Supporting Information

A Synthetic Analogue of Rocaglaol Displays a Potent and Selective Cytotoxicity in Cancer cells: Involvement of Apoptosis Inducing Factor and Caspase-12

Frédéric Thuaud, Yohann Bernard, Gülen Türkeri, Ronan Dirr, Geneviève Aubert, Thierry Cresteil, Aurélie Baguet, Catherine Tomasetto, Yuri Svitkin, Nahum Sonenberg, Canan Nebigil and Laurent Désaubry*
desaubry@chimie.u-strasbg.fr

Table of Contents

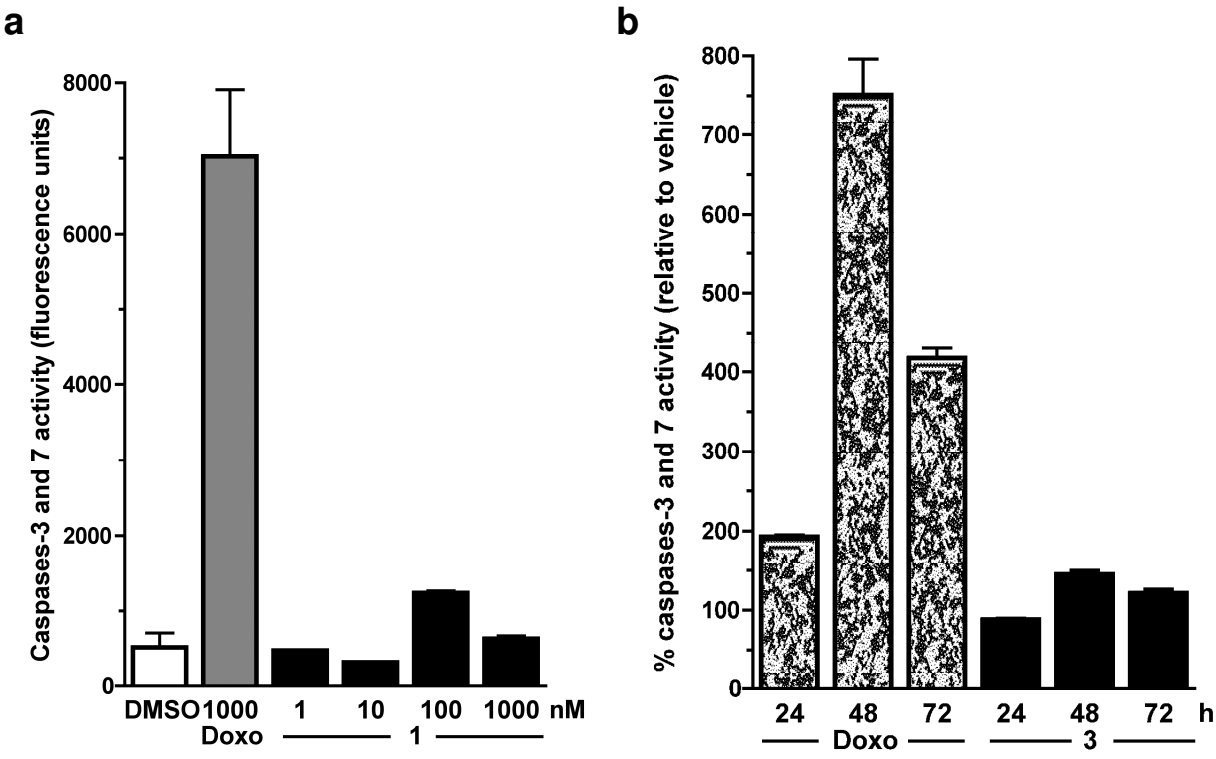


Figure S1 Flavaglines do not activate caspases-3/7 in HL60 cells **(a)** A 48 h treatment with racemic rocaglaol **1** does not activate caspases-3/7 in HL60 cells. HL60 cells were treated with **1** or doxorubicine (1 μM) as a positive control, for 48 h. Whole cell extracts were prepared and assayed for their ability to cleave the caspase-3-specific substrate (DEVD-AMC). Relative fluorescence release was measured spectroscopically. Reaction rates were calculated from the slope of the linear time-dependent reaction and expressed as the fold-activation over the control (HL60 with DMSO alone). **(b) 3** does not activate caspases-3/7 in HL60 cells after 24, 48 and 72 h.

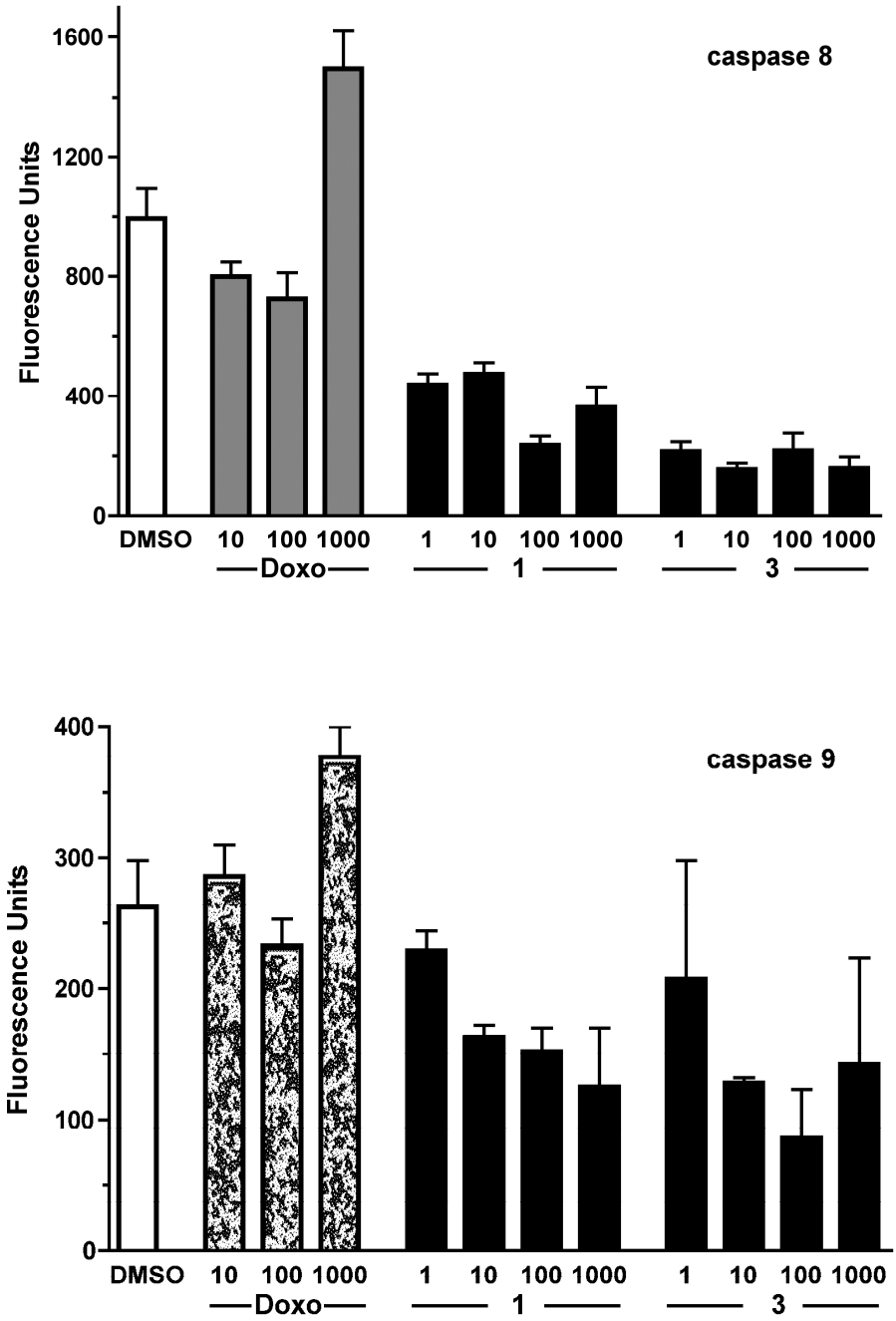


Figure S2 Flavaglines **1** and **3** (10^{-9}-10^{-6} M) do not activate caspases 8 and 9 in HL60 cells. HL60 cells were treated with **1**, **3** or doxorubicin as a positive control. Whole cell extracts were prepared and assayed for their ability to cleave the substrate of caspases 8 and 9 (LEHD-AMC and IETD-AMC). Relative fluorescence release was measured spectroscopically. Reaction rates were calculated from the slope of the linear time-dependent reaction and expressed as the fold-activation over the control (HL60 with DMSO alone).

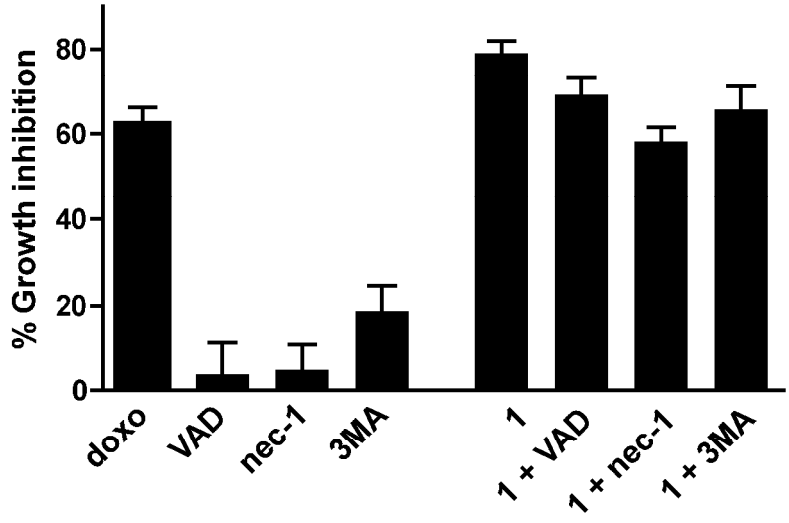


Figure S3 Inhibitors of caspases, necroptosis and autophagy do not block the antiproliferative effects of racemic rocaglaol **1** in HL60 cells. HL60 cells were treated for 72 h with **1** (50 nM) or vehicle in presence or not of Z-VAD-FMK (50 μM), necrostatin-1 (50 μM) and 3-methyladenine (2 mM), which inhibit caspases, autophagy and necroptosis respectively. Growth inhibition was not significantly altered by any of these inhibitors. Doxorubicine (1 μM) was used as a positive control.

