

A NOVEL COVALENT mTOR INHIBITOR, DHM25, SHOWS *IN VIVO* ANTI-TUMOR ACTIVITY AGAINST TRIPLE-NEGATIVE BREAST CANCER CELLS

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SUPPORTING INFORMATION

EXPERIMENTAL PROCEDURE

S3

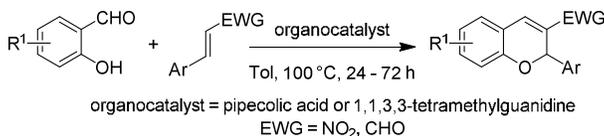
SUPPORTING FIGURES

S12

GENERAL INFORMATION. All reagents and solvents were used as purchased from commercial suppliers or were purified/dried according to Armarego W. L. F. and Chai C. L. L. (Purification of Laboratory Chemicals, 6th edition, Elsevier). ¹H and ¹³C-NMR spectra were recorded on a Bruker Avance 300 or 500 instrument using TMS and CDCl₃ respectively as internal standard. Chemical shifts (δ values) are given in parts per million (ppm), coupling constants (*J* values) are given in Hertz (Hz), and multiplicity of signals are reported as usual. HRMS analyzes were obtained using a Waters Q-TOF 2 or a Bruker Micro-TOF Q II instrument for ESI. Optical rotations were recorded on a Perkin Elmer Model 341 polarimeter. The purity of final tested compounds was typically determined to be $\geq 95\%$ by HPLC, conducted on a Shimadzu Prominence system using a Grace Prevail C18 column and diode array detector. Chiral HPLC was performed using a Daicel Chiralpak IA column. Elementary analysis of the injected DHM25 was performed on a Thermo-fisher ICAP 6500 Duo instrument. Melting points were obtained on a hot bench. VCD spectra were measured on a Bruker Optics Vertex 70 spectrometer and ECD spectra using a Jasco J815 spectrometer. TLC analyzes were performed using precoated Merck TLC Silica Gel 60 F₂₅₄ plates. Purifications by column chromatography on silica gel were performed using Grace Davisil Silica Gel 60 (70-200 mesh and 40-60 mesh) and purifications by preparative thin layer chromatography on silica gel using Merck Silica Gel 60 PF₂₅₄. Petroleum ether (PE) used for purifications was the low boiling point fraction (40-60 °C).

EXPERIMENTAL PROCEDURE

General procedure for the synthesis of chromene



The mixture of Michael acceptor derivative (1.0 mmol), salicylaldehyde compound (1.0 mmol, 1.0 eq.) and organocatalyst (0.2 mmol, 0.2 eq.) in 1.5 mL of dry toluene was heated at 80-100 °C for 48-72 h under inert atmosphere (conversion followed by TLC). After cooling to room temperature, the mixture was charged directly on the silica gel column for purification.

8-Ethoxy-2-(4-fluorophenyl)-3-nitro-2*H*-chromene (**Pichromene**)

Mp = 118-120 °C. ¹H NMR (500 MHz, CDCl₃) δ 1.37 (t, *J* = 7.0, 3H), 3.93-4.11 (m, 2H), 6.64 (s, 1H), 6.92-6.95 (m, 3H), 6.98 (t, *J* = 8.7 Hz, 2H), 7.30-7.44 (m, 2H), 8.04 (s, 1H). ¹³C NMR (125 MHz, CDCl₃) δ 14.8, 65.2, 73.4, 115.9 (d, *J* = 21.8), 118.8, 119.0, 122.4, 122.7, 129.0 (d, *J* = 8.4), 129.6, 132.7 (d, *J* = 3.2), 141.4, 143.1, 148.1, 163.3 (d, *J* = 248.1). HRMS [M+Na]⁺ calculated for C₁₇H₁₄NO₄FNa 338.0805, found 338.0807.

2-(4-Fluorophenyl)-8-methoxy-3-nitro-2*H*-chromene (**PWPHT33**)

Mp = 103-105 °C. ¹H NMR (500 MHz, CDCl₃) δ 3.82 (s, 3H), 6.63 (s, 1H), 6.95-7.02 (m, 5H), 7.35-7.41 (m, 2H), 8.03 (s, 1H). ¹³C NMR (125 MHz, CDCl₃) δ 56.5, 73.7, 115.9 (d, *J* = 21.8), 117.0, 118.8, 122.2, 122.7, 129.1 (d, *J* = 8.4), 129.4, 132.7 (d, *J* = 3.3), 141.4, 142.6, 148.8, 163.4 (d, *J* = 248.6). HRMS M⁺ calculated for C₁₆H₁₂NO₄F 301.0750, found 301.0752.

