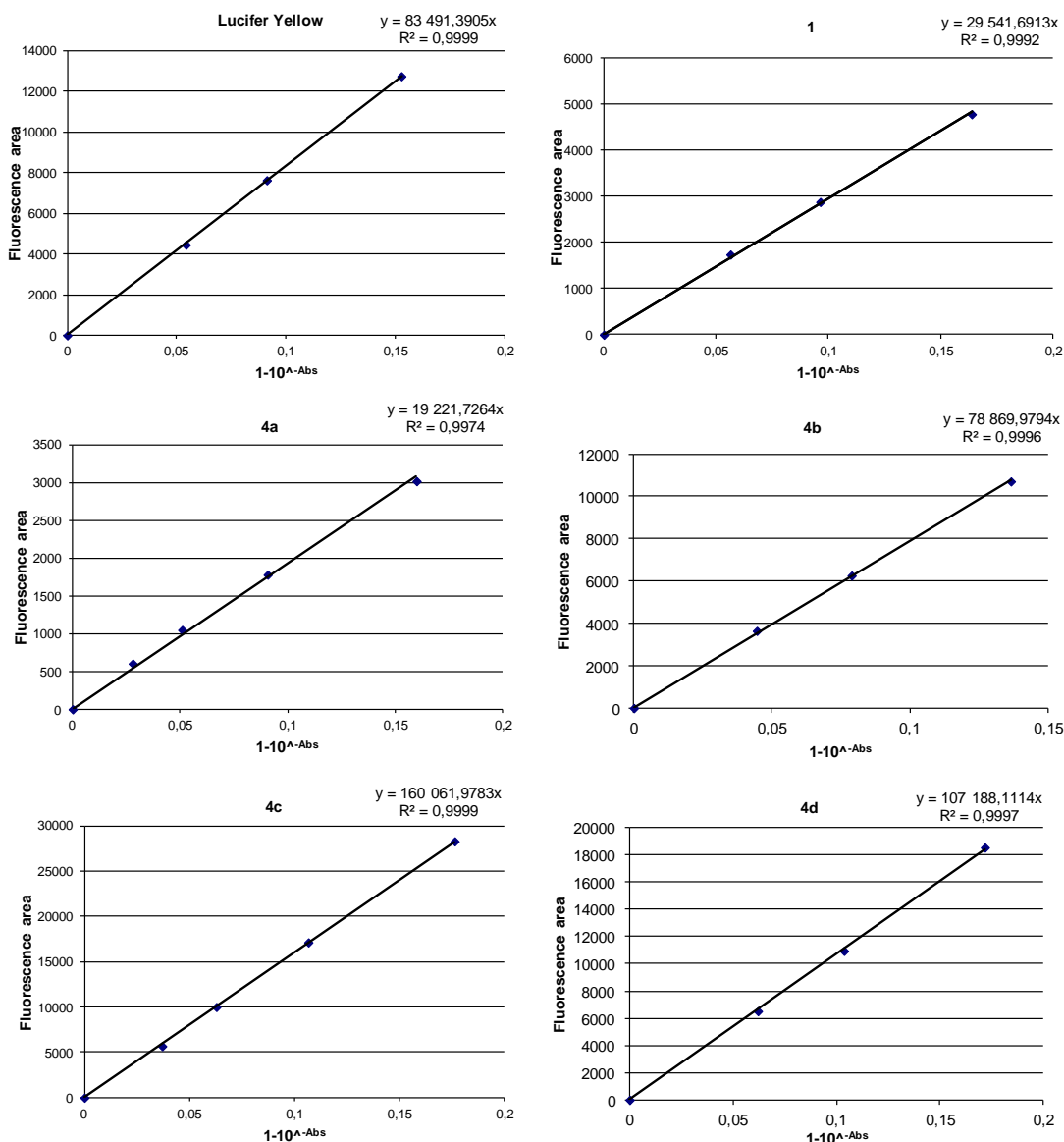
Quantum Yields in Water (pH = 2.5) with SDS (0.1 % w/v)

Relative quantum yields were measured on the above solutions at 25 °C using rhodamine 6G ($\Phi_F = 0.76$ in water) as a standard for the BSA-reacted dyes and Lucifer Yellow CH ($\Phi_F = 0.21$) for the native dyes (Table 2).

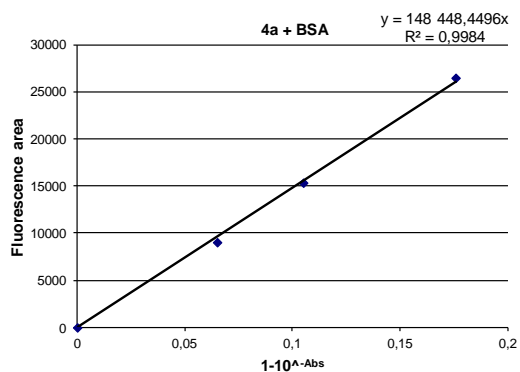
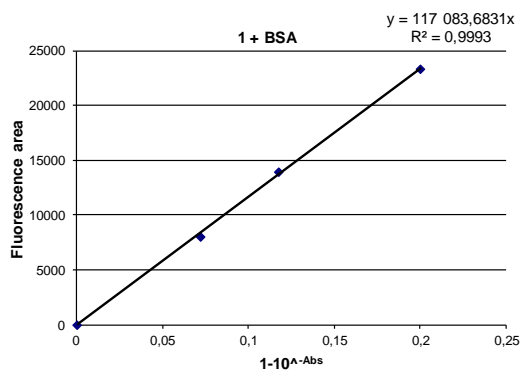
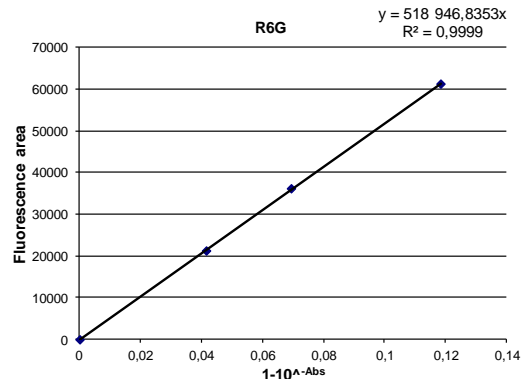
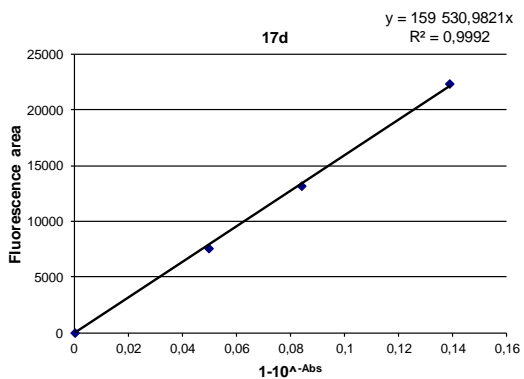
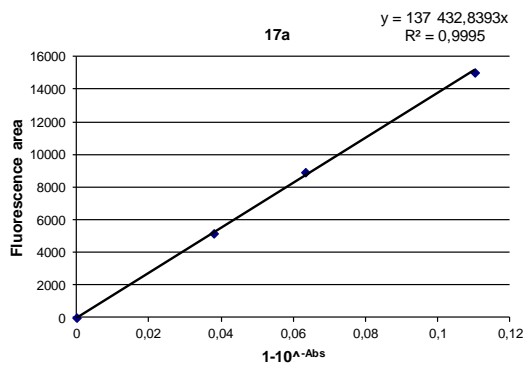
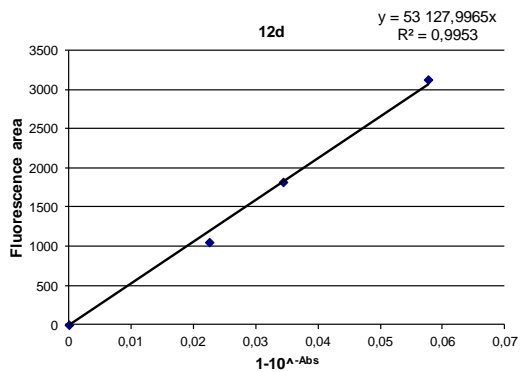
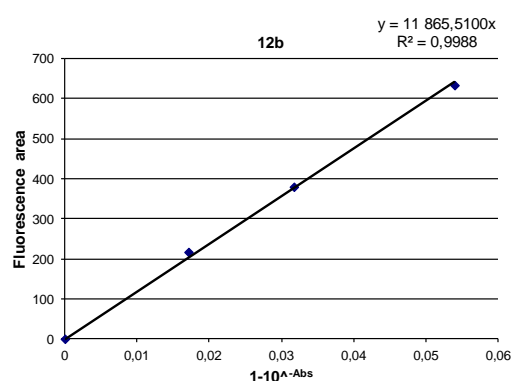
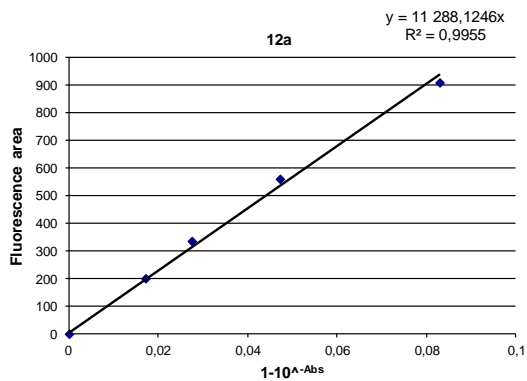
The following equation was used to determine the relative fluorescence quantum yield:³

$$\Phi_F(x) = (f_s/f_x)(F_x/F_s)(n_x/n_s)^2\Phi_F(s)$$

Where f is the absorption factor ($f = 1 - 10^{-A}$, where A = Absorbance at the excitation wavelength), F is the area under the emission curve, n is the refractive index of the solvents (at 25 °C) used in measurements ($n = 1.333$ for buffer), and the subscripts s and x represent standard and unknown, respectively.



³ A. M. Brouwer, *Pure Appl. Chem.* **2011**, *83*, 2213-2228.