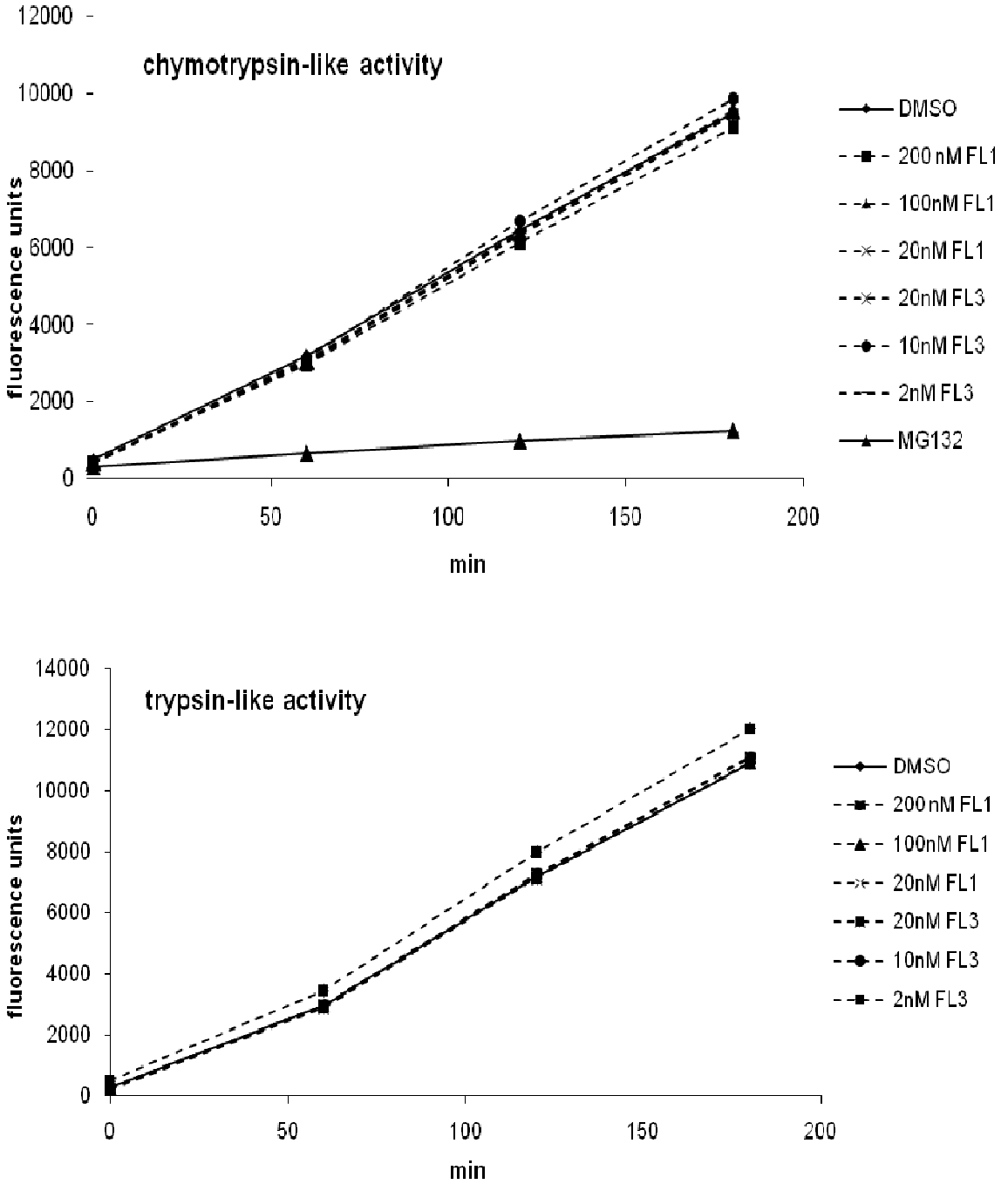


Figure S4 Flavaglines do not inhibit the proteasome. Neither **1** (20-200 nM) nor **3** (2-20 nM) were capable to elicit a reduction of protease activities. 100 nM MG132 (bortezomib) was used as positive control for chymotrypsin like activity.

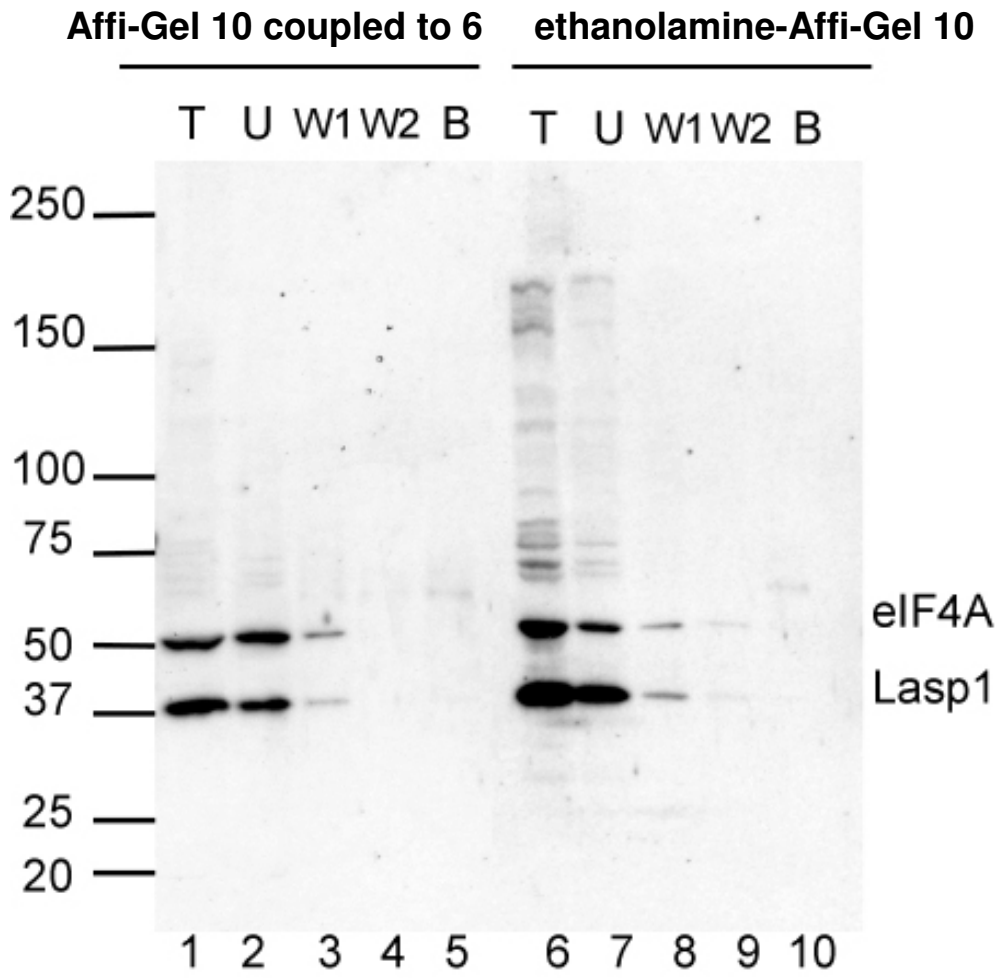


Figure S5 Pull-down experiment does not reveal any interaction between flavagline **6** and eIF4A. 500 μg of proteins from HeLa whole cell extracts (T, lanes 1and 6) were incubated with beads of Affi-Gel 10 coupled to **6** or blocked with ethanolamine, the supernatant was collected as the unadsorbed material (U, lanes 2 and 7). After washing (W1 and W2, lanes 3-4 and 8-9), bound proteins were recovered by 5 min at 100 °C using SDS- and β-mercaptoethanol-containing elution buffer (B, lanes 5 and 10). Proteins present in every collected fraction were analyzed by Western blot using anti-eIF4A and anti-Lasp1 antibodies. The proteins detected using the various antibodies and the protein molecular size markers (kilo Daltons) are indicated on the right on the left, respectively.