6,8-Dibromo-2-(4-fluorophenyl)-3-nitro-2*H*-chromene (**PWPHT45**)

Mp = 134-136 °C. ¹H NMR (500 MHz, CDCl₃) δ 6.69 (s, 1H), 7.03 (t, *J* = 8.6, 2H), 7.32-7.38 (m, 2H), 7.41 (d, *J* = 2.3, 1H), 7.67 (d, *J* = 2.2, 1H), 7.95 (s, 1H). ¹³C NMR (125 MHz, CDCl₃): δ 74.2, 112.6, 114.9, 116.2 (d, *J* = 21.8), 120.8, 127.6, 129.0 (d, *J* = 8.7), 131.7, 131.7, 139.3, 142.7, 149.4, 163.6 (d, *J* = 249.7). HRMS [M+Na]⁺ calculated for C₁₅H₈NO₃FBr₂Na 449.8753, found 449.8757.

2-(4-Fluorophenyl)-7-methoxy-3-nitro-2*H*-chromene (**PWPHT47**)

Mp = 128-130 °C. ¹H NMR (500 MHz, CDCl₃) δ 3.79 (s, 3H), 6.39 (dd, *J* = 0.8, 2.4, 1H), 6.53 (s, 1H), 6.57 (dd, *J* = 2.4, 8.5, 1H), 7.00 (t, *J* = 8.6, 2H), 7.24 (d, *J* = 8.5, 1H), 7.35 (dd, *J* = 5.2, 8.5, 2H), 8.04 (d, *J* = 0.8, 1H). ¹³C NMR (125 MHz, CDCl₃) δ 55.8, 74.0, 102.4, 110.0, 111.1, 116.0 (d, *J* = 21.8), 129.1 (d, *J* = 8.4), 130.0, 131.9, 133.1 (d, *J* = 3.6), 138.3, 155.5, 163.4 (d, *J* = 248.7), 165.4. HRMS [M+Na]⁺ calculated for C₁₆H₁₂NO₄FNa 324.0648, found 324.0646.

2-(4-Fluorophenyl)-3-nitro-2*H*-benzo[*g*]chromene (**PWPHT54**)

¹H NMR (300 MHz, CDCl₃) δ 6.55 (s, 1H), 6.86 (d, *J* = 8.7, 1H), 6.93-7.05 (m, 3H), 7.28-7.39 (m, 4H), 8.05 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 73.7, 116.0 (d, *J* = 21.7), 117.4, 117.9, 122.8, 127.2, 128.7, 129.1 (d, *J* = 8.5), 129.5, 130.6, 132.8 (d, *J* = 3.3), 134.6, 141.1, 153.4, 163.4 (d, *J* = 248.7).

6-Bromo-2-(4-fluorophenyl)-8-methoxy-3-nitro-2*H*-chromene (**PWPHT88**)

Mp = 134-136 °C. ¹H NMR (500 MHz, CDCl₃) δ 3.81 (s, 3H), 6.62 (s, 1H), 7.00 (t, *J* = 8.6, 2H), 7.03 (d, *J* = 2.1, 1H), 7.09 (d, *J* = 2.1, 1H), 7.36 (dd, *J* = 5.2, 8.7, 2H), 7.94 (s, 1H). ¹³C NMR (125 MHz, CDCl₃) δ 56.7, 73.8, 114.5, 116.1 (d, *J* = 21.6), 119.7, 119.9, 124.0, 128.0, 129.1 (d, *J* = 8.8), 132.2 (d, *J* = 3.1), 141.8, 142.1, 149.5, 163.5 (d, *J* = 249.1). HRMS [M+Na]⁺ calculated for C₁₆H₁₁NO₄FBrNa 401.9753, found 401.9753.

6-Bromo-2-(4-fluorophenyl)-3-nitro-2*H*-chromene (**PWPHT89**)

Mp = 134-136 °C. ¹H NMR (500 MHz, CDCl₃) δ 6.54 (s, 1H), 6.75 (d, *J* = 8.6, 1H), 7.01 (t, *J* = 8.6, 2H), 7.30-7.35 (m, 2H), 7.40 (dd, *J* = 2.4, 8.7, 1H), 7.45 (d, *J* = 2.4, 1H), 7.96 (s, 1H). ¹³C NMR (125 MHz, CDCl₃) δ 73.9, 114.8, 116.2 (d, *J* = 21.8), 119.2, 119.7, 128.0, 129.2 (d, *J* = 8.8), 132.3 (d, *J* = 3.5), 132.5, 136.9, 142.0, 152.4, 163.5 (d, *J* = 249.6). HRMS [M+H]⁺ calculated for C₁₅H₁₀NO₃FBr 349.9828, found 349.9825.