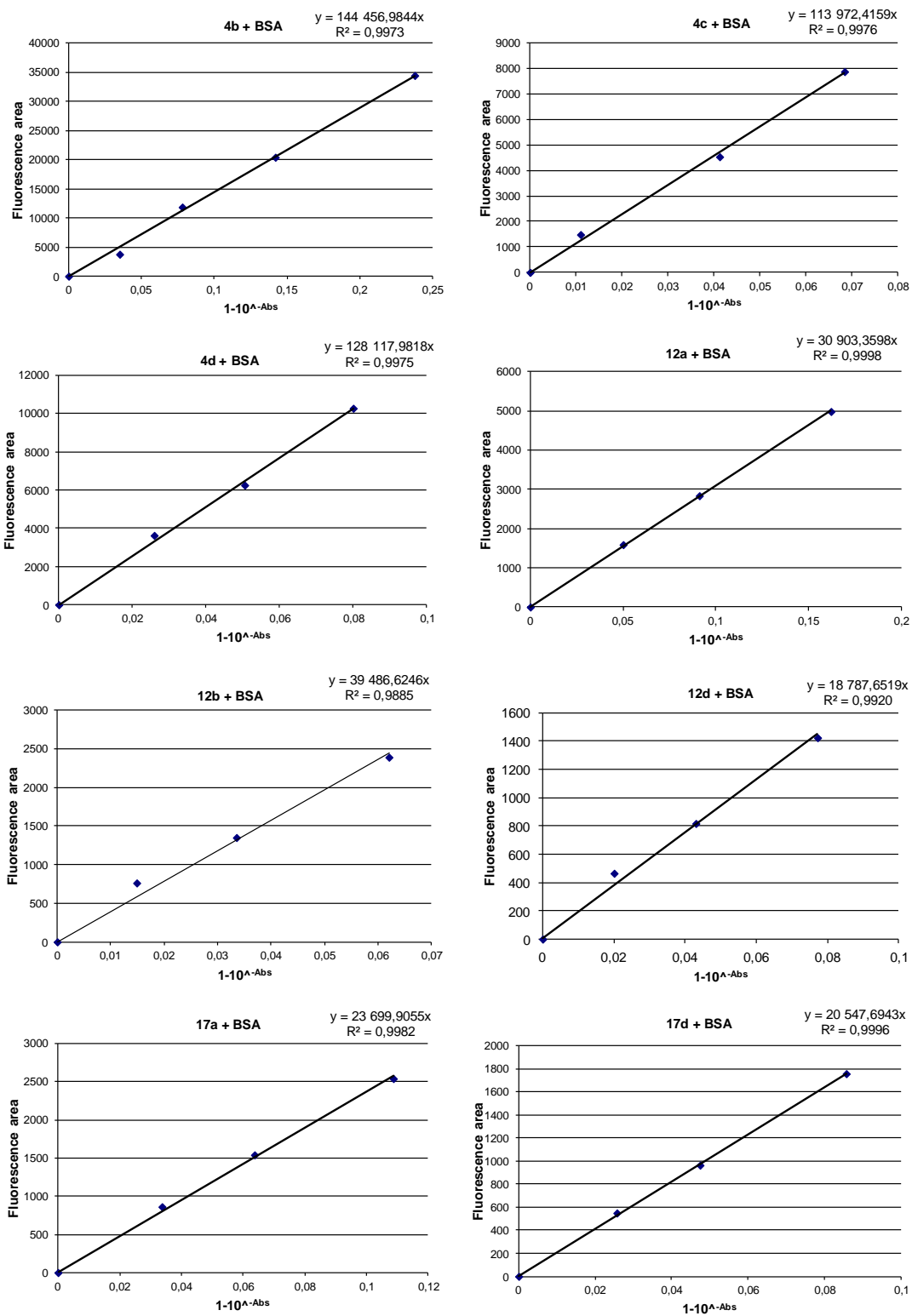


Figure S4. Calculations of quantum yield for each analogue and epicocconone (**1**) in 0.1% SDS (pH 2.5) or with excess protein (BSA, pH 2.5, 0.1% SDS).

III) 1D Gels

Limit of Detection

Epicoconone analogs (**4a-d**) were dissolved in DMSO (10 mg/mL) and further diluted to 1 mg/mL with water and stored at -80°C . One vial of GE low molecular weight markers (GE Healthcare; 17-0446-01, 575 μg of total protein) was diluted with $1\times$ LDS buffer (250 μL Invitrogen $4\times$ LDS buffer (NP0008), 100 μL 1 M DTT, 650 μL RO water) to achieve a final concentration of 51.2 $\mu\text{g}/\text{mL}$ of soybean trypsin inhibitor (STI). The stock solution was serially diluted ($2\times$) from 12.8 $\mu\text{g}/\text{mL}$ to 0.125 $\mu\text{g}/\text{mL}$ (based on STI), Table S1.

Table S1. Precise quantities of protein in each lane of 1D gel electrophoresis (Figs 2, S3 & S4)

lane (pg/5 μL)	1	2	3	4	5	6	7	8	9	10	11	12
Phosphorylase B	0	107305	53652	26826	13413	6707	3353	1677	838	419	210	105
Albumin	0	132930	66465	33232	16616	8308	4154	2077	1039	519	260	130
Ovalbumin	0	235430	117715	58857	29429	14714	7357	3679	1839	920	460	230
Carbonic Anhydrase	0	132930	66465	33232	16616	8308	4154	2077	1039	519	260	130
Soybean Trypsin Inh	0	128125	64063	32031	16016	8008	4004	2002	1001	500	250	125
α-Lactoglobulin	0	185781	92891	46445	23223	11611	5806	2903	1451	726	363	181

The practical limit of detection was obtained by measuring the volume of the peaks (Figure S5) and an identical area just outside the measured area. These volumes were plotted against protein concentration and the line of best fit determined (Figure S6). The slope of the line is an indication of contrast and the goodness of fit (R^2) an indication of linearity of response over the concentration range. A steep slope and $R^2 = 1$ would indicate a dye with the best performance, being easy to see slight variations in concentration and being readily quantifiable.