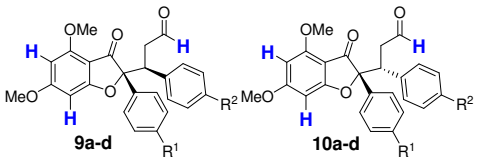
Synthesis of 1 (racemic rocaglaol)

3-(2,3-Dihydro-4,6-dimethoxy-2-(4-methoxyphenyl)-3-oxobenzofuran-2-yl)-3-phenylpropanal (9a). A suspension of benzofuranone **7** (R^1 = OMe, 4.4 g, 14.6 mmol) in *t*-BuOH (250 mL) was heated at 50 °C under argon. Benzyltrimethylammonium hydroxide in MeOH (40%, 333 µL, 0.80 mmol) and, immediately after, cinnamaldehyde **8** (3.70 mL, 29.2 mmol), were added. The mixture was stirred for 2 h at 50 °C, cooled to rt, concentred and acidified with 1 M HCl (30 mL), extracted with CH_2Cl_2, dried over $MgSO_4$ and concentrated to dryness. Purification of the resulting yellow oil by chromatography (Et_2O/Pentane 6:4) yielded 1.55 g (25%) of ketoaldehyde **9a** (1.55 g, 25%) as a white solid: R_f = 0.4 (Et_2O/ Hept 9:1). NMR ^1H ($CDCl_3$): 2.68 (1H, ddd, *J* = 1.3, 4.3, 17.3 Hz), 3.03 (1H, ddd, *J* = 2.4, 10.0, 17.2 Hz), 3.69 (3H, s), 3.78 (3H, s), 3.85 (3H, s), 4.20 (1H, dd, *J* = 4.4, 10.6 Hz), 5.79 (1H, d, *J* = 1.8 Hz), 6.21 (1H,d, *J* = 1.8 Hz), 6.88 and 7.65 (4H, AA'BB', *J* = 8.9 Hz), 7.07-7.17 (3H, m), 7.30-7.33 (2H, m), 9.39 (1H, dd, *J* = 1.3, 2.4 Hz). NMR ^{13}C ($CDCl_3$): 44.3, 47.0, 55.3, 55.9, 55.9, 88.4, 92.8, 93.6, 104.0, 114.1, 126.4, 127.3, 128.2, 128.5, 129.6, 136.8, 159.1, 159.6, 169.7, 174.2, 195.0, 200.3. IR (thin film): 2942, 2839, 1717, 1700, 1618, 1593, 1216, 1155 cm^{-1}.

Determination of the stereochemistry of compounds 9 and 10:

The structure of compounds **9a** and **10a** had been unambiguously established by Taylor and colleagues. The most significant differences in ^1H chemical shifts occurred at the dimethoxyphenyl moiety and the aldehyde. We observed the same trend with **9b**-**c** and **10b**-**c**.

Chemical shift or representative protons	
9a: 5.79, 6.21, 9.39	**10a:** 5.99, 6.30, 9.47
(5.80, 6.21, 9.39)*	(5.99, 6.30, 9.47)*
9b: 5.80, 6.23, 9.39	**10b:** 5.99, 6.32, 9.47
9c: 5.81, 6.21, 9.41	**10c:** 6.02, 6.33, 9.48
9d: 5.82, 6.23, 9.38	**10d:** 6.03, 6.32, 9.47



9-10a: R^1 = OMe, R^2 = H
9-10b: R^1 = H, R^2 = H
9-10c: R^1 = Br, R^2 = H
9-10d: R^1 = H, R^2 = OMe

* Davey, A. E.; Schaeffer, M. J.; Taylor, R. J. K. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2657.

6,8-Dimethoxy-8b-hydroxy-3a-(4-methoxyphenyl)-3-phenyl-2,3-3a,8b-tetrahydro-cyclopenta[*b*]benzofuran-1-one (12a). Trimethylsilyl cyanide (0.63 mL, 4.71 mmol) was added dropwise to a solution of aldehyde **9a** (680 mg, 1.57 mmol) in CH_3CN (8 mL) at room temperature under argon. Immediately after, zinc iodide (10 mg) was added and the resulting mixture was stirred for 1 h, filtered and concentrated to afford crude cyanohydrin **11a** (850 mg, 34%). LDA (1.73 mmol, 0.77 M in THF) was added dropwise at -78 °C under argon to a solution of cyanhydrin **11** (834 mg, 1.57 mmol) in dry THF (7 mL). After stirring for 2 h at -78 °C, the solution was heated to -50 °C for 10 min. The reaction was quenched by the addition of saturated aqueous ammonium chloride (10 mL). Standard extractive work-up (CH_2Cl_2) gave a yellow solid (690 mg). This solid was directly treated by tetra-*n*-butylammonium fluoride (1.43 mL, 1 M in THF) added dropwise at room temperature in dry THF (5 mL). The solution was stirred for 5 h and quenched by addition of water. Standard extractive work-up (AcOEt) and purification by flash chromatography (Et_2O) afforded **12a** (90 mg, 16%) as a white solid: R_f = 0.4 (Et_2O/ Hept 9:1). NMR ^1H ($CDCl_3$): 3.00 (1H, d, *J* = 7.4 Hz), 3.04 (1H, d, *J* = 5.2 Hz), 3.10 (1H, br s), 3.70 (3H, s), 3.83 (3H, s), 3.85 (3H, s), 3.86 (1H, d, *J* = 5.0 Hz), 6.11 (1H, d, *J* = 1.9 Hz), 6.34 (1H, d, *J* = 1.9 Hz), 6.68 (2H, d, *J* = 8.8 Hz), 6.95 (4H, dd, *J* = 4.2, 7.8 Hz), 7.11 (3H, dd, *J* = 1.7, 4.9 Hz). NMR ^{13}C ($CDCl_3$): 39.8, 48.6, 55.1, 55.6, 55.7, 88.5, 89.7, 92.6, 101.2, 106.5, 113.1, 125.8, 126.8, 127.8, 127.9, 128.0, 137.2, 158.4, 158.8, 161.2, 164.7, 210.7. HR-MS calcd for $C_{26}H_{24}K_1O_6$: 471.1210, found : 471.1204. IR (thin film): 3502, 2935, 2839, 1748, 1596, 1146.

6,8-Dimethoxy-3a-(4-methoxyphenyl)-3-phenyl-1,2,3,3a-tetrahydro-cyclopenta[*b*]benzofuran-1,8b(1H)-diol (*rac* rocaglaol, 1). CH_3CN (1.2 mL) was added at room temperature under argon to $Me_4NBH(OAc)_3$ (526 mg, 2 mmol) followed by the addition of glacial acetic acid (1.2 mL). After stirring for 30 min at room temperature a solution of ketone **12a** (90 mg, 0.21 mmol) in CH_3CN (2 mL) was added dropwise. The resulting mixture was stirred overnight at room temperature. The reaction was quenched by addition of saturated aqueous sodium hydrogen carbonate (40 mL). Standard extractive work-up (AcOEt) and purification by flash chromatography (Et_2O) afforded **1** (47 mg, 52%) as a white solid: R_f = 0.4 (Et_2O/Hept 9:1). NMR ^1H ($CDCl_3$): 1.80 (1H, br s), 2.20 (1H, d, *J* = 6.9 Hz), 2.73 (1H, ddd, *J* = 6.2, 13.8, 13.9 Hz), 3.31 (1H, br s), 3.69 (3H, s), 3.83 (3H, s), 3.89 (3H, s), 3.99 (1H, dd, *J* = 6.6, 14.1 Hz), 4.80 (1H, d, *J* = 6.2 Hz), 6.13 (1H, d, *J* = 1.9 Hz), 6.28 (1H, d, *J* = 1.9 Hz), 6.67 (2H, d, *J* = 8.9 Hz), 6.97-7.00 (2H, m), 7.08-7.13 (5H, m). NMR ^{13}C ($CDCl_3$): 36.4, 53.2, 55.0, 55.6, 55.7, 79.0, 89.4, 92.4, 94.8, 103.5, 107.8, 112.6, 126.2, 126.8, 127.6, 128.1, 128.9, 138.6, 157.0, 158.5, 160.9, 163.8. IR (thin film): 3500, 2937, 2838, 1599, 1513, 1498, 1147. HR-MS calcd for $C_{26}H_{26}Na_1O_6$: 457.1627, found : 457.1622.

Synthesis of 2

3-(2,3-Dihydro-4,6-dimethoxy-2-phenyl-3-oxobenzofuran-2-yl)-3-phenylpropanal (9b). A suspension of benzofuranone **7** (R^1 = H, 4.05 g, 15.0 mmol) in *t*-BuOH (250 mL) was heated at 50 °C under argon. Benzyltrimethylammonium hydroxide in MeOH (40%, 341 µL, 0.82 mmol) and, immediately after, cinnamaldehyde **8** (3.77 mL, 30.0 mmol), were added. After stirring for 5 min, the solution was cooled to rt, concentred and acidified with 1 M HCl (35 mL), extracted with CH_2Cl_2, dried over $MgSO_4$ and concentrated to dryness. Purification of the resulting yellow solid residue by chromatography (Et_2O/pentane 6:4) yielded 1.94 g (33%) of ketoaldehyde **9b** (1.90 g, 35%) as a white solid: R_f = 0.4 (Et_2O/ Hept 9:1). NMR ^1H ($CDCl_3$): 2.64 (1H, dd, *J* = 4.2, 17.3 Hz), 3.06 (1H, ddd, *J* = 2.1, 10.8, 17.3 Hz), 3.70 (3H, s), 3.86 (3H, s), 4.25 (1H, dd, *J* = 4.2, 10.8 Hz), 5.80 (1H, br s), 6.23 (1H, br s), 7.07-7.14 (3H, m), 7.30-7.39 (5H, m), 7.75 (2H, d, *J* = 7.7 Hz), 9.39 (1H, s). NMR ^{13}C ($CDCl_3$): 44.2, 47.0, 55.9, 55.9, 88.4, 92.9, 93.7, 107.3, 125.0, 127.4, 128.2, 128.3, 128.7, 129.6, 136.5, 136.6, 158.9, 169.7, 174.2, 194.7, 200.2. IR (thin film): 2842, 1719, 1700, 1616, 1589, 1153, 728 cm^{-1}.