8-Bromo-2-(4-fluorophenyl)-3-nitro-2*H*-chromene (**PWPHT90**)

Mp = 143-145 °C. ¹H NMR (500 MHz, CDCl₃) δ 6.69 (s, 1H), 6.90 (t, *J* = 7.8, 1H), 7.01 (t, *J* = 8.6, 2H), 7.28 (dd, *J* = 1.5, 7.6, 1H), 7.35-7.39 (m, 2H), 7.55 (dd, *J* = 1.5, 8.0, 1H), 8.02 (s, 1H). ¹³C NMR (125 MHz, CDCl₃) δ 74.0, 111.6, 116.1 (d, *J* = 21.8), 119.6, 123.7, 128.9, 128.9 (d, *J* = 8.8), 129.6, 132.2 (d, *J* = 3.2), 137.6, 141.9, 150.2, 163.5 (d, *J* = 249.3). HRMS [M+Na]⁺ calculated for C₁₅H₉NO₃FBrNa 371.9647, found 371.9646.

6-Bromo-2-(4-bromophenyl)-3-nitro-2*H*-chromene (**DHM9**)

Mp = 136-138 °C. ¹H NMR (300 MHz, CDCl₃): δ 6.52 (s, 1H), 6.77 (d, *J* = 8.6, 1H), 7.23 (d, *J* = 8.3, 2H), 7.41 (dd, *J* = 2.4, 8.6, 1H), 7.44-7.46 (m, 1H), 7.46 (d, *J* = 8.5, 2H), 7.97 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 73.8, 114.8, 119.1, 119.6, 124.0, 128.0, 128.7, 132.2, 132.5, 135.2, 136.9, 141.7, 152.2. HRMS [M+H]⁺ calculated for C₁₅H₁₀NO₃Br₂ 409.9027, found 409.9029.

6-Bromo-2-(4-chlorophenyl)-3-nitro-2*H*-chromene (**DHM10**)

Mp = 130-132 °C. ¹H NMR (500 MHz, CDCl₃): δ 6.53 (s, 1H), 6.76 (dd, *J* = 0.7, 8.7, 1H), 7.27-7.31 (m, 4H), 7.41 (dd, *J* = 2.4, 8.7, 1H), 7.45 (d, *J* = 2.4, 1H), 7.96 (s, 1H). ¹³C NMR (125 MHz, CDCl₃): δ 73.8, 114.8, 119.2, 119.7, 128.1, 128.5, 129.3, 132.6, 134.8, 135.9, 137.0, 141.8, 152.3. HRMS [M+H]⁺ calculated for C₁₅H₁₀NO₃ClBr 365.9533, found 365.9531.

6-Bromo-2-(4-nitrophenyl)-3-nitro-2*H*-chromene (**DHM12**)

Mp = 121-123 °C. ¹H NMR (300 MHz, CDCl₃) δ 6.66 (s, 1H), 6.81 (d, *J* = 8.6, 1H), 7.45 (dd, *J* = 2.4, 8.6, 1H), 7.49 (d, *J* = 2.3, 1H), 7.54 (d, *J* = 8.4, 2H), 8.02 (s, 1H), 8.20 (d, *J* = 8.8, 2H). ¹³C NMR (75 MHz, CDCl₃) δ 73.3, 115.3, 119.1, 119.4, 124.3, 128.1, 128.7, 132.8, 137.3, 141.1, 143.0, 148.7, 152.1. HRMS [M+H]⁺ calculated for C₁₅H₁₀N₂O₅Br 376.9773, found 376.9771.

8-Bromo-2-(4-bromophenyl)-3-nitro-2*H*-chromene (**DHM18**)

Mp = 139-141 °C. ¹H NMR (300 MHz, CDCl₃) δ 6.68 (s, 1H), 6.91 (t, *J* = 7.8, 1H), 7.23-7.32 (m, 3H), 7.46 (d, *J* = 8.5, 2H), 7.56 (dd, *J* = 1.5, 8.0, 1H), 8.03 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 73.9, 111.6, 119.5, 123.8, 124.0, 128.6, 129.0, 129.7, 132.2, 135.3, 137.6, 141.6, 150.2. HRMS [M+H]⁺ calculated for C₁₅H₁₀NO₃Br₂ 409.9027, found 409.9024.