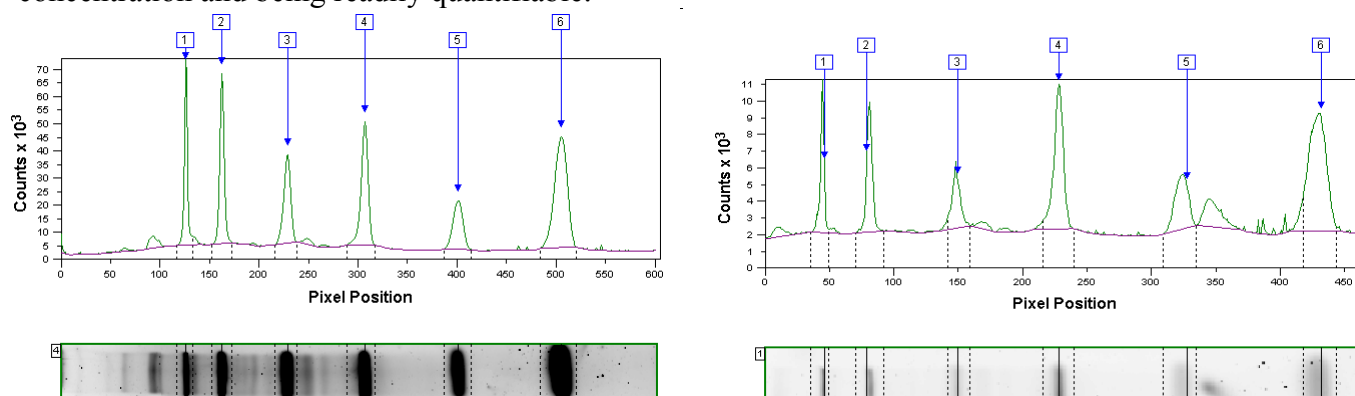
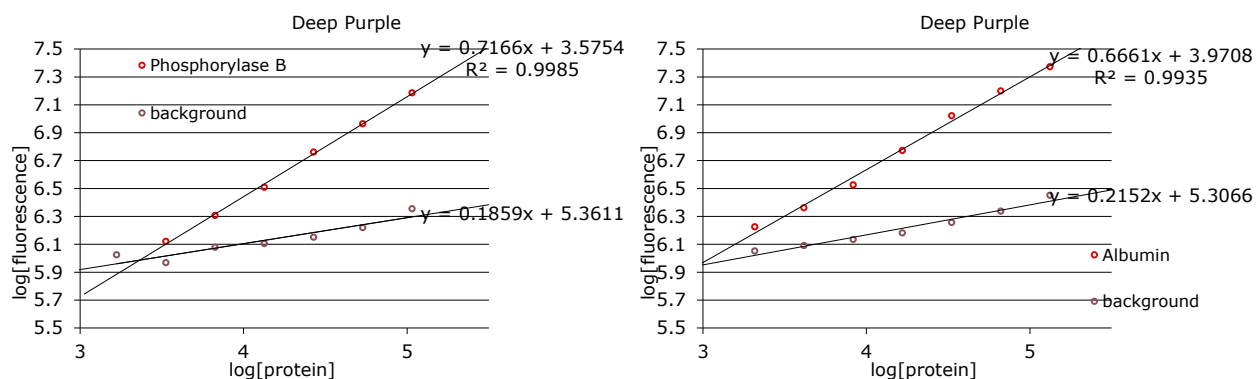
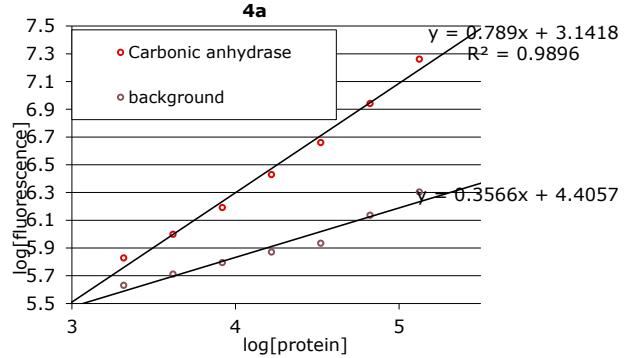
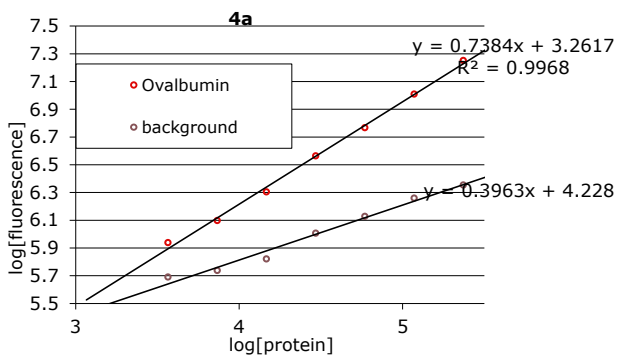
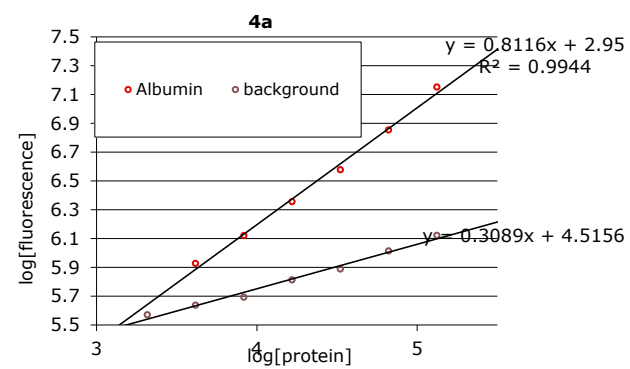
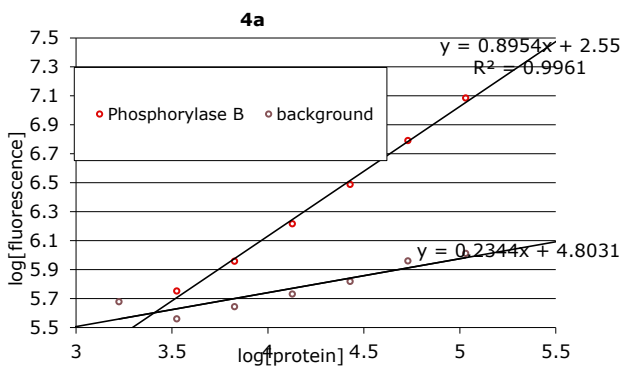
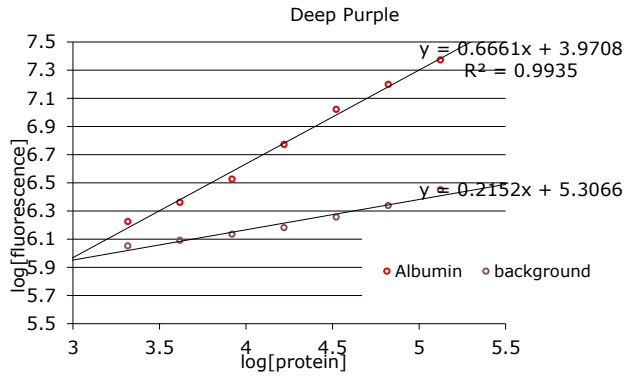
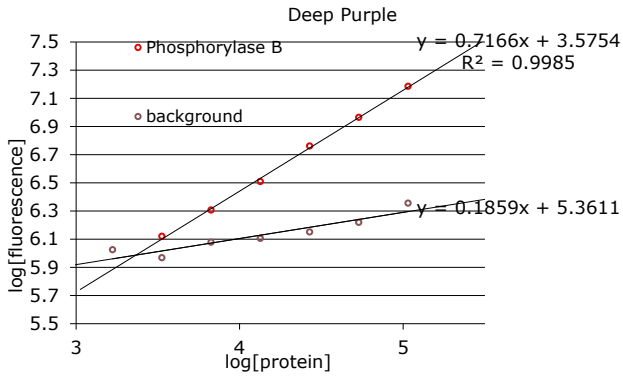
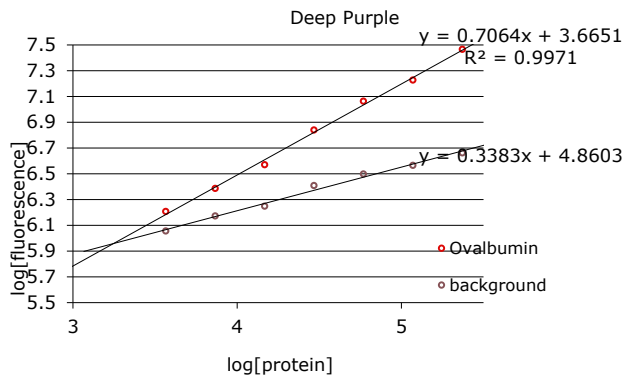
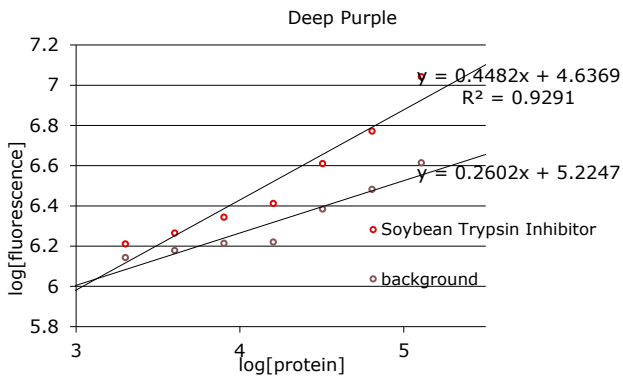
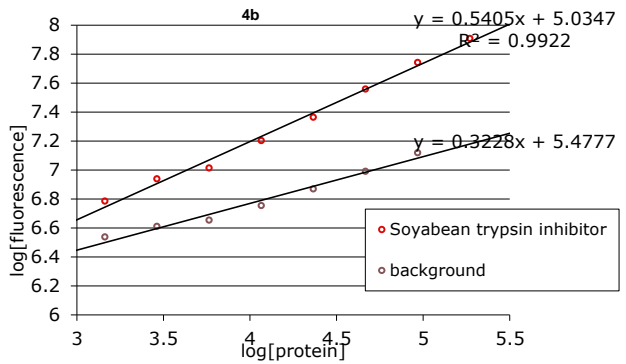
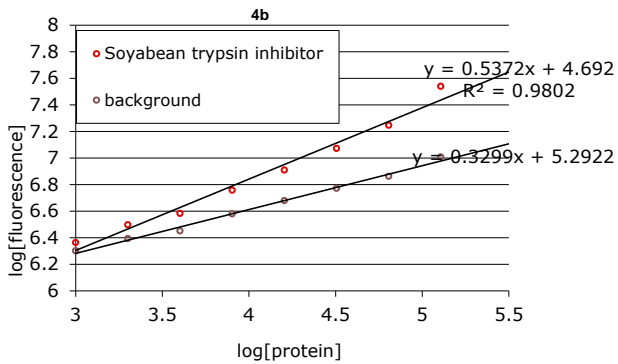
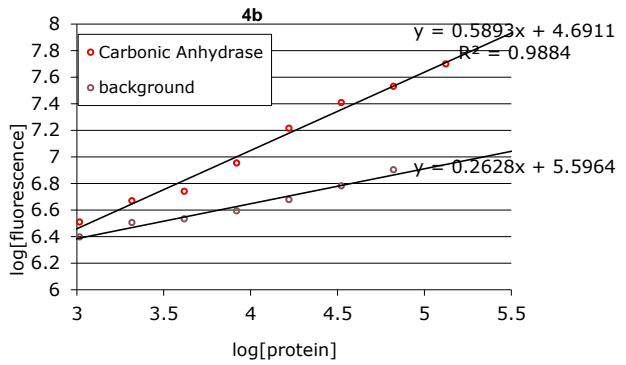
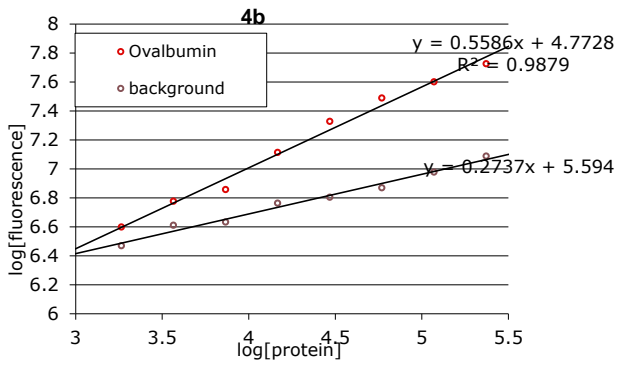
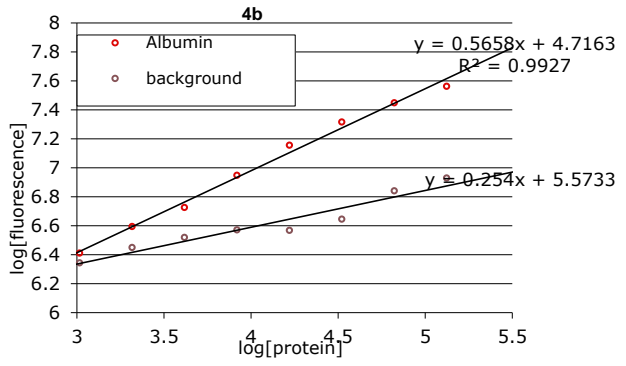
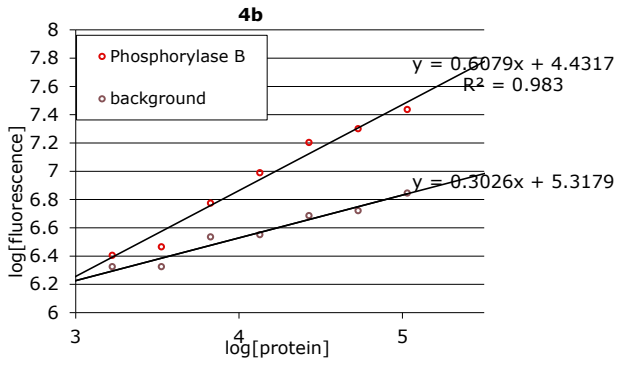
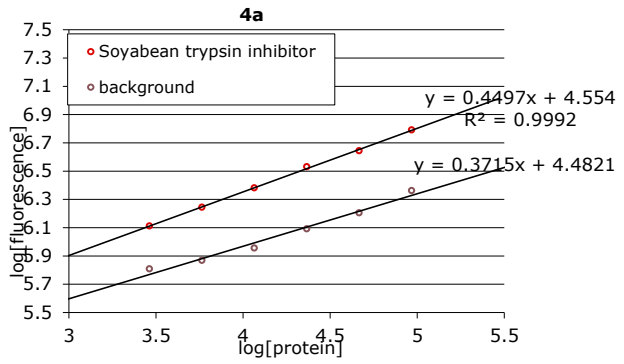
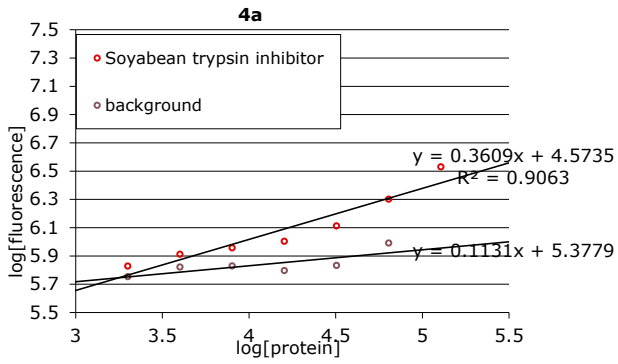
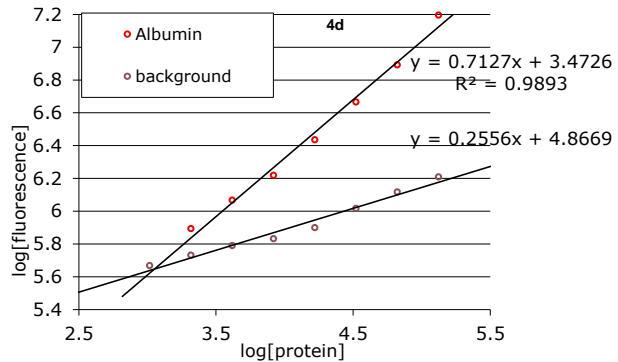
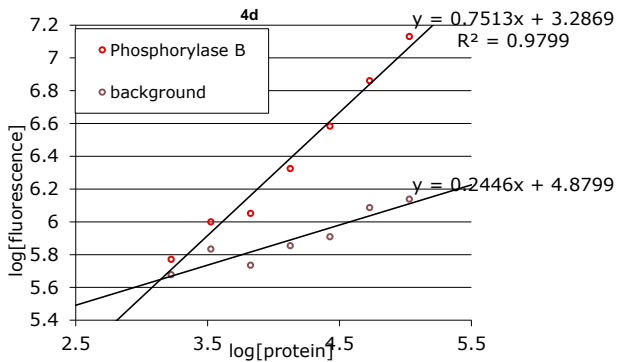
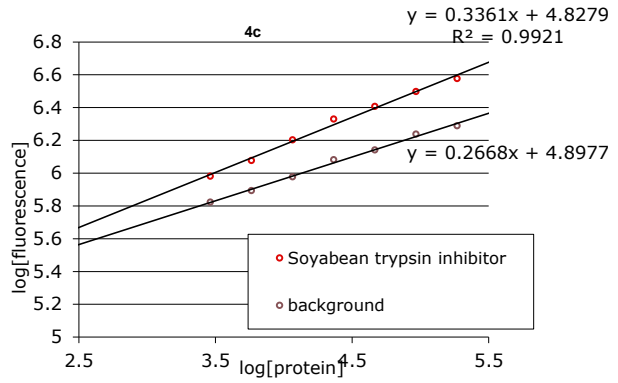
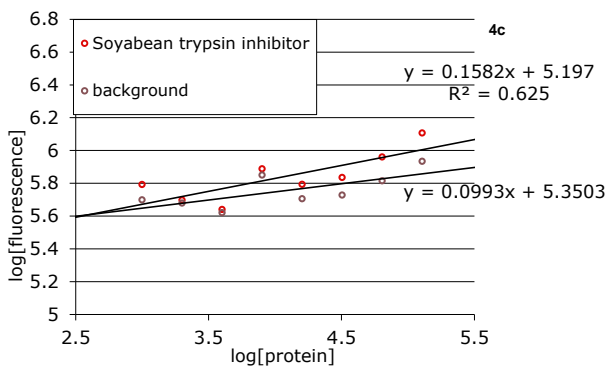
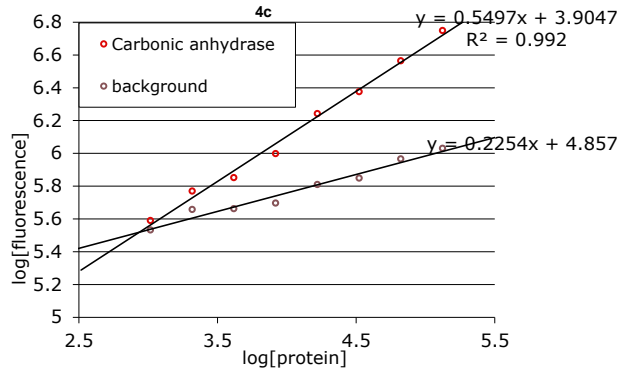
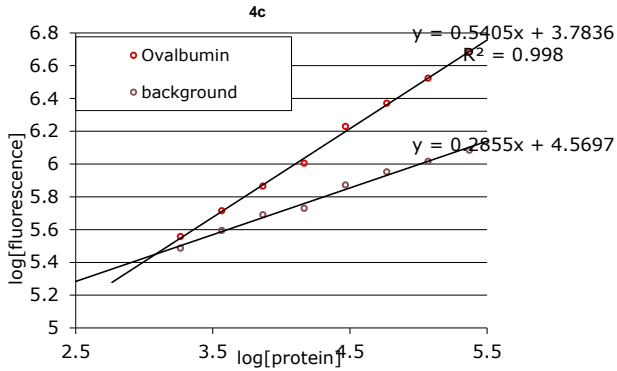
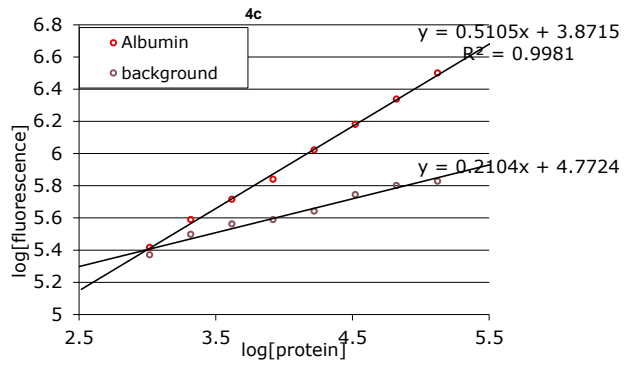
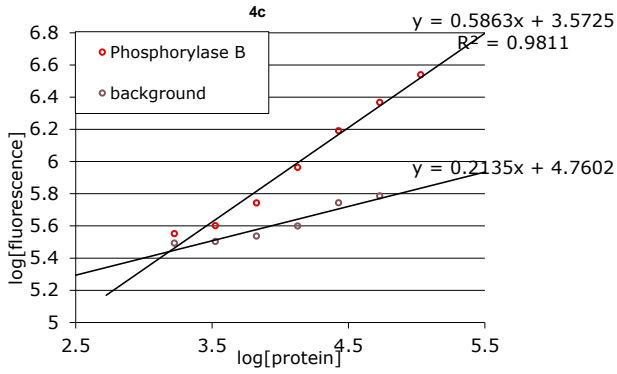