6,8-Dimethoxy-3,3a-diphenyl-8b-hydroxy-2,3-3a,8b-tetrahydro-cyclopenta[*b*]benzofuran-1-one (12b). Trimethylsilyl cyanide (961 mg, 9.69 mmol) was added dropwise to a solution of aldehyde **9b** (1.3 g, 3.23 mmol) in CH_3CN (17 mL) at room temperature under argon. Immediately after, zinc iodide (10 mg) was added and the resulting mixture was stirred for 1 h, filtered and concentrated to afford crude cyanohydrin **11b** (1.76 g, 36%), which was used without purification. LDA (3.41 mmol, 1 M in THF) was added dropwise at -78 °C under argon to a solution of cyanohydrin **11b** (1.55 g, 3.10 mmol) in dry THF (15 mL). After stirring for 2 h at -78 °C the solution was heated to -50 °C for 10 min. The reaction was quenched by the addition of saturated aqueous ammonium chloride (20 mL). Standard extractive work-up (CH_2Cl_2) gave a yellow solid (1.49 g).This solid was directly treated by tetra-*n*-butylammonium fluoride (2.7 mL, 1 M in THF) added dropwise at room temperature in dry THF (13 mL). The solution was stirred overnight and quenched by addition of methanol. Standard extractive work-up (AcOEt) and purification by flash chromatography (Et_2O) afforded **12b** (440 mg, 35%) as a white solid: R_f = 0.4 (Et_2O/Hept 8:2). NMR ^1H ($CDCl_3$): 3.03 (1H, d, *J* = 1.8 Hz), 3.06 (1H, s), 3.16 (1H, br s), 3.83 (3H, s), 3.85 (3H, s), 3.94 (1H, d, *J* = 11.6 Hz), 6.12 (1H, d, *J* = 1.9 Hz), 6.37 (1H, d, *J* = 1.9 Hz), 6.92-6.95 (2H, m), 7.04-7.10 (5H, m), 7.13-7.15 (3H, m). NMR ^{13}C ($CDCl_3$): 39.7, 48.7, 55.5, 55.7, 88.8, 89.7, 92.6, 101.3, 106.4, 126.5, 126.8, 127.4, 127.6, 127.8, 127.9, 133.7, 137.0, 158.4, 161.1, 164.7, 210.6. IR (thin film): 3468, 3040, 2937, 2836, 1741, 1594, 1125. HR-MS calcd for $C_{25}H_{22}Na_1O_5$: 425,1365, found : 425.1359.

6,8-Dimethoxy-3,3a-diphenyl-1,2,3,3a-tetrahydro-cyclopenta[*b*]benzofuran-1,8b(1H)-diol (2). CH_3CN (4 mL) was added at room temperature under argon to $Me_4NBH(OAc)_3$ (1.7 g, 6.58 mmol) followed by the addition of glacial acetic acid (4 mL). After stirring for 30 min at room temperature a solution of ketone **12b** (380 mg, 0.94 mmol) in CH_3CN (10 mL) was added dropwise. The resulting mixture was stirred for 24 h at room temperature and quenched by addition of saturated aqueous ammonium chloride (50 mL). Standard extractive work-up (AcOEt) and purification by flash chromatography (Et_2O) afforded **2** (266 mg, 70%) as a white solid: R_f = 0.4 (Et_2O/Hept 9:1). NMR ^1H ($CDCl_3$): 1.93 (1H, br s), 2.16 (1H, dd, *J* = 7.4, 12.9 Hz), 2.76 (1H, ddd, *J* = 6.4, 13.9, 13.9 Hz), 3.37 (1H, br s), 3.82 (3H, s), 3.96 (3H, s), 4.01 (1H, dd, *J* = 6.6, 14.0 Hz), 4.80 (1H, d, *J* = 6.1 Hz), 6.13 (1H, d, *J* = 1.9 Hz), 6.30 (1H, d, *J* = 1.9 Hz), 6.96-7.00 (2H, m), 7.08-7.15 (6H, m), 7.20-7.23 (2H, m). NMR ^{13}C ($CDCl_3$): 36.2, 53.3, 55.6, 55.6, 79.1, 89.3, 92.4, 94.8, 103.4, 107.7, 124.8, 126.2, 127.1, 127.5, 127.6, 128.0, 134.7, 138.4, 156.9, 160.8, 163.8. IR (thin film): 3486, 3324, 3059, 3000, 2936, 1593, 1139 cm^{-1}. HR-MS calcd for $C_{25}H_{24}Na_1O_5$: 427,1521, found: 427.1516.

Synthesis of 4

3-(2,3-Dihydro-4,6-dimethoxy-2-phenyl-3-oxobenzofuran-2-yl)-3-(4-methoxyphenyl)-propanal (9d). Benzyltrimethylammonium hydroxide in MeOH (40%, 125 µL, 0.30 mmol) was added to a solution of benzofuranone **7** (R^1 = H, 500 mg, 1.85 mmol) and 4-methoxycinnamaldehyde **8** (450 mg, 2.77 mmol) in THF (35 mL) under argon. The mixture was stirred for 2 h at rt, concentred and quenched with a saturated aqueous ammonium chloride solution (10 mL), extracted with CH_2Cl_2, dried over $MgSO_4$ and concentrated to dryness. Purification of the resulting yellow oil by chromatography (Et_2O/Pentane 6:4) yielded 210 mg (26%) of ketoaldehyde **9d** as a white solid: R_f = 0.3 (Et_2O/ Hept 8:2). NMR ^1H ($CDCl_3$): 2.6 (1H, ddd, *J* = 1.2, 4.2, 17.2 Hz), 3.01 (1H, ddd, *J* = 2.6, 11.0, 17.2 Hz), 3.70 (3H, s), 3.71 (3H, s), 3.86 (3H, s), 4.21 (1H, dd, *J* = 4.2, 11.0 Hz), 5.82 (1H, d, *J* = 1.8 Hz), 6.23 (1H, d, *J* = 1.8 Hz), 6.69, 7.74 (4H, AA'BB', *J* = 8.8 Hz), 7.23-7.36 (5H, m), 9.38 (1H, dd, *J* = 1.2, 2.5 Hz). NMR ^{13}C ($CDCl_3$): 44.0, 47.1, 55.0, 55.9, 56.0, 88.8, 93.3, 94.3, 113.4, 125.2, 128.0, 128.6, 129.5, 130.5, 136.5, 158.5, 159.3, 170.3, 174.6, 195.6, 200.2. IR (thin film): 2941, 2838, 1700, 1617, 1591, 1216, 1155 cm^{-1}.

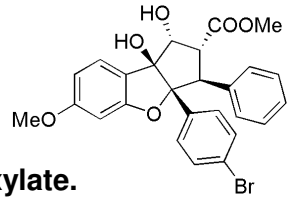
6,8-Dimethoxy-8b-hydroxy-3-(4-methoxyphenyl)-3a-phenyl-2,3-3a,8b-tetrahydro-cyclopenta[*b*]benzofuran-1-one (12d). Trimethylsilyl cyanide (674 mg, 6.80 mmol) was added dropwise to a solution of aldehyde **9d** (1.47 g, 3.40 mmol) in CH_3CN (20 mL) at room temperature under argon. Immediately after, zinc iodide (10 mg) was added and the resulting mixture was stirred for 1 h, filtered and concentrated to afford crude cyanohydrin **11d** (1.78 g, 49%) which was used in the next step without purification. LDA (4.2 mmol, 1 M in THF) was added dropwise at -78 °C under argon to a solution of cyanohydrin **11d** (1.75 g, 3.40 mmol) in dry THF (15 mL). After stirring for 2 h at -78 °C the solution was heated to -50 °C for 10 min. The reaction was quenched by the addition of saturated aqueous ammonium chloride (20 mL). Standard extractive work-up (CH_2Cl_2) gave a yellow solid (1.6 g).This solid was directly treated by tetra-*n*-butylammonium fluoride (3.7 mL, 1 M in THF) added dropwise at room temperature in dry THF (10 mL). The solution was stirred overnight and quenched by addition of methanol. Standard extractive work-up (CH_2Cl_2) and purification by flash chromatography (Et_2O) afforded tricyclic ketone **12d** (200 mg, 15%) as a yellow solid. R_f = 0.45 (Et_2O). NMR ^1H ($CDCl_3$): 2.97 (1H, d, *J* = 6.0 Hz), 3.02 (1H, d, *J* = 3.7 Hz), 3.70 (3H, s), 3.83 (3H, s), 3.85 (3H, s), 3.86-3.91 (1H, m), 6.11 (1H, d, *J* = 2.0 Hz), 6.35 (1H, d, *J* = 2.0 Hz), 6.62 (2H, d, *J* = 8.8 Hz), 6.82 (2H, d, *J* = 8.6 Hz), 7.03–7.18 (5H, m). NMR ^{13}C ($CDCl_3$): 40.1, 48.1, 55.1, 55.6, 55.7, 88.8, 89.7, 92.6, 101.3, 106.4, 113.2, 126.6, 127.5, 127.7, 128.9, 129.0, 133.8, 158.4, 161.1, 164.7, 168.3, 210.7. IR (thin film): 3503, 2932, 2842, 1746, 1596, 1148. HR-MS calcd for $C_{26}H_{24}KO_6$: 471.1209, found: 471.1204.

6,8-Dimethoxy-3-(4-methoxyphenyl)-3a-phenyl-1,2,3,3a-tetrahydro-cyclopenta[*b*]benzofuran-1,8b(1H)-diol (4). CH_3CN (1.8 mL) was added at room temperature under argon to $Me_4NBH(OAc)_3$ (985 g, 3.74 mmol) followed by the addition of glacial acetic acid (1.8 mL). After stirring for 30 min at room temperature a solution of ketone **12d** (180 mg, 0.416 mmol) in CH_3CN (4 mL) was added dropwise. The resulting mixture was stirred for 24 h at room temperature. The reaction was quenched by the addition of saturated aqueous sodium hydrogen carbonate (30 mL). Standard extractive work-up (CH_2Cl_2) and purification by flash chromatography (Et_2O) afforded **4** (100 mg, 55%) as a white solid: R_f = 0.4 (Et_2O). NMR ^1H ($CDCl_3$): 1.82 (1H, br s), 2.26 (1H, s), 2.83 (1H, dd, *J* = 6.9, 12.6 Hz), 3.44 (1H, br s), 3.80 (3H, s), 3.94 (3H, s), 4.00 (3H, s), 4.09 (1H, m), 4.92 (1H, d, *J* = 5.2 Hz), 6.25 (1H, s), 6.39 (1H, s), 6.72 (2H, m), 6.96 (2H, m), 7.24-7.36 (5H, m). NMR ^{13}C ($CDCl_3$): 36.7, 52.8, 55.1, 55.7, 55.8, 79.2, 89.4, 92.5, 94.9, 103.6, 107.9, 113.0, 127.0, 127.2, 127.7, 129.0, 130.6, 134.9, 157.0, 158.0, 160.9, 163.9. IR (thin film): 3504, 2942, 2838, 2359, 2597, 1597, 1513, 1146. HR-MS calcd for $C_{26}H_{26}Na_1O_6$: 457,1627, found : 457.1622.