8-Bromo-2-(4-chlorophenyl)-3-nitro-2*H*-chromene (**DHM19**)

Mp = 131-133 °C. ¹H NMR (300 MHz, CDCl₃) δ 6.69 (s, 1H), 6.91 (t, *J* = 7.8, 1H), 7.28 (dd, *J* = 1.6, 7.7, 1H), 7.28-7.35 (m, 4H), 7.55 (dd, *J* = 1.5, 8.0, 1H), 8.03 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 73.9, 111.6, 119.5, 123.8, 128.3, 129.0, 129.2, 129.7, 134.8, 135.7, 137.6, 141.6, 150.2. HRMS [M+H]⁺ calculated for C₁₅H₁₀NO₃ClBr 365.9533, found 365.9533.

6,8-Dibromo-2-(4-bromophenyl)-3-nitro-2*H*-chromene (**DHM25**)

Mp = 150-152 °C. ¹H NMR (300 MHz, CDCl₃) δ 6.67 (s, 1H), 7.24 (d, *J* = 8.5, 2H), 7.40 (d, *J* = 2.2, 1H), 7.48 (d, *J* = 8.5, 2H), 7.67 (d, *J* = 2.2, 1H), 7.95 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 74.1, 112.6, 115.0, 120.7, 124.3, 127.7, 128.6, 131.7, 132.3, 134.8, 139.3, 142.4, 149.4. HRMS [M+H]⁺ calculated for C₁₅H₉NO₃Br₃ 487.8132, found 487.8133. Anal. calculated for C₁₅H₉NO₃Br₃: C, 36.77; H, 1.65, N, 2.86. found: C, 36.29, H, 1.56, N, 2.73. (*S*)-DHM25: [α]_D = +154.6 (*c* = 1, CHCl₃); (*R*)-DHM25: [α]_D = -157.7 (*c* = 1, CHCl₃).

6,8-Dibromo-2-(4-chlorophenyl)-3-nitro-2*H*-chromene (**DHM26**)

Mp = 140-142 °C. ¹H NMR (300 MHz, CDCl₃) δ 6.68 (s, 1H), 7.31 (s, 4H), 7.41 (d, *J* = 2.2, 1H), 7.67 (d, *J* = 2.2, 1H), 7.96 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 74.0, 112.5, 114.9, 120.7, 127.7, 128.3, 129.3, 131.7, 134.2, 135.9, 139.2, 142.4, 149.3. HRMS [M+H]⁺ calculated for C₁₅H₉NO₃ClBr₂ 443.8638, found 443.8640.

6,8-Dibromo-2-(4-nitrophenyl)-3-nitro-2*H*-chromene (**DHM27**)

Mp = 151-153 °C. ¹H NMR (300 MHz, CDCl₃) δ 6.80 (s, 1H), 7.44 (d, *J* = 2.3, 1H), 7.57 (d, *J* = 8.4, 2H), 7.71 (d, *J* = 2.2, 1H), 8.00 (s, 1H), 8.21 (d, *J* = 8.8, 2H). ¹³C NMR (75 MHz, CDCl₃) δ 73.6, 112.5, 115.5, 120.5, 124.3, 127.9, 128.3, 131.9, 139.6, 141.9, 142.5, 148.7, 149.1. HRMS [M+H]⁺ calculated for C₁₅H₉N₂O₅Br₂ 454.8878, found 454.8878.

6,8-Dibromo-3-nitro-2-(3,4,5-trimethoxyphenyl)-2*H*-chromene (**DHM31**)

¹H NMR (300 MHz, CDCl₃) δ 3.80 (s, 6H), 3.81 (s, 3H), 6.57 (s, 2H), 6.69 (s, 1H), 7.40 (d, *J* = 2.2, 1H), 7.68 (d, *J* = 2.2, 1H), 7.93 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 74.4, 104.0, 112.5, 114.9, 121.0, 127.5, 131.3, 131.7, 139.1, 139.2, 142.9, 149.7, 153.6. HRMS [M+Na]⁺ calculated for C₁₈H₁₅NO₆Br₂Na 521.9164, found 521.9166.

6-Bromo-3-nitro-2-(3,4,5-trimethoxyphenyl)-2*H*-chromene (**DHM37**)

¹H NMR (300 MHz, CDCl₃) δ 3.78 (s, 6H), 3.81 (s, 3H), 6.51 (s, 1H), 6.55 (s, 2H), 6.79 (d, *J* = 8.6, 1H), 7.42 (dd, *J* = 2.5, 8.6, 1H), 7.46 (d, *J* = 2.3, 1H), 7.96 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 56.4, 60.9, 74.7, 104.5, 114.6, 119.2, 119.7, 127.8, 131.8, 132.4, 136.9, 139.4, 142.0, 152.6, 153.7. HRMS [M+Na]⁺ calculated for C₁₈H₁₆NO₆BrNa 444.0059, found 444.0062.