Figure S5. Representative 1D electrophoresis gels for Deep Purple at 32 and 1 ng of protein/lane. Density volumes were calculated (IMageQuant IT) for phosphorylase B (1), albumin/BSA (2), ovalbumin (3), carbonic anhydrase (4), soybean trypsin inhibitor (5) and α -lactoglobulin (6). Background measurements were taken from an identical area between peaks.









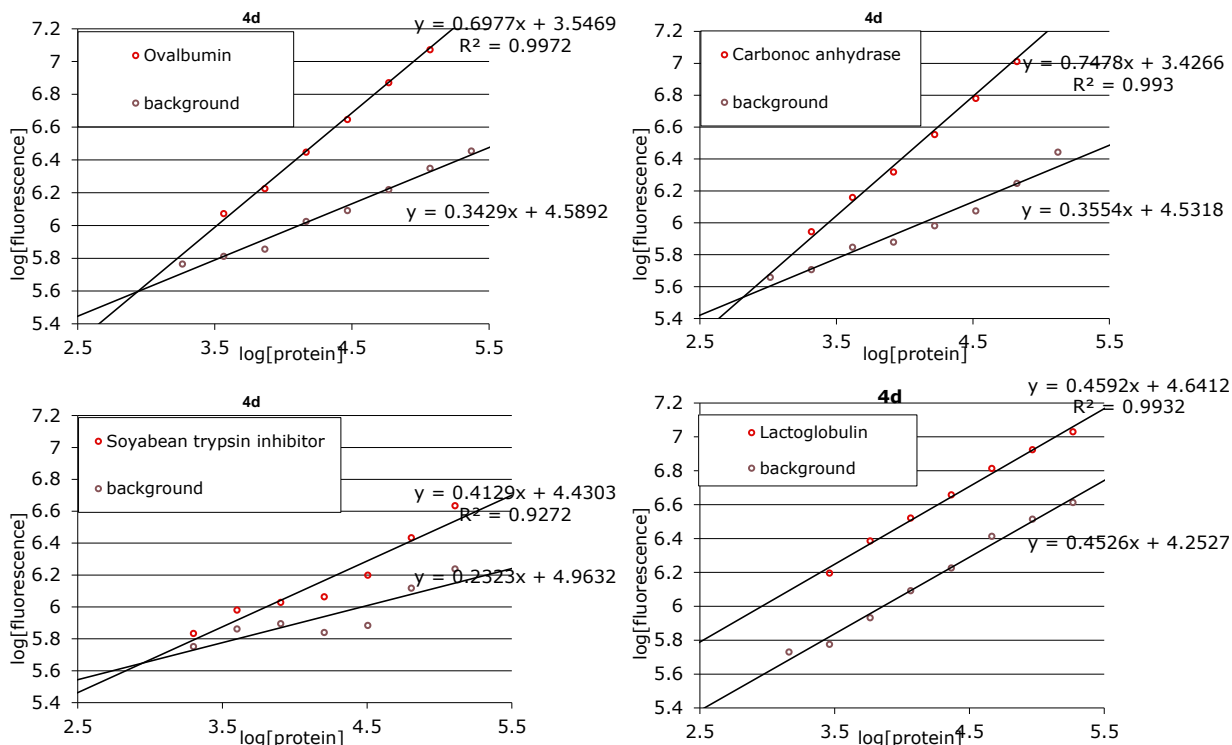


Figure S6. Determination of LOD was made by linear regression of background versus signal for six proteins as in Figure S4 for compounds **1** and **4a-d**.

Figure S6 shows the plots of spot intensity vs. protein concentration and background counts. The point of intersection of the two lines is where signal can no longer be differentiated from background and used here to define the LOD. For α -lactoglobulin, no lower limit could be determined as the band was clearly visible even at the lowest concentration (180 pg) for all stains. Compound **4a**, gave the largest slope. The difference in slope of the signal (red circles) and background (green circles) is an indication of contrast and the goodness of fit (R^2) and indication of linearity of response. 4As all stains gave good linearity over the range of concentrations used all could be used to quantify protein concentrations over at least 3 orders of magnitude. It was generally not possible to determine the detection limit for α -lactoglobulin as it was clearly visible at even the lowest concentration (180 pg). The limits of detection are summarized in Table 3.

Photo-bleaching

Stains, gels and running conditions were exactly as described for the limit of detection studies. Standard gels were run to compare the performance of compounds **4a-d** with **1** (Fig. S7). Gels were then placed on a UV transilluminator for 16 minutes and rescanned (Fig. S8).

Signal to noise (S/N) was calculated from the pixel volumes used for the photobleaching studies by dividing the peak volume by a nearby area of background at each time point for each of six proteins (Fig. S8). Analog **4d** gave the best S/N (~8:1) for 4 out of the 6 proteins. In general the S/N did not change significantly during the experiment except for **4b**, which increased from 3:1 to ~7:1, indicating that the background bleached faster than the protein bands. A useful characteristic. The half-life of the stain under continuous UV irradiation was measured by fitting a first order exponential decay (Graphpad Prism v4) to the band intensities over time (Fig. 5, Table S2).