Synthesis of fluorescent probes 5 and 21

Methyl 2-(4-bromophenyl)-5-hydroxy-2,5-methano-8-methoxy-10-oxo-2,3,4,5-tetrahydro-1-benzoxepin-4-carboxylate (15). A solution of hydroxyflavone **13** (1.0 g, 3 mmol) and methyl cinnamate (5.15 g, 30 mmol) in 90 mL of CH_2Cl_2/MeOH (3:1) was degassed with argon for 10 min in a pyrex tube. This mixture was then irradiated (450 W Iwasaki UV lamp) for 15 h at 0 °C under an argon atmosphere. The solution was concentrated *in vacuo*, purified by flash chromatography (heptane/AcOEt 4:6), heated to reflux in EtOAc (20 mL) for 4 h and concentrated *in vacuo* to give 560 mg (38%) of adduct **15** as a white solid. NMR ^1H ($CDCl_3$): 3.80 (3H, s), 3.82 (3H, m), 3.70 (1H, d, J = 8.3 Hz), 4.65 (1H, d, J = 8.3 Hz), 6.56 (2H, m), 7.19-7.40 (9H, m), 7.52 (1H, d, *J* = 8.4 Hz). NMR ^{13}C ($CDCl_3$): 52.8, 54.4, 55.8, 60.9, 88.6, 97.8, 97.3, 101.9, 108.4, 117.0, 122.5, 125.7, 127.4, 128.5, 130.0, 130.8, 134.7, 139.3, 152.6, 161.7, 171.5, 191.5.

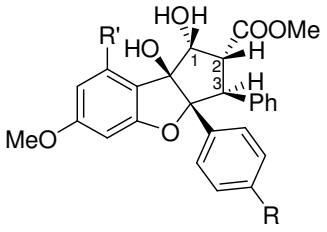
Methyl 3a-(4-bromophenyl)-8b-hydroxy-8-methoxy-1-oxo-3-phenyl-2,3-3a,8b-tetrahydro-cyclopenta[*b*]benzofuran-2-carboxylate (16) To a solution of aglain **15** (400 mg, 0.8 mmol) in MeOH (75 mL) was added a solution of NaOMe in MeOH (0.4 M, 5 mL) at 0 °C. The resulting solution was stirred for 20 min at 60 °C, cooled to rt, and quenched with saturated NH_4Cl, extracted with AcOEt (40 mL), and the organic layer was washed with water (2x20 mL) and brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo* to afford 370 mg of crude β-ketoester **16** as a white solid which was used without further purification. NMR ^1H ($CDCl_3$): 3.67 (3H, s), 3.87 (3H, s), 4.11 (1H, s), 4.54 (1H, s), 6.64 (1H, dd, *J* = 2.3, 8.4 Hz), 6.72 (1H, d, *J* = 2.3 Hz), 6.90-7.40 (10H, m). NMR ^{13}C ($CDCl_3$): 51.8, 53.2, 55.8, 55.9, 87.7, 96.9, 100.6, 108.4, 115.3, 118.0, 126.0, 127.8, 128.3, 128.4, 128.7, 129.0, 132.8, 138.5, 145.0, 159.9, 172.2, 203.9.



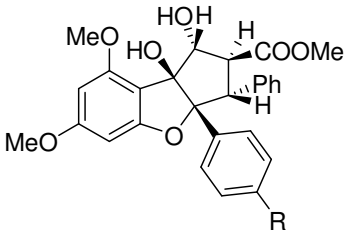
Methyl 3a-(4-bromophenyl)-1,8b-dihydroxy-8-methoxy-1-oxo-3-phenyl-2,3-3a,8b-tetrahydro-cyclopenta[*b*]benzofuran-2-carboxylate. Glacial acetic acid (230 μL, 3.93 mmol) was added to a solution of $Me_4NBH(OAc)_3$ (620 mg, 2.35 mmol) in CH_3CN (20 mL). After stirring for 5 min at room temperature, a solution of ketone **16** (200 mg, 0.39 mmol) in CH_3CN (10 mL) was added dropwise. The resulting mixture was stirred for 3 h at rt, successively quenched with saturated aqueous NH_4Cl (20 mL) and a 3 M aqueous solution of sodium/potassium tartrate (10 mL) and stirred for 30 min. The aqueous solution was extracted with CH_2Cl_2 (2x30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. Purification by HPLC (Symetry shield RP18, 7 µm, 19x300 mm, with a flow rate of 10 mL/min using a 50 min gradient from water (0.1% TFA) to CH_3CN (0.1% TFA)) yielded the title diol (100 mg, 50%) as a white solid. NMR ^1H ($CDCl_3$): 3.67 (3H, s), 3.87 (3H, s), 3.92 (1H, dd, *J* = 14, 6.2 Hz),

4.45 (1H, d, *J* = 14 Hz), 4.83 (1H, d, *J* = 6.2 Hz), 6.60 (2H, m), 6.90-7.15 (7H, m), 7.23 (2H, d, *J* = 8.6 Hz), 7.33 (1H, d, *J* = 8.1 Hz). NMR ^{13}C ($CDCl_3$): 51.1, 52.8, 56.2, 79.5, 93.6, 97.3, 101.8, 109.1, 119.1, 122.2, 127.5, 128.3, 128.7, 129.8, 131.0, 134.4, 137.0, 160.9, 163.6, 172.0.

Determination of the stereochemistry of compounds 17 and 23:
The structure of these compounds was based on the chemical shifts of H-1, H-2 and H-3 of the corresponding methyl esters which were compared to those of similar compounds described by Porco and colleagues (ref 19):



Endo rocaglic derivatives



Exo rocaglic derivatives

	R = Br, R' = H (Methyl ester of **17**)	R = Br, R' = OMe (Methyl ester of **23**)	R = R' = OMe (ref 19)	R = Br	R = OMe (ref 19)
H-1	4.83	4.98	5.01	4.78	4.76
H-2	3.92	3.85	3.80	3.24	3.23
H-3	4.45	4.32	4.28	3.96	4.02

The structure of the methyl ester of **13** was further confirmed by NOE (Figure S5). No NOE was observed between H-2 and H-3.

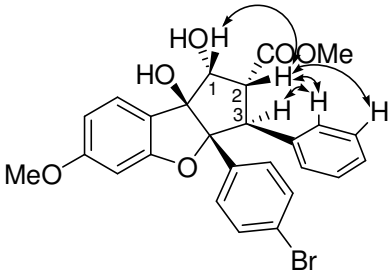


Figure S5 Key NOE correlations of the methyl ester of **17**.

3a-(4-Bromophenyl)-1,8b-dihydroxy-8-methoxy-1-oxo-3-phenyl-2,3-3a,8b-tetrahydro-cyclopenta[*b*]benzofuran-2-carboxylic acid (17). To a solution of the previous ester (20 mg, 0.04 mmol) in MeOH/H_2O (4:1, 10 mL) was added solid KOH (8 mg, 16 μmol). This solution was stirred for 12 h at 45 °C, cooled to rt and quenched with 4 mL of 1 N HCl. The aqueous solution was then extracted with EtOAc (2x5 mL). The combined organic layer was dried over $MgSO_4$, concentrated *in vacuo* to afford 19 mg (95%) of acid **17** as a white solid. NMR ^1H ($CDCl_3$): 3.80 (3H, s), 3.92 (1H, dd, *J* = 5.5, 13.4 Hz), 4.41 (1H, d, *J* = 13.4 Hz), 4.84 (1H, d, *J* = 5.5 Hz), 6.54 (2H, m), 6.90-7.30 (10H, m). NMR ^{13}C ($CDCl_3$): 53.6, 55.7, 78.9, 93.1, 97.3, 101.4, 108.7, 118.8, 121.8, 127.2, 128.0, 128.6, 129.4, 130.5, 134.0, 136.6, 160.3, 163.0, 174.8.

N-(2-(2-(2-(*tert*-Butoxycarbonylamino)ethoxy)ethoxy)ethyl)-2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamide (Boc-protected form of 20). To a solution of **18** (80 mg, 0.32 mmol) in anhydrous CH_2Cl_2 (2 mL) and DMF (0.5 mL), were added EDCI (75 mg, 0.39 mmol) and HOBt (55 mg, 0.39 mmol) at 0 °C. After 10 minutes, a solution of amine **19** (80 mg, 0.32 mmol) and of DIPEA (107 mg, 0.83 mmol) in anhydrous CH_2Cl_2 (1 mL) was added. After 10

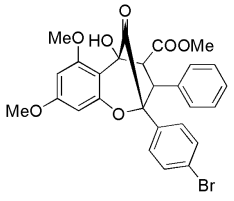
minutes at 0 °C, the solution was stirred for 12 h at rt. The solution was concentrated and the residue was purified on silica gel (2% Et_3N in CH_2Cl_2) to afford 110 mg (72%) of the Boc-protected form of **20**. NMR ^1H ($CDCl_3$): 1.41 (9H, s), 3.02 (6H, s), 3.10-3.55 (12H, m), 3.61 (2H, s), 6.05 (1H, s), 6.47 (1H, d, *J* = 2.4 Hz), 6.58 (1H, dd, *J* = 2.4, 8.9 Hz), 7.47 (1H, d, *J* = 8.9 Hz). NMR ^{13}C ($CDCl_3$): 28.4, 38.8, 39.6, 40.2, 40.5, 69.8, 70.0, 70.2, 70.4, 98.4, 107.6, 109.5, 110.7, 126.1, 150.5, 153.2, 156.1, 156.8, 159.4, 172.5.