6,8-Dibromo-2-(4-bromophenyl)-2*H*-chromene-3-carbaldehyde (**LR20**)

¹H NMR (300 MHz, CDCl₃) δ 6.42 (s, 1H), 7.19 (d, *J* = 8.2, 2H), 7.33 (s, 1H), 7.34 (d, *J* = 2.2, 1H), 7.42 (d, *J* = 8.2, 2H), 7.65 (d, *J* = 2.2, 1H), 9.72 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 74.1, 112.2, 114.0, 122.6, 123.1, 128.2, 130.6, 131.8, 134.8, 136.8, 138.5, 138.6, 150.4, 189.3. HRMS [M+H]⁺ calculated for C₁₆H₁₀O₂Br₃ 470.8231, found 470.8233.

6,8-Dibromo-2-(4-bromophenyl)-2*H*-chromene-3-carboxylic acid (**LR24**)

To a solution of 6,8-dibromo-2-(4-bromophenyl)-2*H*-chromene-3-carbaldehyde **LR20** (456 mg, 1.16 mmol) in a mixture of *tert*-butanol and water (5/1, 12 mL) were added potassium dihydrogenophosphate (236 mg, 1.73 mmol, 1.5 eq.), amylene (735 μL, 6.94 mmol, 6.0 eq.) and sodium chlorite (314 mg, 3.47 mmol, 3.0 eq.) at room temperature. The reaction mixture was stirred overnight then concentrated under reduced pressure and diluted with water. The aqueous phase was extracted with EtOAc and the combined organic layers were washed with brine, dried over MgSO₄, filtered and the filtrate concentrated under reduced pressure. The title compound **LR24** was purified by column chromatography on silica gel using PE/AcOEt/HCO₂H 4/1/0.05 as eluent (413 mg, 87% yield). Mp = 256-258 °C. ¹H RMN (300 MHz, DMSO-*d*₆) δ 7.71 (d, *J* = 2.3, 1H), 6.37 (s, 1H), 7.29 (d, *J* = 8.4, 2H), 7.56 (d, *J* = 8.5, 2H), 7.71 (d, *J* = 2.3, 1H), 7.73 -7.78 (m, 2H). ¹³C RMN (75 MHz, DMSO-*d*₆) δ 75.1, 111.5, 113.8, 122.8, 124.1, 127.3, 129.6, 131.2, 131.5, 132.2, 136.8, 137.8, 149.1, 165.5. HRMS M⁺ calculated for C₁₆H₉O₃Br₃ 485.8102, found 485.8099.

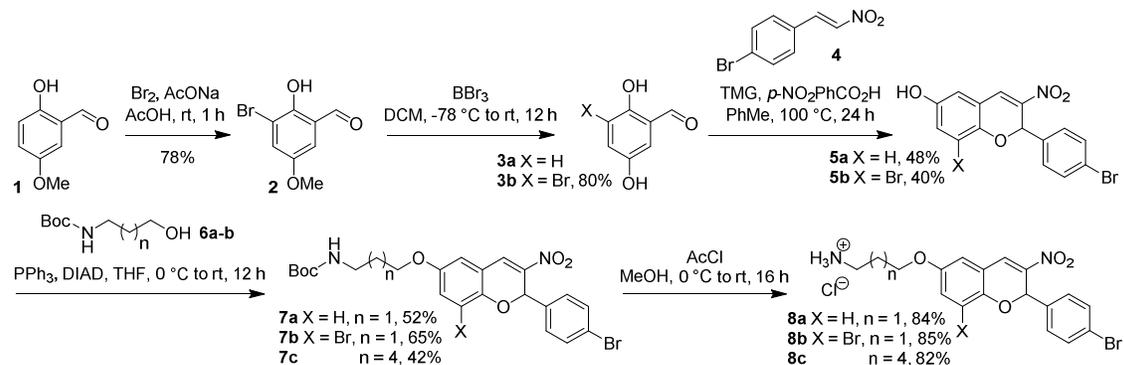
6,8-Bromo-2-(4-bromophenyl)-*N,N*-dimethyl-2*H*-chromene-3-carboxamide (**LR28**)