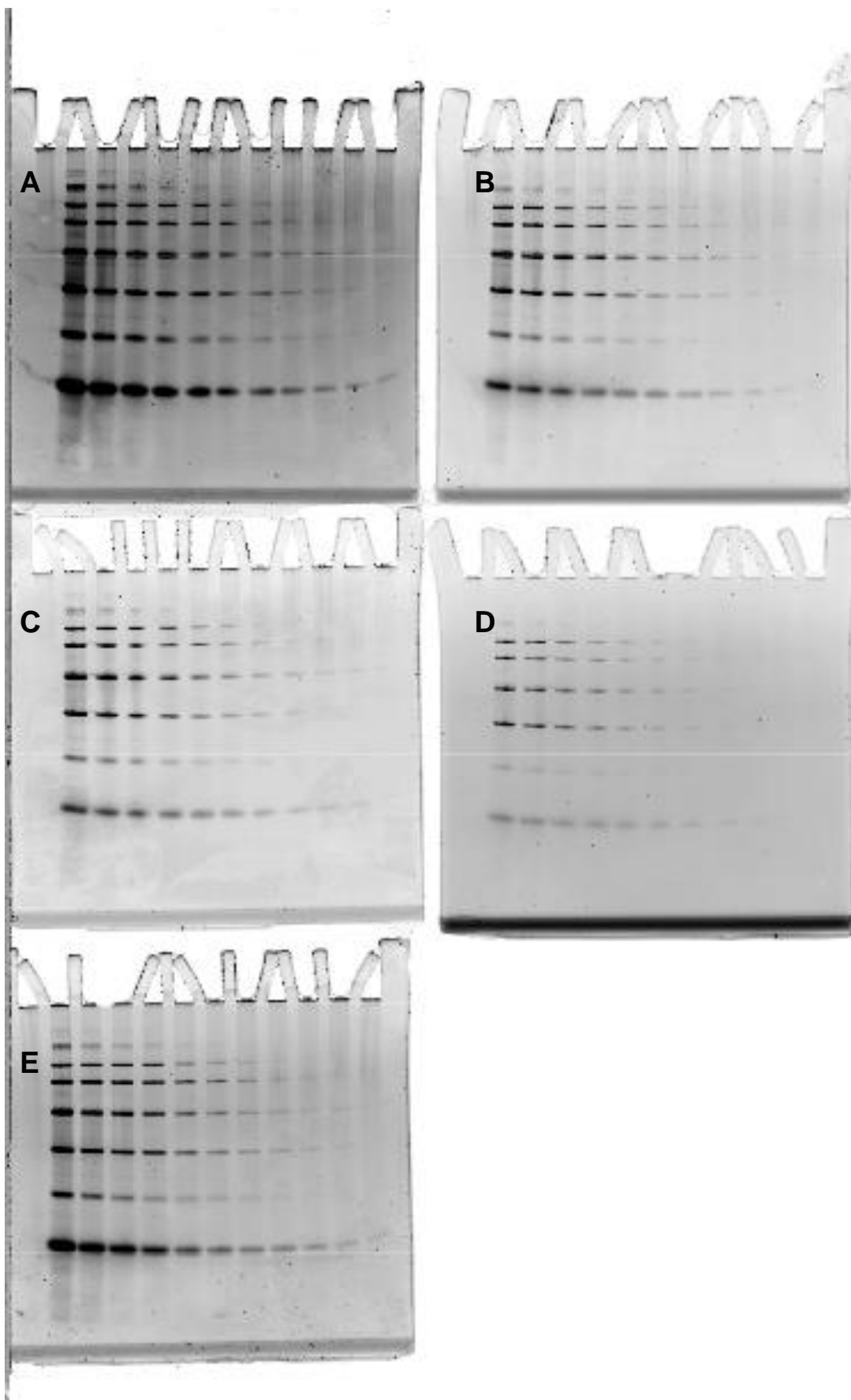


Figure S7. Serial dilution of 6 proteins from 128 ng to 128 pg/spot and stained with 1 $\mu\text{g/mL}$ (100 μL) of **4b** (A), **4d** (B), **4a** (C), **4c** (D) and Deep Purple (E). Gels were scanned together on a Typhoon laser scanner (532 nm) and contrast and brightness optimized automatically (ImageQuant)

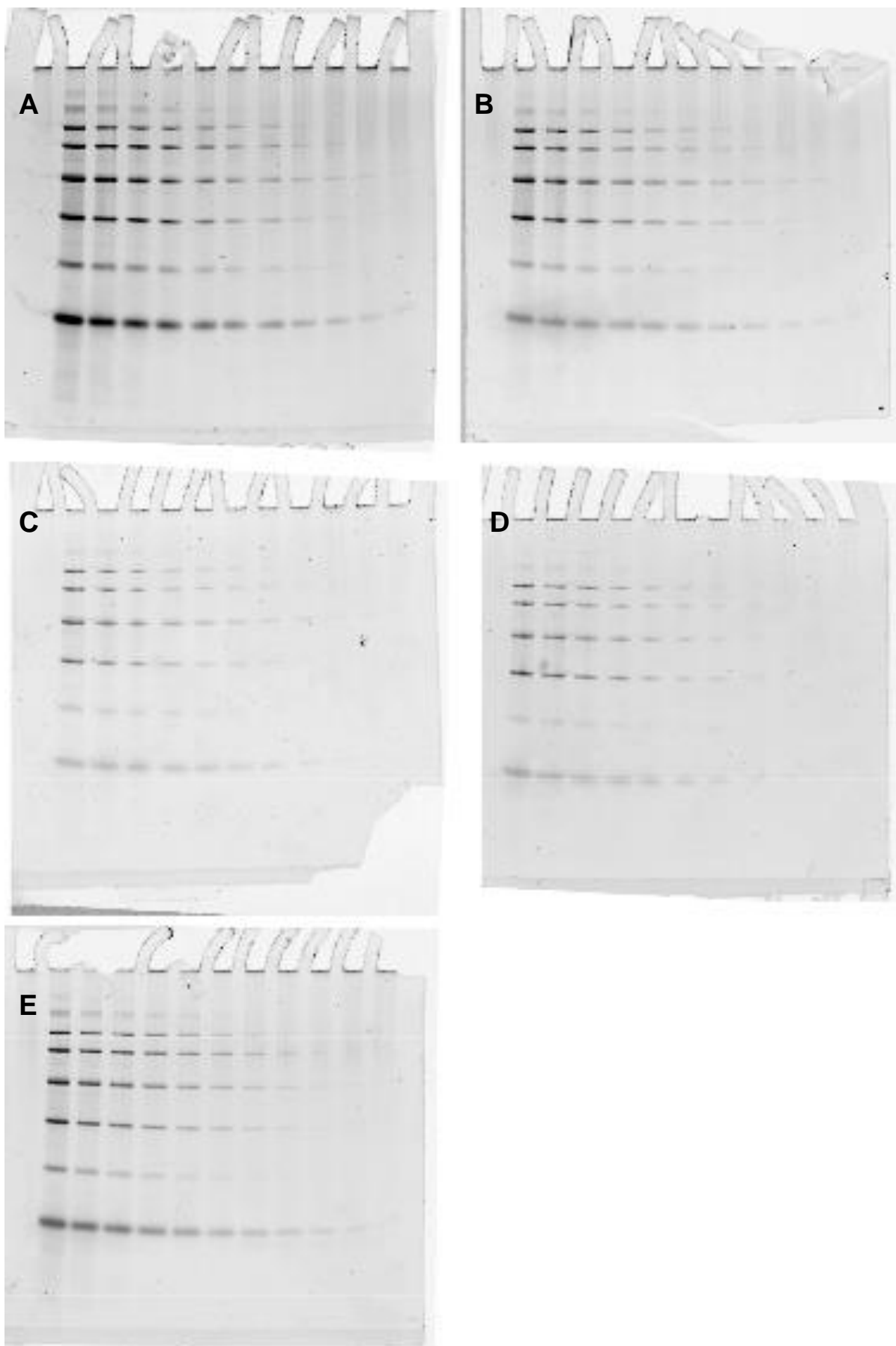


Figure S8. After exposure to intense UV irradiation ($4 \times 15\text{W}$, 312 nm) gels were rescanned at regular intervals. Here are the images from Figure S7 scanned under identical conditions and contrast settings after 990 s exposure.

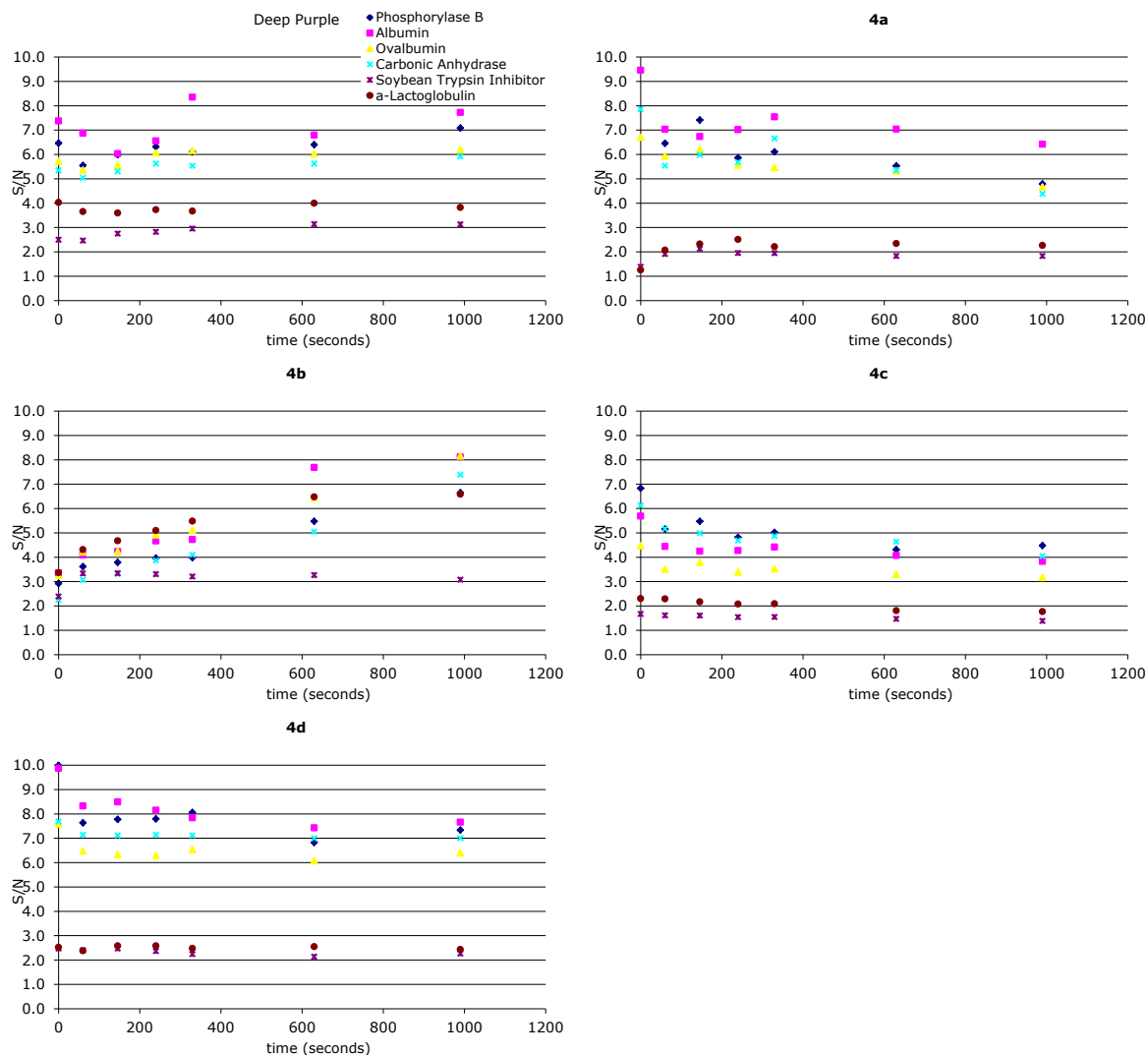


Figure S9. Signal to noise (S/N) calculations for Deep Purple and four analogs during photobleaching experiments.

Table S2. Photobleaching rates and half-lives for 1D gel staining using Deep Purple and epicocconone analogs (**4a-d**) calculated from Fig. 5 (Graphpad Prism; first order decay).