N-(2-(2-(2-(Amino)ethoxy)ethoxy)ethyl)-2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamide (**20**). A mixture of TFA/ CH_2Cl_2 (1:4, 5 mL) was added dropwise to a solution of the previous N-Boc compound (100 mg, 0.21 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The solution was stirred at room temperature for 12 h and concentrated *in vacuo*. The solid was diluted three times in EtOAc (20 mL), dried over $MgSO_4$ and concentrated *in vacuo* to afford 80 mg of amine **20** (98%) as an yellow oil. NMR ^1H ($CDCl_3$): 2.94 (6H, s), 3.10-3.55 (12H, m), 3.62 (2H, s), 6.08 (1H, s), 6.51 (1H, d, *J* = 2.4 Hz), 6.68 (1H, dd, *J* = 2.4, 8.9 Hz), 7.41 (1H, d, *J* = 8.9 Hz). NMR ^{13}C ($CDCl_3$): 39.3, 39.8, 40.3, 40.5, 69.9, 70.2, 70.4, 98.4, 107.6, 109.5, 110.7, 126.1, 150.5, 153.2, 156.1, 159.4, 172.5.

Fluorescent probe 5. To a solution of flavagline **17** (50 mg, 0.10 mmol) in anhydrous CH_2Cl_2 (2 mL) were added EDCI (23 mg, 0.12 mmol) and HOBt (17 mg, 0.12 mmol) at 0 °C. After 10 minutes, a solution of coumarin **20** (48 mg, 0.10 mmol) and of DIPEA (33 mg, 0.25 mmol) in anhydrous CH_2Cl_2 (1 mL) was added dropwise. After 10 minutes at 0 °C, the solution was stirred for 12 h at rt. The solution was concentrated and purified by flash chromatography (2% Et_3N in CH_2Cl_2 followed by CH_2Cl_2/EtOH 8:2) to afford 88 mg of a mixture of diastereomers. A pure sample of fluorescent probe **5** (19 mg, 22%) was isolated by RP-HPLC (Symetry shield RP18, 7 µm, 19x300 mm, with a flow rate of 10 mL/min using a 50 min gradient from water (0.1% TFA) to CH_3CN (0.1% TFA)). NMR ^1H ($CDCl_3$): 3.03 (6H, s), 3.05-3.45 (12H, m), 3.60 (2H, s), 3.81 (3H, s), 3.95 (1H, dd, *J* = 5.5, 14 Hz), 4.48 (1H, d, *J* = 14 Hz), 4.81 (1H, d, *J* = 5.5 Hz), 6.08 (1H, s), 6.50-6.70 (4H, m), 7.04-7.10 (7H, m), 7.19 (2H, d, *J* = 9.0 Hz), 7.32 (1H, d, *J* = 7.9 Hz), 7.52 (1H, d, *J* = 8.9 Hz). NMR ^{13}C ($CDCl_3$): 39.4, 39.8, 40.4, 40.8, 51.7, 55.8, 56.3, 69.5, 69.6, 70.2, 70.3, 79.3, 93.3, 96.7, 98.5, 101.6, 108.2, 108.7, 109.8, 110.1, 119.9, 121.7, 126.1, 127.0, 127.1, 128.2, 128.6, 129.6, 130.6, 134.5, 136.7, 153.3, 153.4, 156.2, 160.6, 162.7, 162.8, 168.4, 171.7.

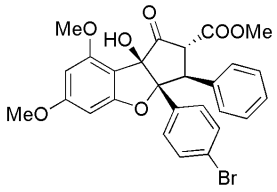
N-(2-(2-(2-(Acetamido)ethoxy)ethoxy)ethyl)-2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetamide (**21**). To a solution of coumarin **20** (30 mg, 0.08 mmol) in 5 mL of were added Ac_2O (9 µL, 95 µmol) and a catalytic amount of DMAP. This solution was stirred at rt for 2 h. The mixture was then quenched with 1 N HCl (3 mL). The aqueous layer was extract with EtOAc (2x15 mL) and the combined organic layers was washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (CH_2Cl_2/EtOH 9:1) to afford 30 mg (88%) of amide **21** as a yellow solid. NMR ^1H ($CDCl_3$): 1.97 (3H, s), 3.03 (6H, s), 3.30-3.60 (12H, m), 3.62 (2H, s), 6.05 (1H, s), 6.47 (1H, d, *J* = 2.5 Hz), 6.59 (1H, dd, *J* = 2.5, 8.9 Hz), 7.49 (1H, d, *J* = 8.9 Hz). NMR ^{13}C ($CDCl_3$): 23.4, 39.5, 39.8, 40.3, 40.6, 69.7, 70.0, 70.3, 70.5, 98.3, 108.6, 109.4, 110.2, 126.0, 150.5, 153.3, 156.2, 162.1, 168.3, 170.7.

Synthesis of flavagline 6

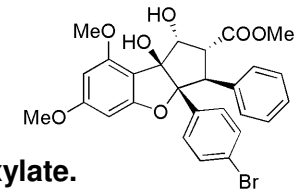


Methyl 2-(4-bromophenyl)-6,8-dimethoxy-5-hydroxy-2,5-methano-10-oxo-2,3,4,5-tetrahydro-1-benzoxepin-4-carboxylate.
A solution of hydroxyflavone **22** (1.0 g, 2.7 mmol) and methyl cinnamate **14** (3.43 g, 21 mmol) in 90 mL of CH_2Cl_2/MeOH (3:1) was degassed with argon for 10 min in a pyrex tube. This mixture was then irradiated (450 W Iwasaki UV lamp) for 30 h at 0 °C under an argon atmosphere. The solution was concentrated *in vacuo*, purified by flash chromatography

(heptane/AcOEt 8:2 to 4:6), heated to reflux in EtOAc (20 mL) for 4 h and concentrated *in vacuo* to give 400 mg (29%) of the title compound as a white solid. NMR ^{1}H ($CDCl_3$): 3.56 (3H, s), 3.76 (3H, s), 3.83 (3H, s), 4.17 (1H, d, *J* = 9.1 Hz), 4.49 (1H, d, *J* = 9.1 Hz), 6.09 (1H, d, *J* = 2.1 Hz), 6.19 (1H, d, *J* = 2.1 Hz), 6.90-7.20 (5H, m), 7.21 (2H, d, *J* = 9.1 Hz), 7.50 (2H, d, *J* = 9.1 Hz). NMR ^{13}C ($CDCl_3$): 51.9, 52.4, 53.5, 54.6, 55.4, 55.6, 56.0, 56.3, 57.2, 62.3, 81.0, 81.3, 83.8, 87.5, 92.9, 93.9, 94.6, 98.0, 103.7, 104.7, 121.9, 122.1, 126.9, 127.5, 128.1, 128.5, 128.9, 129.0, 129.3, 129.8, 130.4, 130.5, 130.7, 131.3, 132.6, 134.8, 137.5, 139.4, 152.7, 153.5, 158.2, 158.6, 161.5, 162.2, 170.6, 171.7, 202.9.



Methyl 3a-(4-bromophenyl)-6,8-dimethoxy-8b-hydroxy-1-oxo -3-phenyl-2,3-3a,8b-tetrahydro-cyclopenta[*b*]benzofuran-2-carboxylate. To a solution of the previous photochemical adduct (120 mg, 0.2 mmol) in MeOH (15 mL) was added a solution of NaOMe in MeOH (0.1 M, 5 mL) at 0 °C. The resulting solution was stirred for 20 min at 60 °C, cooled to rt, and quenched with saturated NH_4Cl, extracted with AcOEt (20 mL), and the organic layer was washed with water (2x20 mL) and brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo* to afford 120 mg of crude β-ketoester as white solid which was used without further purification. NMR ^{1}H ($CDCl_3$): 3.65 (3H, s), 3.77 (3H, s), 3.84 (3H, s), 4.05 (1H, d, J = 12.7 Hz), 4.24 (1H, d, J = 12.7 Hz), 4.47 (1H, s), 6.10 (1H, d, J = 2.0 Hz), 6.33 (1H, d, J = 2.0 Hz), 6.90-7.20 (5H, m), 7.27 (2H, d, J = 8.7 Hz), 7.50 (2H, d, J = 8.7 Hz). NMR ^{13}C ($CDCl_3$): 51.9, 53.1, 55.7, 55.8, 56.9, 88.5, 89.2, 92.7, 99.1, 105.9, 122.0, 127.0, 127.8, 128.2, 128.5, 130.9, 134.3, 135.1, 158.7, 160.7, 165.1, 170.0, 203.1.