To a solution of 6,8-dibromo-2-(4-bromophenyl)-2*H*-chromene-3-carboxylic acid **LR24** (120 mg, 0.24 mmol) in methylene chloride (1 mL) in the presence of one drop of DMF, oxalyl chloride (25 μL, 0.29 mmol, 1.15 eq.) was added. The reaction mixture was stirred 20 h at room temperature and Me₂NH.HCl (40 mg, 0.49 mmol, 2.00 eq.) and Et₃N (170 μL, 1.22 mmol, 5.00 eq.) were added. The reaction mixture was stirred 4 h at room temperature and after addition of HCl 1 M, the aqueous phase was extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO₄, filtered and the filtrate concentrated under reduced pressure. The title compound **LR28** was purified by column chromatography on silica gel using CH₂Cl₂/AcOEt 4/1 as eluent (120 mg, 95% yield). ¹H NMR (500 MHz, CDCl₃) δ 2.99 (s, 6H), 6.29 (s, 1H), 6.56 (s, 1H), 7.15 (d, *J* = 2.2, 1H), 7.22-7.34 (m, 2H), 7.44 (d, *J* = 8.5, 2H), 7.52 (d, *J* = 2.2, 1H). ¹³C NMR (125 MHz, CDCl₃) δ 35.3, 38.5, 111.3, 113.7, 122.9, 123.0, 123.1, 128.4, 129.2, 131.3, 131.8, 136.0, 137.3, 148.6, 162.3, 167.5.

Methyl 6,8-dibromo-2-(4-bromophenyl)-2*H*-chromene-3-carboxylate (**LR29**)

To a solution of 6,8-dibromo-2-(4-bromophenyl)-2*H*-chromene-3-carboxylic acid **LR24** (120 mg, 0.24 mmol) in methanol, thionyl chloride (20 μL, 0.27 mmol, 1.1 eq.) was added. The reaction mixture was heated at reflux for 20 h then cooled to room temperature. The solvent was removed under reduce pressure and the title compound **LR39** was purified by column chromatography on silica gel using PhMe/PE 3/1 as eluant (114 mg, 93% yield). ¹H NMR (300 MHz, CDCl₃) δ 3.81 (s, 3H), 6.36 (s, 1H), 7.21-7.31 (m, 3H), 7.38-7.48 (m, 2H), 7.55 (d, *J* = 2.2, 1H), 7.57 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 52.4, 75.0, 111.9, 113.9, 123.1, 123.2, 126.1, 128.7, 130.2, 131.8, 131.9, 137.0, 137.3, 149.4, 164.5. HRMS [M+Na]⁺ calculated for C₁₇H₁₁O₃Br₃Na 522.8156, found 522.8153.

Synthesis of DHM25-Biotin, DHM9-Biotin and DHM25-FITC:



3-Bromo-5-methoxysalicylaldehyde (**2**)

To a solution of 5-methoxysalicylaldehyde **1** (2.0 mL, 16.0 mmol) in glacial acetic acid (75.0 mL) was added sodium acetate (2.1 g, 25.6 mmol, 1.6 eq.) followed by dibromine (1.0 mL, 19.4 mmol, 1.2 eq.). After 1 h at room temperature, the mixture was concentrated under reduced pressure and the residue dissolved in dichloromethane and washed with water. The organic phase was dried over MgSO_4 , filtered and the filtrate concentrated under reduced pressure leading to the expected 3-bromo-5-methoxysalicylaldehyde **2** (2.9 g, 78% yield) after purification by recrystallization in ethanol. ^1H NMR (300 MHz, CDCl_3) δ 3.82 (s, 3H), 7.04 (d, $J = 3.0$, 1H), 7.42 (d, $J = 2.9$, 1H), 9.83 (s, 1H), 11.13 (s, 1H).

3-bromo-5-hydroxysalicylaldehyde (**3b**)

To a solution of 3-bromo-5-methoxysalicylaldehyde **2** (1.34 g, 6.06 mmol) in dry dichloromethane (20.0 mL) was added a solution of boron tribromide (1 M in dichloromethane, 25.0 mL, 25.0 mmol, 4.1 eq.) at -50°C under inert atmosphere. This solution was slowly warmed to room temperature and stirred for 12 h before water was added to quench the reaction. The reaction mixture was extracted with ethyl acetate and the combined organic phase washed successively with saturated aqueous sodium thiosulfate, water and brine then dried over MgSO_4 . After filtration and concentration of the filtrate under reduced pressure, 3-bromo-5-hydroxysalicylaldehyde **3b** was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 4/1 to 3/2 as eluent (1.05 g, 80% yield). ^1H NMR (500 MHz, CDCl_3) δ 5.16 (bs, 1H), 6.61 (s, 1H), 6.77 (d, $J = 2.9$, 1H), 7.08 (d, $J = 2.9$, 1H), 7.24 (d, $J = 8.5$, 2H), 7.45 (d, $J = 8.5$, 2H), 8.05 (s, 1H). ^{13}C NMR (125 MHz, CDCl_3) δ 73.5, 111.6, 115.5, 119.7, 123.7, 124.4, 128.4, 128.7, 132.0, 135.0, 142.3, 144.1, 151.0. HRMS $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_7\text{H}_5\text{O}_3\text{BrNa}$ 238.9320, found 238.9320.