One phase exponential decay	Deep Purple	4a	4b	4c	4d
Best-fit values					
K	0.05991	0.09777	0.02128	0.01538	0.03281
HalfLife	11.57	7.089	32.57	45.06	21.13
95% Confidence Intervals					
HalfLife	10.61 to 12.72	6.509 to 7.783	28.05 to 38.82	30.77 to 84.09	18.21 to 25.15
Goodness of Fit					
R square	0.9959	0.9967	0.983	0.8705	0.9841

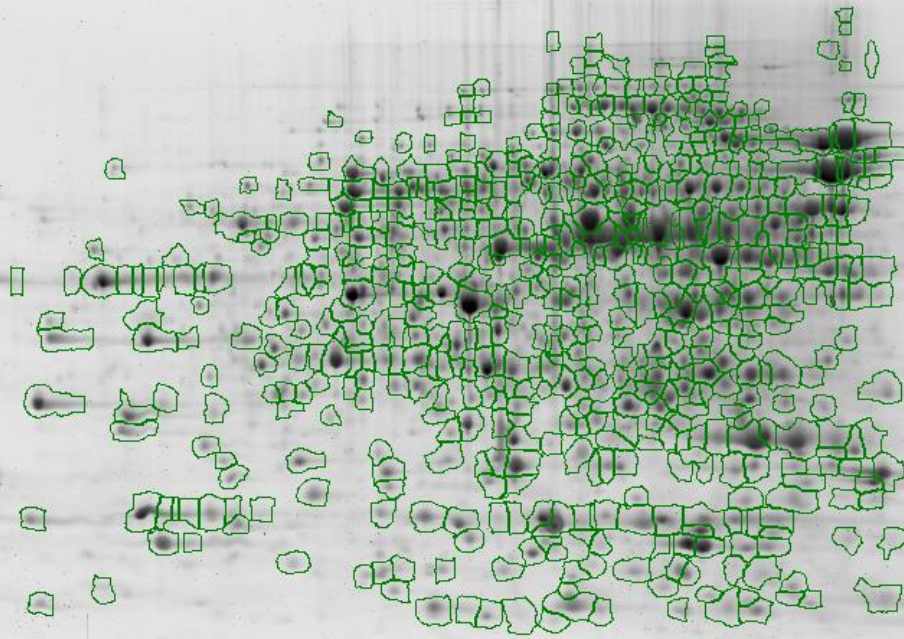
IV) 2D Gels:

Four 24 cm pI 3-10 Imobiline dry strips (GE Healthcare, 17-1234-01) rehydrated into IEF buffer (40 mM Tris-HCl, 2M thiourea, 5M urea, 2% CHAPS, 2% sulfobetaine 3-10, 0.002% bromophenol blue; 180 μ L per IPG strip) containing whole cell lysate from *E. coli* (100 μ g per IPG strip) for 6 h by laying the strips gel down in a rehydration tray. The strips were then focused for 48,000 Vh using an IPGphor 3 power pack (GE-Healthcare, 11-0033-64) before being stored at -80 °C. The strips were equilibrated using equilibration buffer (GelCompany 1019-20; 2×10 mL) for 2×15 min; the first equilibration also contained 1% (w/v) DTT while the second contained 5% (w/v) iodoacetamide. Separation in the second dimension was performed using a flatbed Tower electrophoresis unit (Serva HPE-TS1.01) using the standard manufacturer's protocol. 2DGel flatbed 12.5% acrylamide gels ($250 \times 200 \times 0.65$ mm) from GelCompany (1009-02) were used following the standard protocol. Briefly, focused IPG strips were removed from the IAA solution, placed gel side down onto the stacking gel portion of the 2D gels. Proteins were transferred to the 2D gel (100 V/28 mA/30 min., 200 V/52 mA/30 min., 300 V/80 mA/ 10 min.) and then the IPG strip removed. The 2D gel was run at 15 °C (1500 V/160 mA) for 3.5 hours. The gels were then removed and fixed in 15% ethanol (v/v), 1% citric acid (500 mL) on a rocker for 90 min and then stained in 100 mM sodium borate buffer (350 mL; pH 10.9) with either Deep Purple Total Protein Stain (GE Healthcare, RPN6306; Gel A 1.00 mL; Gel B 1.75 mL) or analog **4b** (Gel C 350 μ L; Gel D 100 μ l of a 1 mg/mL solution in DMSO). After 1 h staining the gels were destained in 15% ethanol (500 mL) for 45 minutes and transferred to fixative (500 mL; 15% ethanol (v/v), 1% citric acid (w/v)) for 45 minutes. All gels were imaged using a Typhoon Trio (GE, 63-0055-87) using the 532 nm Nd-YAG laser, 540 PMT, 610BP30 filter, 100 μ m resolution and normal sensitivity (Fig. 3, Fig. S9).

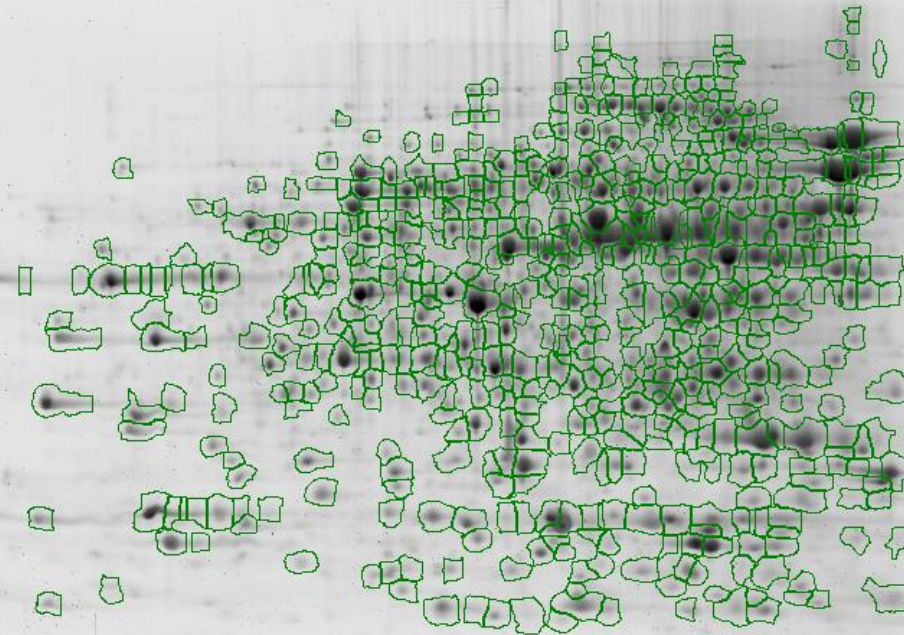
Analysis of the gels was performed initially using ImageQuant TL (v7.01, GE Healthcare) and spot counts and topographic analysis determined using Progenesis SameSpot software (v4.1 Non Linear Dynamics) using the following filter settings: $P < 0.05$, fold > 2 , spot area > 400 , automatic spot detection and vectors, automatic background subtraction (Fig. S9). The Deep Purple low concentration (standard GE protocol) stain was used as the reference gel as this is the standard manufacturer's protocol.

Both stains resulted in spots in all four quadrants of the gel and similar spot patterns were observed for both stains however **4b** was generally more intense and had improved staining of acidic (left half) proteins than Deep Purple. In the case of the high abundance proteins spot boundaries were overlaid in many cases, potentially reducing overall spot counts for **4b** at the higher concentration (Fig. S9C).