Methyl 3a-(4-bromophenyl)-1,8b-dihydroxy-6,8-dmethoxy-1-oxo -3-phenyl-2,3-3a,8b-tetrahydro-cyclopenta[*b*]benzofuran-2-carboxylate. Glacial acetic acid (128 μL, 2.22 mmol) was added to a solution of $Me_4NBH(OAc)_3$ (351 mg, 1.33 mmol) in CH_3CN (3 mL). After stirring for 5 min at room temperature, a solution of the previous compound (120 mg, 0.22 mmol) in CH_3CN (10 mL) was added dropwise. The resulting mixture was stirred was stirred for 3 h at rt, successively quenched with saturated aqueous NH_4Cl (15 mL) and a 3 M aqueous solution of sodium/potassium tartrate (3 mL) and stirred for 30 min. The aqueous solution was extracted with AcOEt (2x30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. Purification by HPLC (Symetry shield RP18, 7 μm, 19x300 mm, with a flow rate of 10 mL/min using a 50 min gradient from water (0.1% TFA) to CH_3CN (0.1% TFA)) yielded the title diol (60 mg, 50%) as a white solid. NMR ^{1}H ($CDCl_3$): 3.64 (3H, s), 3.82 (3H, s), 3.86 (3H, s), 3.92 (1H, dd, *J* = 14.1, 6.6 Hz), 4.33 (1H, d, *J* = 14.1 Hz), 5.01 (1H, d, *J* = 6.6 Hz), 6.12 (1H, d, J = 2.0 Hz), 6.27 (1H, d, J = 2.0 Hz), 6.87 (2H, m), 7.07 (5H, m), 7.25 (2H, d, *J* = 8.3 Hz). NMR ^{13}C ($CDCl_3$): 50.4, 52.1, 55.1, 55.8, 60.5, 79.7, 89.6, 92.8, 93.8, 101.7, 107.6, 121.7, 126.9, 127.8, 128.0, 129.6, 130.3, 134.1, 136.7, 157.1, 160.7, 164.3, 170.5.

3a-(4-Bromophenyl)-1,8b-dihydroxy-6,8-dimethoxy-1-oxo-3-phenyl-2,3-3a,8b-tetrahydro-cyclopenta[*b*]benzofuran-2-carboxylic acid (23). To a solution of the previous ester (240 mg, 0.44 mmol) in MeOH (8 mL) was added solid KOH (149 mg, 0.89 mmol). This solution was stirred for 12 h at 45 °C, cooled to rt and quenched with 5 mL of 1 N HCl. The aqueous solution was then extracted with EtOAc (2x15 mL). The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to afford 230 mg (95%) of acid **23** as a white solid. NMR ^{1}H ($CDCl_3$): 3.83 (3H, s), 3.84 (3H, s), 3.9 (1H, dd, *J* = 5.6, 13.9 Hz), 4.29 (1H, d, *J* = 13.9 Hz), 5.02 (1H, d, *J* = 5.6 Hz), 6.12 (1H, d, *J* = 2.1 Hz), 6.27 (1H, d, *J* = 2.1 Hz), 6.88 (2H, m), 7.05 (5H, m), 7.25 (2H, d, *J* = 8.6 Hz). NMR ^{13}C ($CDCl_3$):

50.4, 55.1, 56.0, 79.7, 89.7, 93.0, 93.9, 101.8, 107.3, 121.9, 127.1, 127.9, 128.1, 129.7, 130.5, 133.9, 136.4, 157.1, 160.9, 164.5, 174.7.

Azide 25. To a solution of flavagline **23** (230 mg, 0.44 mmol) in anhydrous CH_2Cl_2 (15 mL) were added EDCI (105 mg, 0.55 mmol) and HOBt (74 mg, 0.55 mmol) at 0 °C. After 10 minutes, a solution of amine **24** (160 mg, 0.46 mmol) and DIPEA (153 mg, 1.18 mmol) in anhydrous CH_2Cl_2 (4 mL) was added. After 10 minutes at 0 °C, the solution was stirred for 12 h at rt. The solution was concentrated and purified by flash chromatography (CH_2Cl_2/EtOH 95:5) to afford 250 mg (64%) of adduct **25** as a white solid. NMR ^{1}H ($CDCl_3$): 3.20-3.70 (28H, m), 3.80-3.90 (7H, m), 4.48 (1H, d, *J* = 14.0 Hz), 4.81 (1H, d, *J* = 5.7 Hz), 6.11 (1H, d, *J* = 2.1 Hz), 6.27 (1H, d, *J* = 2.1 Hz), 6.90-7.15 (7H, m), 7.22 (2H, d, *J* = 8.6 Hz). NMR ^{13}C ($CDCl_3$): 39.5, 50.9, 51.7, 55.8, 56.3, 69.9, 70.2, 70.3, 70.7, 70.8, 70.9, 79.3, 89.0, 92.5, 93.9, 101.9, 107.0, 121.5, 126.9, 128.1, 128.4, 129.7, 130.2, 134.9, 136.7, 157.6, 161.3, 164.1, 170.9.

Amine 6. Thiophenol (150 µL, 1.4 mmol) and Et_3N (150 µL, 1.4 mmol) were added to a solution of $SnCl_2$ (66 mg, 0.3 mmol) in CH_3CN (25 mL) at rt. After 5 minutes, azide **25** (200 mg, 0.2 mmol) was added in 4 mL of CH_3CN. The solution was stirred at rt for 3 h and quenched with saturated $NaHCO_3$ (50 mL). The mixture was extracted with CH_2Cl_2 (4x40 mL) and the combined organic phase was washed with brine (150 mL), dried over $MgSO_4$ and concentrated *in vacuo*. HPLC purification (Symetry shield RP18, 7 µm, 19x300 mm, with a flow rate of 10 mL/min using a 50 min gradient from water (0.1%TFA) to CH_3CN (0.1% TFA)) afforded 140 mg (72%) of amine **6**. NMR ^{1}H ($CDCl_3$): 2.92 (2H, t, J = 5.2 Hz), 3.40-3.80 (26H, m), 3.86 (6H, m), 4.12 (1H, dd, J = 4.6, 14.1 Hz), 4.55 (1H, d, *J* = 14.1 Hz), 4.88 (1H, d, *J* = 4.6 Hz), 6.13 (1H, d, *J* = 1.9 Hz), 6.30 (1H, d, *J* = 1.9), 6.00-7.15 (9H, m). NMR ^{13}C ($CDCl_3$): 39.1, 39.8, 50.8, 55.6, 55.7, 69.4, 69.7, 69.8, 69.9, 70.0, 70.1, 70.6, 79.6 , 88.7, 92.1, 94.2, 102.2, 107.4, 121.0, 126.4, 127.8, 128.2, 129.6, 130.0, 135.6 , 137.5, 157.9, 161.8, 162.4, 172.0.

Flavagline 6-conjugated matrix

A volume of 1 mL of packed Affigel 10 (Bio-Rad) was washed on a glass filter with CH_3CN and added to a solution of **6** (12 mg, 20 µmol) in CH_3CN (0.8 mL) and triethylamine (20 µL). This suspension was gently rotated for 12 h at room temperature. HPLC analysis of the supernatant indicated that the coupling was quantitative. Ethanolamine (50 µL) was added and after 4 h, the resin was thoroughly washed with CH_3CN, EtOH and water. HPLC analysis of the washing solution did not reveal any trace of amine **6**, indicating a quantitative coupling.

Supplementary Biological Assay Methods

Cell culture. The human cell lines KB (nasopharyngeal epidermoid carcinoma), HepG2 (hepatocarcinoma), HCT116 and HCT15 were purchased from ECACC (Salisbury, UK) and HL60 cells from ATCC. MCF7 were given by Dr Matthias Kassack (Bonn University, Germany). KB and HepG2 cells were grown in D-MEM medium supplemented with 10% fetal calf serum, in the presence of penicillin, streptomycin and fungizone in 75 cm^2 flask under 5% CO_2, whereas HCT116 (colon adenocarcinoma), HCT15 (colon adenocarcinoma), MCF7 (breast adenocarcinoma), HL60 (promyeocytic leukaemia) were grown in RPMI medium. Resistant MCF7 cells were obtained by prolonged treatment with doxorubicin. H9c2 cells (rat heart myoblast) were obtained from ATCC and grown in D-MEM medium supplemented with 10% fetal calf serum.

Cell proliferation assay. Cells were plated in 96-well tissue culture plates in 200 µL medium and treated 24 h later with compounds dissolved in DMSO; compound concentrations ranged 0.5 nM to 10 µM and were prepared by use of a Biomek 2000 (Beckman). Control cells received the same volume of DMSO (1% final volume). After 72 h exposure to the drug, MTS reagent (Promega) was added and incubated for 3 h at 37 °C: the absorbance was monitored at 490 nm and results are expressed as the inhibition of cell proliferation calculated as the ratio [(OD_{490} treated/OD_{490} control)×100]. For IC_{50} determinations (50% inhibition of cell proliferation) experiments were performed in duplicate.

Cell culture and experimental design of *in vitro* cardiotoxicity assay. H9c2 cells were cultured in Dulbecco's modified Eagle's medium at 37 °C in 5% CO_2, and the medium was changed every 2–3 days. Sub-confluent H9c2 (106) cells were synchronized overnight in a serum and glucose free medium (Gibco, DMEM w- L-glutamine, w/o D-glucose, sodium pyruvate) and were then treated with **1**, **3**, or their vehicle for 24, 48 or 72 h. Cells were washed, and subsequently FACS or TUNEL analyses were performed. Cell viability was determined by Cell-titer 96 aqueous one solution cell proliferation assay kit (Promega, Madison, WI, USA). The process of the experiment is completely according to the instruction. Briefly, aliquots of 2x103 cells/well were cultured in 96-well plates for 24 h and then treated with **3** or its vehicle (DMSO) for additional 24, 48 and 72 h. 40 mL of Cell-titer 96 aqueous one solution were added to each well and incubated for an additional 3 h. The absorbance at 490 nm was recorded with a 96-well plate reader (Termoscience). Each experiment was performed in triplicate and repeated at least three times. Results are expressed as percent of cell viability.

***In vivo* acute toxicity assay.** Eleven-week-old male B6 mice were obtained from Charles River Laboratories (L'Arbresle, France). All mice were treated and housed in accordance with the French animal protection laws. A stock solution of **3** in ethanol (2 mg/mL) was diluted 100 times by an aqueous solution of PEG400 (40%) and sterilized by filtration (0.22 µm). This solution was administrated intraperitoneally twice a day with an interval of 8 h for 4 days to ten mice at the dose of 0.1 mg/kg. Body weights were measured once a day for 13 days.