8-Bromo-2-(4-bromophenyl)-6-hydroxy-3-nitro-2H-chromene (**5b**)

To a solution of 3-bromo-5-hydroxysalicylaldehyde **3b** (1.0 g, 4.6 mmol) and 1-bromo-4-(2-nitrovinyl)benzene **4** (1.1 g, 4.8 mmol, 1.05 eq.) in dry toluene (10.0 mL) was added 1,1,3,3-tetramethylguanidine (115.0 μL , 0.92 mmol, 0.2 eq.) and 4-nitrobenzoic acid (154.0 mg, 0.92 mmol, 0.2 eq.). The reaction mixture was heated at 100°C for 2 days then cooled and concentrated under reduced pressure. The expected chromene **5b** was obtained after purification by column chromatography on silica gel using dichloromethane/toluene 9/1 to 7/3 as eluent (0.79 g, 40% yield). ^1H NMR (500 MHz, CDCl_3) δ 5.16 (bs, 1H), 6.61 (s, 1H), 6.77 (d, $J = 2.9$, 1H), 7.08 (d, $J = 2.9$, 1H), 7.24

(d, $J = 8.5$, 2H), 7.45 (d, $J = 8.5$, 2H), 8.05 (s, 1H). ^{13}C NMR (125 MHz, CDCl_3) δ 73.5, 111.6, 115.5, 119.7, 123.7, 124.4, 128.4, 128.7, 132.0, 135.0, 142.3, 144.1, 151.0. HRMS $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{15}\text{H}_{10}\text{NO}_4\text{Br}_2$ 425.8977, found 425.8983.

2-(4-Bromophenyl)-6-hydroxy-3-nitro-2H-chromene (**5a**)

Prepared in the same way as 8-bromo-2-(4-bromophenyl)-6-hydroxy-3-nitro-2H-chromene **5b** starting from 5-hydroxysalicylaldehyde **3a**. Mp = 170-174 °C. ^1H -NMR (300 MHz, CDCl_3) δ 6.47 (s, 1H), 6.71-6.86 (m, 2H), 6.80 (s, 1H), 7.22 (d, $J = 8.5$, 2H), 7.44 (d, $J = 8.5$, 2H), 7.97 (s, 1H). ^{13}C -NMR (75 MHz, CDCl_3) δ 73.2, 115.6, 118.1, 118.4, 121.6, 123.5, 128.7, 129.6, 131.9, 135.6, 141.3, 146.6, 151.6. HRMS $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{15}\text{H}_{11}\text{NO}_4\text{Br}$ 347.9871, found 347.9869.

8-Bromo-2-(4-bromophenyl)-6-(3-*tert*-butoxycarbonylamino)propoxy)-3-nitro-2H-chromene (**7b**)

To a solution of 3-aminopropanol (5.0 mL, 65.4 mmol) in dry dichloromethane (50.0 mL) was added dry trimethylamine (11.0 mL, 78.9 mmol, 1.20 eq.) followed by di-*tert*-butyl dicarbonate (15.0 g, 68.7 mmol, 1.05 eq.) at 0 °C under inert atmosphere. The solution was stirred at room temperature for 4 h then water was added and the reaction mixture was extracted with dichloromethane. The combined organic phase was washed with aqueous HCl 1 M then brine, dried over MgSO_4 , filtered and the filtrate concentrated under reduced pressure leading to the required *tert*-butyl (3-hydroxypropyl)carbamate **6a** which was used without further purification.