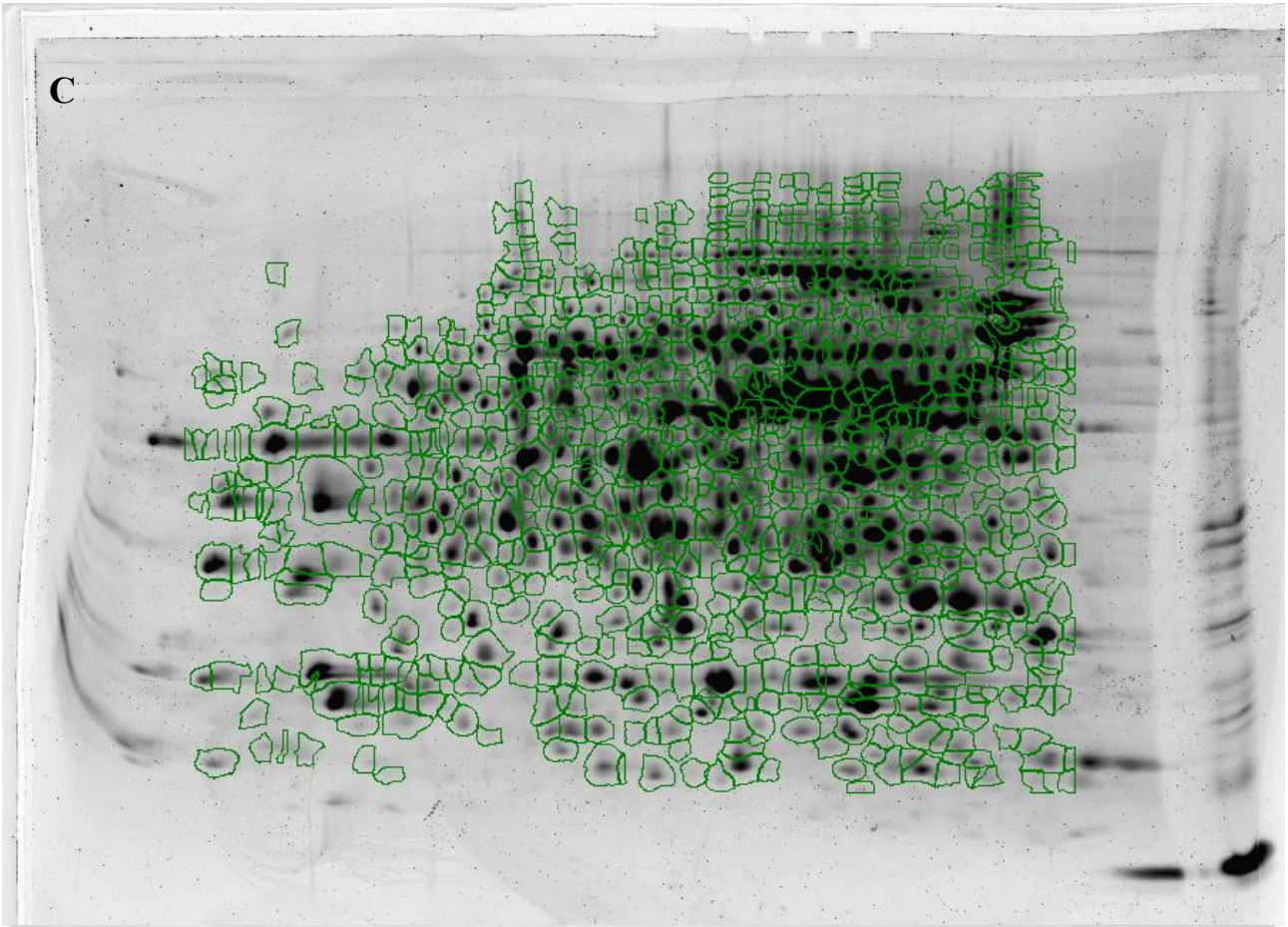
A



B



C



D

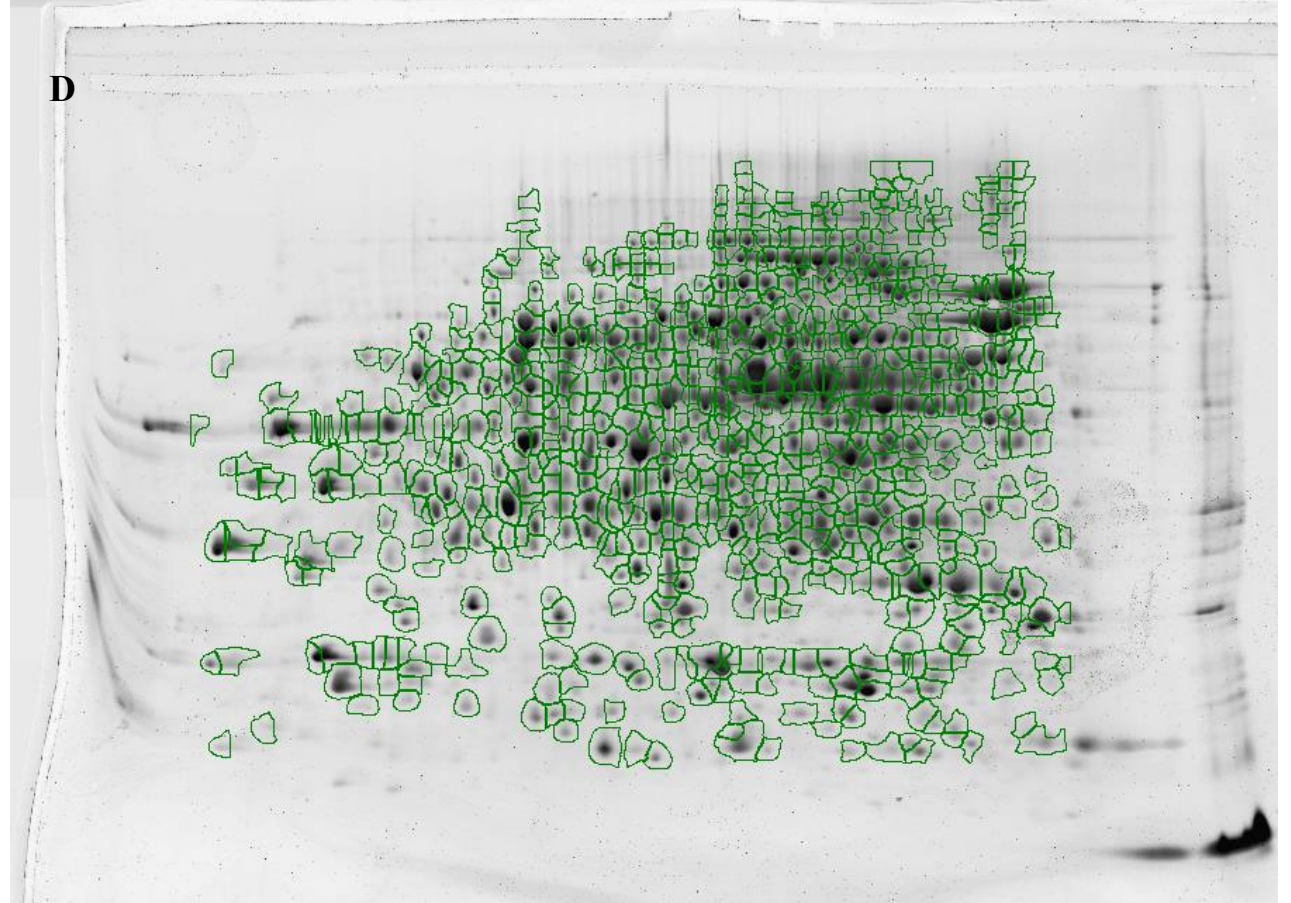


Figure S10. Spot counting using ImageQuant IT. (A) Deep Purple Total Protein Stain (1 mL), standard protocol. (B) Higher concentration of Deep Purple (1.75 mL of stain in 350 mL buffer). (C) Compound **4b** (350 µg in 350 mL) and (D) Compound **4b** (100 µg). The green borders indicate individual spots identified by the software.

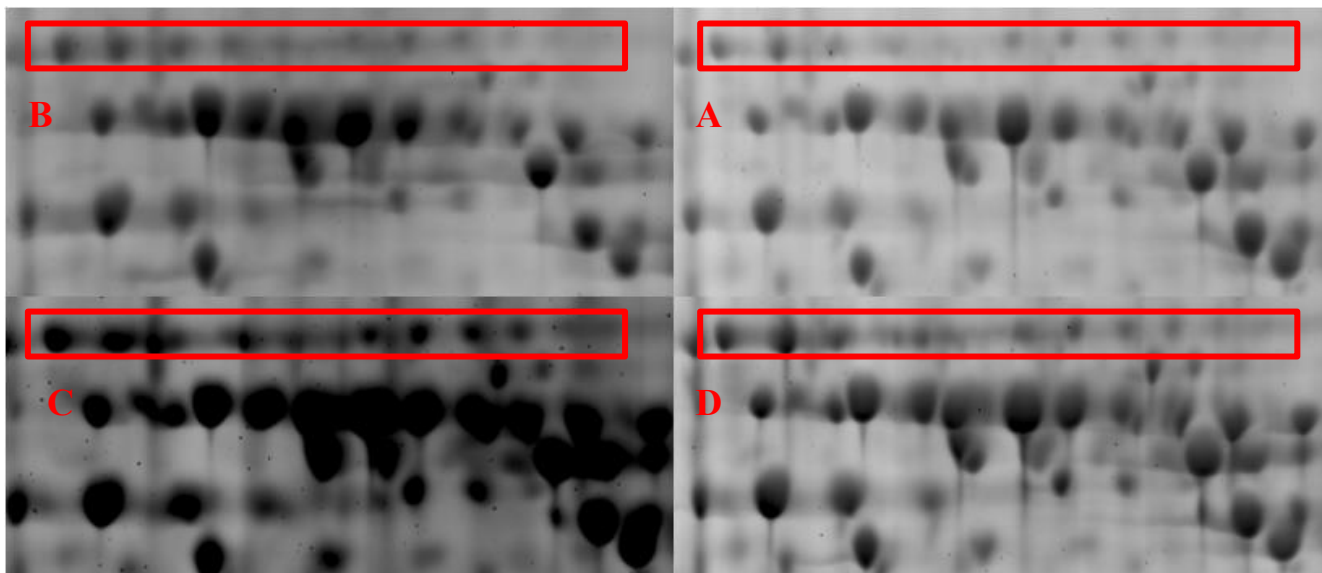


Figure S11. Enlarged detail of Fig. S10. (A) Deep Purple (1 mL), standard protocol. (B) Higher concentration of Deep Purple (1.75 mL). (C) Compound **4b** (350 µg) and (D) Compound **4b** (100 µg). The red rectangles show a trail of charge homologs of one protein (probably carbamylation) that is best seen in C or D.

The better peak shape observed with **4b** (Fig. 4), allowed the software to continue differentiating spots even at very high intensity. This gives the dye a better dynamic range as well as being overall more sensitive.

Analysis of the spot boundaries supports the supposition that blurred boundaries occur in Deep Purple staining, as there are a number of regions that were not separated into distinct spots with the Deep Purple stained gels that were resolved in the **4b** stained gels. Topographical analysis of the spots shows much steeper edges to the spots in the **4b** stained gels compared to the Deep Purple stained gels. Additionally peak analysis indicates that the staining intensity of the low **4b** concentration was similar to the staining intensity of the High Deep Purple stain concentration. This results in darker spots without loss of spot definition making the **4b** stain more suitable for 2D gel analysis.

In the fine detail (Fig. S11), the boxed region shows a trail of charge homologs that are easier to see in the gels stained with **4b**. In general the gels were of similar quality with Deep Purple being less sensitive than **4b**.

Table S3. Spot counts for gels stained with Deep Purple or **4b** (Fig S8) at two concentrations.

	Gel 1 -DP 1.75 mL	Gel 2 - DP 1 mL	Gel 3 - 4b 350 ug	Gel 4 - 4b 100 ug
Total spots detected	799	630	940	814
# Unmatched Spots	142	— ^a	229	133
# Matched spots	603	— ^a	549	497
% change from ref.	+21	— ^a	+33	+23

[a] not applicable as this is the reference gel

Looking at the total spot counts as measured with the SameSpot software (Table S3) it can be seen that **4b** was able to detect one-third more spots. In general these were new spots as almost all the spots detected with Deep Purple were also detected with **4b** (matched spots). This indicates that **4b** had similar staining characteristics but was just more sensitive.

V) Differential staining between 4b and Sypro Ruby

E. coli (strain MG1655) were maintained as glycerol stocks and stored at $-80\text{ }^{\circ}\text{C}$. Cultures were performed in LB medium (20 mL) inoculated with single colonies from LB agar plates under agitation (150 rpm). Bacteria were collected by centrifugation at ($4000 \times g$; 10 min.) and the pellet washed with Tris ($20 \times 50\text{ mL}$; 100 mM, pH 7). The pellet was freeze-dried and re-suspended in iso-electric focusing (IEF) buffer of the following composition: 7 M urea, 2 M thiourea, 0.1% amidosulfobetaine (ASB14), 20 mM Dithiotheritol (DTT) and 2% (v/v) carrier ampholytes (pH 4-7; GE Healthcare). The mixture was incubated twice during 10 min successively at $37\text{ }^{\circ}\text{C}$ and $-80\text{ }^{\circ}\text{C}$, and subsequently submitted to two cycles of 1 min. sonication on ice. Protein extracts were then harvested in the supernatant after elimination of cellular debris by centrifugation ($10,000 \times g$ for 30 min.). Protein concentrations were evaluated using the Bio-Rad protein assay and protein extracts were stored at $-20\text{ }^{\circ}\text{C}$ until further analysis.

For IEF, an additional volume of IEF buffer was added to the protein solution containing 50 μg of proteins in a final volume of 400 μL (with 0.4% of Coomassie blue). IEF was carried out with immobilized pH gradients (Immobiline DryStrips pH 4-7, 18 cm NL GE Healthcare). After passive rehydration for 24 h, IEF was performed by using an Multiphor II (GE Healthcare) as follows: 150 V for 1 h, 350V for 15 min., 750V for 45 min., 1.5 kV for 1 h, and 3.5kV for 17 h (1mA, constant) for a total of 70 kVh.

After two equilibration steps dedicated to reduction/alkylation of cysteines (using DTT (20 mM) and iodoacetamide (50 mM) respectively), the second dimension was obtained by SDS-PAGE, carried out with a Protean[®] II xi cell (Bio-Rad), and using a 12.5% (w/v) polyacrylamide resolving gel (width, 16 cm; length, 20 cm; thickness, 0.75 mm). Triplicate gels were fixed overnight and proteins were stained using either analogue **4b** or Sypro Ruby staining (Fig. S12). Gels were stored in water at $4\text{ }^{\circ}\text{C}$ before spot excision. For each condition, three independent cultures were obtained and analyzed.