Cellular localization studies. HeLa cells were seeded on glass coverslips in 24-well plates in DMEM supplemented with 5% of fetal calf serum. After 24 h of culture, cells were treated with 50 µM of probe **5** or coumarin-control **21** for 2 h. For labelling of mitochondria and ER, cells were incubated 15 min at 37 °C with 1 µM of Mito-Tracker Red dye (Molecular Probe) or 1 µM of ER-Tracker Red dye (Molecular Probe) or BODIPY 558/568 brefeldin A (Molecular Probe). After treatment, cells were washed with PBS and then were fixed for 5 min at room temperature in 4% paraformaldehyde in PBS. Slides were mounted in Vectashield (Polysciences Inc., Warrington, PA). Observations were made with a confocal microscope (Leica SP2-UV, Leica Microsystem).

Assay of mRNA translation. Synthesis of capped and polyadenylated luciferase mRNA was described previously (Svitkin YV, Sonenberg N (2004) *Methods Mol Biol* 257:155-170). Translation in rabbit reticulocyte lysates was carried out as recommended by the manufacturer (Promega). The reaction mixtures (10 µL) included rabbit reticulocyte lysate (70% v/v), amino acids, capped and polyadenylated luciferase mRNA (2 µg/mL) and increasing concentrations of **3** as indicated (serial dilutions of **3** were made up in 0.1% DMSO, and 0.5 µL of each dilution was added to reaction mixtures). Incubation was at 30 °C for 1 h. Luciferase levels were determined in 3 µL aliquots of 300-fold diluted samples by enzymatic assay (Promega). A Lumat LB 9507 bioluminometer (EG&G Bertold) was used for the measurements. Data (relative luciferase units determined in 1 µL aliquots of translation samples, RLU) are averages of three assays.

Detection of stress-granules by immunofluorescence. HeLa cells were seeded on glass coverslips in 24-well plates in DMEM supplemented with 5% of fetal calf serum. After 24 h of culture, cells were treated with cycloheximide (10 µg/mL) or puromycin (100 µg/mL) for 1 h or with different concentrations of **3** (50 nM to 500 µM) for 30 min to 8 h. After treatment, cells were washed with PBS, fixed for 5 min at room temperature in 4% paraformaldehyde in PBS, and then permeabilized for 10 min with 0.1% Triton X-100 in PBS. After blocking in 1% bovine serum albumin in PBS, cells were incubated 1 h at room temperature with the rabbit anti-MLN51 and mouse anti-FMRP antibodies (a kind gift from B. Bardoni). Cells were washed three times in PBS and incubated 30 min with Cy3-conjugated affinity-purified donkey anti-mouse (Interchim, Montluçon, France) or AlexaFluor 488-conjugated donkey anti-rabbit (Molecular Probes, Eugene, OR) (1:500) for 30 min. Cells were washed three times in PBS and nuclei were counterstained with Hoechst-33258 dye. Slides were mounted in Vectashield (Polysciences Inc., Warrington, PA). Observations were made with a fluorescence microscope (Leica DMLB 30T, Leica Microsystem, Wetzlar Germany) or confocal microscopes (Leica SP1 and Leica SP2-MP, Leica Microsystem).

Pull-down assay. For the flavagline **6**-agarose pull down assay, sub-confluent HeLa cells were collected and washed in phosphate-buffered saline 1X (PBS). Lysis was performed by freezing the cells in liquid N_2 in 500 µL of lysis buffer (50 mM Tris.HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, protease inhibitor cocktail 1X). Cellular debris was removed by centrifugation at 10,000 x *g* for 10 min. Five hundred µg of total protein extract was incubated for 10-12 h at 4 °C with 40 µL of flavagline **6**-agarose or uncoupled agarose. The beads were washed extensively with lysis buffer and bound-proteins were eluted by SDS sample buffer (50 mM Tris.HCl pH 6.8, 2% SDS, 10% glycerol, 1.4 M β-mercaptoethanol, and bromophenol blue). Eluted proteins were recovered from the beads by centrifugation.

Total proteins, unbound proteins, or eluted proteins were resolved by 12% to 8% SDS-PAGE and electro-transferred to nitrocellulose sheets (Schleicher and Schuell, Dassel, Germany). The membrane was blocked in PBS containing 3% nonfat dry milk and 0.1% Tween 20. Goat anti-eIF4A1 antibody, rabbit anti-eiF4A and anti-Lasp1 were used as primary antibodies at dilutions of 1/1000. After washing, the blots were incubated with appropriate secondary antibodies. Horseradish peroxidase-conjugated (HRP) AffiniPure donkey anti-rabbit at 1/10000 (Jackson ImmunoResearch) and HRP donkey anti-goat at 1/1000 (Santa Cruz biotechnology, Santa Cruz, CA) were used. Finally, protein-antibody complexes were visualized by an enhanced chemiluminescence detection system (SuperSignal West Pico, Pierce).

Proteasomal assay. HL60 cells were suspended in a buffer consisting in 25 mM Hepes (pH 7.5), 0.5 mM EDTA, 0.05% NP40, 0.01% SDS, and 0.2 mM ATP. Cells at a concentration of 15,000 cells in 180 µL of lysis buffer were added to each well of black 96-well microplates and kept on ice for 20 min; proteosomal activity was assayed after addition of 50 µM LLVY-AMC (chymotrypsin-like activity) or RLR-AMC (trypsin-like activity). Microplates were

incubated at 37 °C in the dark and fluorescence was monitored every hour over a 3 h-incubation period (exc 360 nm, em 435 nm). Compounds were added to the incubation mixture at concentrations ranging from 10^{-9} M to 10^{-6} M with a fixed volume of DMSO. Controls were performed with the same conditions with vehicle only. Bortezomib (100 nM) was used as a positive control in chymotrypsin-like samples and elicited more than 95% inhibition. Results are expressed as the residual activity in the presence of chemicals.

Caspase activity assay. Caspase activities were assayed in HL60 cell after treatment with chemicals for 48 h. HL60 cells (20,000 cells per well in 180 µL RPMI medium) were plated in black 96-well culture microplates and treated with chemicals dissolved in DMSO (1% v/v, final concentration). Plates were kept under 5% CO_2 for 48 h. Lysis buffer (20 µL of a 10x stock solution) consisting in 250 mM Hepes (pH 7.5) 5 mM EDTA, 0.5% NP40, 0.1% SDS and 50 mM dithiothreitol, was added before caspase substrates, which were dissolved in water (DEVD-AMC and LEHD-AMC) or DMSO (IETD-AMC) at a final concentration of 50 µM. Plates were incubated at 37 °C and fluorescence was recorded (exc 360 nm, em 435 nm) after 0, 30, 60, 120, and 180 min. Reaction rates were calculated from the slope of the linear time-dependent reaction and are expressed as the fold-activation relative to the control (HL60 with DMSO alone). Doxorubicin 10^{-8} to 10^{-6} M was used as a positive control. **1** and **3** were added at concentrations ranging from 10^{-9} to 10^{-6} M.

Flow cytometry. HL60 cells were incubated in 6-well plates with **3** (20 nM) for 48 h. Control cells received the same volume of DMSO (1% v/v, final concentration). Cells were harvested and treated for cell cycle analysis as previously described (Venot C, *et al.* (1998) *EMBO J* 17:4668-4679).

Inhibition of P-gp mediated efflux. MCF7-adriamycin resistant cells were plated in black 96-well microplates at a density of 60,000 cells per well. All steps were performed with a Biomek 2000 robot. After 24 h, the culture medium was removed, wells were washed twice with PBS, and 100 µL RPMI medium, without fetal calf serum, containing 12.5 µM rhodamine 123 was added per well together with test compounds dissolved in methanol (1% v/v, final concentration). Microplates were kept for 1 h at 37 °C under 5% CO_2. Cells were washed 3 times with 200 µL PBS and lysed with 100 µL Tris 20 mM pH 7.7-0.2% SDS. Cells were kept under agitation for 15 min at room temperature before being read in a fluorescence microplate reader (ex 507 nm, em 528 nm).

Flow cytometric detection of apoptosis. Double staining for FITC-annexin V (Molecular Probes) binding and for cellular DNA with propidium iodide (PI) was performed as follows. After washing twice with 1x PBS, ~10^6 cells were resuspended in binding buffer (10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl, and 2.5 mM $CaCl_2$). FITC-Annexin V was added to a final concentration of 1 µg Annexin V/mL. A 0.1 volume of PI (10 µg/mL in binding buffer) was added, resulting in a final concentration of 1 µg PI/mL of cell suspension. The mixture was incubated for 10 min in the dark at room temperature, and then cellular fluorescence was measured by flow cytometry with a FACSCalibur, Becton Dickinson apparatus.

Immunohistochemical analysis of AIF and caspase-12 translocation. HeLa cells were cultured in Dulbecco modified Eagle medium (DMEM) supplemented as RPMI. HeLa cells grown on cover slips (104) were pretreated with inhibitors 1 h before 3 treatment. 72 h after treatment, cells were fixed with 4% (w/v) para-formaldehyde at 37 °C and permeabilized with PBS including 0.5% Triton X100 and 10% donkey serum. Cells were stained with monoclonal Abs against rabbit anti-AIF (1/100, Tebu Bio, Le Perray en Yvelines, France), rat anti-caspase 12 (1/220, Sigma-Aldrich, Schnelldorf, Germany) for overnight at 4 °C and rinsed in PBS and stained with Alexa 488 Goat anti-rabbit- IgG and Alexa 594 Donkey anti-rat-IgG (1/200), Invitrogen, Cergy Pontoise, France), respectively, DNA was stained with DAPI

(Sigma). All images were acquired using a X40 objective on an microscope (Leica). Images were cropped, sized and placed using Adobe Photoshop CS version 8.0.