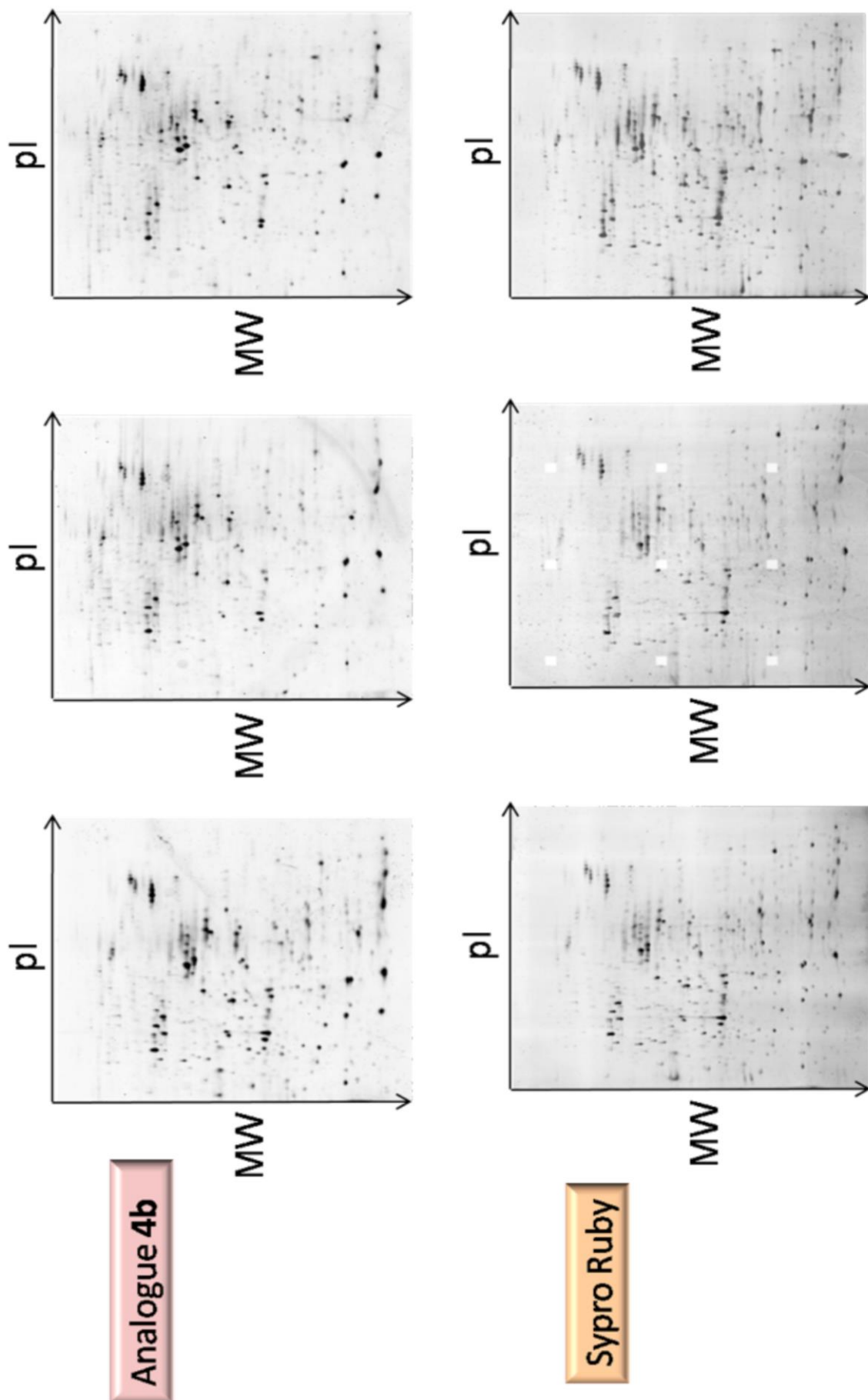


Figure S12: Triplicate gels of *E. coli* whole proteome stained with **4b** or Sypro Ruby.

Statistical analysis

Gels were scanned using the Fluorimager ProXpress from PerkinElmer and analyzed using the SameSpots 4.0 software (Nonlinear Dynamics, Newcastle upon Tyne, UK). Images were primarily tested for similar dynamic range and absence of saturation, and entirely pre-aligned before detection, resulting in optimal matching between all gels. The statistical tool integrated into the software, combines calculation of p values (ANOVA), q values, power, correlation analysis and Principal Component Analysis (PCA). PCA was used to interpret spot quantity variation among the different experimental conditions tested. This analysis is based upon the generation of a covariance matrix describing how each spot varies with respect to every other spot in the data set. For this analysis, ANOVA was calculated using a variance stabilization technique based on the logarithm of normalized volume. The spots with ANOVA < 0.05 , p value < 0.05 and a power > 0.8 were selected and analysed by peptide mass fingerprinting (Tables S4, S5).

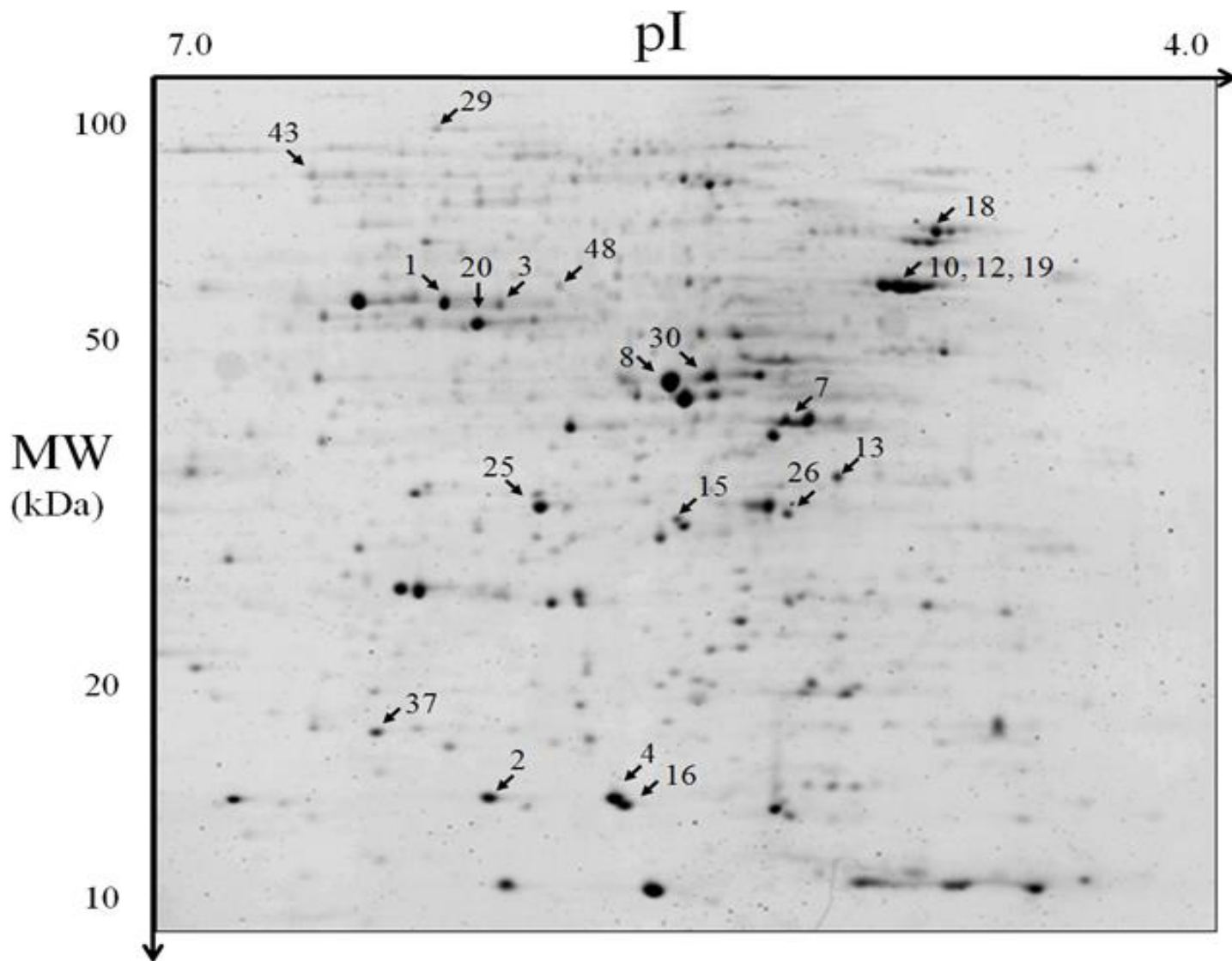


Figure S13: Differential staining between synthetic analogue **4b** versus Sypro Ruby. 2D-Gel of an *E. coli* proteome (protein loading 50 μ g; IPG strip pH 4-7) stained with Sypro Ruby and compound **4b**. Spots highlighted correspond to proteins that present enhanced intensity with **4b**.

Table S4: Proteins more intensely stained by 4b.

Id	Spot	Mass	pI	Score	Protein sequence coverage	Protein Id	Protein Name
A1	1	58665	6.66	476	73	b1676	Pyruvate kinase I
B1	2	15922	5.72	233	89	b2665	Protein ygaU
C1	3	58665	6.66	264	69	b1676	Pyruvate kinase I
D1	4	15399	5.43	205	68	b1237	DNA-binding protein H-NS
G1	7	41093	5.08	686	84	b2926	Phosphoglycerate kinase
H1	8	45626	5.32	273	79	b2779	Phosphopyruvate hydratase
B2	10	57293	4.85	580	93	b4143	Chaperonin groEL
D2	12	57293	4.85	714	93	b4143	Chaperonin groEL
E2	13	35049	5.03	330	29	b0008	Transaldolase
G2	15	31547	5.42	120	38	b2143	Cytidine deaminase
H2	16	15399	5.43	177	58	b1237	DNA-binding protein H-NS
B3	18	68941	4.83	365	76	b0014	Chaperone protein dnaK
C3	19	57293	4.85	560	90	b4143	Chaperonin groEL
D3	20	55188	5.80	115	32	b3734	H ⁺ -transporting two-sector ATPase
A4	25	32317	5.61	329	97	b3236	Malate dehydrogenase
B4	26	33347	5.25	126	73	b2150	Galactose/Glucose-binding protein
E4	29	97290	5.80	125	38	b3128	Translation initiation factor IF-2
F4	30	45626	5.32	354	73	b2779	Phosphopyruvate hydratase
E5	37	18333	5.96	271	74	b0426	Phi6 host factor
C6	43	87383	6.01	199	57	b0439	Endopeptidase La
H6	48	57293	5.53	419	59	b4006	PurH bifunctional enzyme

Table S5: Proteins more intensely stained by Sypro Ruby

Id	Spot	Mass	pI	Score	Protein sequence coverage	Protein Id	Name
E1	5	11525	5.79	73	45	b3029	Protein YgiN
A2	9	22071	4.85	279	40	b4207	FKBP-type 22 kDa peptidyl-prolyl cis-trans isomerase
C2	11	34820	5.47	295	55	b3916	6-phosphofructokinase
G3	23	38183	5.87	282	28	b4269	Putative oxidoreductase
H3	24	43090	5.64	276	50	b3617	Glycine C-acetyltransferase
C4	27	22071	4.85	82	17	b4207	FKBP-type 22 kDa peptidyl-prolyl cis-trans isomerase
D5	36	11994	6.19	75	56	b0435	BolA protein
A6	41	27275	5.21	68	57	b4401	Dye resistance protein
D6	44	26943	5.79	111	41	b0863	Arginine-binding periplasmic protein 1
E6	45	38803	5.77	84	55	b1062	Dihydro-otase

VI) ¹H and ¹³C NMR spectra