To a solution of 8-bromo-6-hydroxy-2-(4-bromophenyl)-3-nitro-2H-chromene **5b** (370.0 mg, 0.87 mmol), *tert*-butyl (3-hydroxypropyl)carbamate **6a** (167.0 mg, 0.95 mmol, 1.1 eq.) and triphenylphosphine (250.0 mg, 0.95 mmol, 1.1 eq.) in dry tetrahydrofuran (5.0 mL) was added diisopropyl azodicarboxylate (187.0 μL , 0.95 mmol, 1.1 eq.) at 0 °C under inert atmosphere. The reaction mixture was warmed to room temperature and stirred for 12 h before being concentrated under reduced pressure. The expected chromene **7b** was purified by column chromatography on silica gel using dichloromethane as eluent (330.0 mg, 65% yield). ^1H NMR (500 MHz, CDCl_3) δ 1.43 (s, 9H), 1.92-1.97 (m, 2H), 3.25-3.34 (m, 2H), 3.95 (t, $J = 5.9$, 2H), 4.70 (bs, 1H), 6.60 (s, 1H), 6.80 (s, 1H), 7.12 (d, $J = 2.8$, 1H), 7.24 (d, $J = 8.4$, 2H), 7.44 (d, $J = 8.5$, 2H), 7.96 (s, 1H). ^{13}C NMR (125 MHz, CDCl_3) δ 28.5, 29.7, 37.8, 66.8, 73.6, 79.6, 111.8, 114.7, 119.7, 123.8, 123.9, 128.6, 129.1, 132.1, 135.2, 142.4, 144.3, 154.3, 156.1. HRMS $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{23}\text{H}_{24}\text{N}_2\text{O}_6\text{Br}_2\text{Na}$ 604.9899, found 604.9893.

2-(4-Bromophenyl)-6-(3-*tert*-butoxycarbonylamino)propoxy)-3-nitro-2H-chromene (**7a**)

Prepared in the same way as 8-bromo-2-(4-bromophenyl)-6-(3-*tert*-butoxycarbonylamino)propoxy)-3-nitro-2H-chromene **7b** starting from 6-hydroxy-2-(4-bromophenyl)-3-nitro-2H-chromene **5a**. ^1H NMR (300 MHz, CDCl_3) δ 1.44 (s, 9H), 1.92-2.00 (m, 2H), 3.25-3.38 (m, 2H), 3.97 (t, $J = 6.0$, 2H), 4.70 (bs, 1H), 6.48 (s, 1H), 6.79 (d, $J = 8.8$, 1H), 6.82 (d, $J = 2.9$, 1H), 6.89 (dd, $J = 3.0, 8.9$, 1H), 7.22 (d, $J = 8.4$, 2H), 7.44 (d, $J = 8.5$, 2H), 8.00 (s, 1H). ^{13}C NMR (75 MHz, CDCl_3) δ 28.5, 29.8, 38.1, 66.5, 73.4, 79.6, 114.6, 118.3, 118.5, 121.4, 123.7, 128.8, 129.7, 132.1, 135.7, 141.5, 147.4, 154.3, 156.1. HRMS $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{23}\text{H}_{25}\text{N}_2\text{O}_6\text{BrNa}$ 527.0794, found 527.0792.

8-Bromo-2-(4-bromophenyl)-6-(6-*tert*-butoxycarbonylamino)hexyloxy)-3-nitro-2H-chromene (**7c**)

To a solution of 6-aminohexanol (1.0 g, 8.5 mmol) in dry dichloromethane (10.0 mL) was added dry trimethylamine (1.5 mL, 10.8 mmol, 1.2 eq.) followed by di-*tert*-butyl dicarbonate (2.0 g, 9.2 mmol,

1.05 eq.) at 0 °C under inert atmosphere. The solution was stirred at room temperature for 4 h then water was added and the reaction mixture was extracted with dichloromethane. The combined organic phase was washed with aqueous HCl 1 M then brine, dried over MgSO₄, filtered and the filtrate concentrated under reduced pressure leading to the required *tert*-butyl (6-hydroxyhexyl)carbamate **6b** which was used without further purification

To a solution of 8-bromo-2-(4-bromophenyl)-6-hydroxy-3-nitro-2*H*-chromene **5b** (345.0 mg, 0.8 mmol), *tert*-butyl (6-hydroxyhexyl)carbamate **6b** (195.0 mg, 0.9 mmol, 1.1 eq.) and triphenylphosphine (235 mg, 0.9 mmol, 1.1 eq.) in dry tetrahydrofuran (5.0 mL) was added diisopropyl azodicarboxylate (175.0 μL, 0.9 mmol, 1.1 eq.) at 0 °C under inert atmosphere. The reaction mixture was warmed to room temperature and stirred for 12 h before being concentrated under reduced pressure. The expected chromene **7c** was purified by column chromatography on silica gel using dichloromethane as eluent (213.0 mg, 42% yield). ¹H NMR (500 MHz, CDCl₃) δ 1.35-1.53 (m, 6H), 1.44 (s, 9H), 1.72-1.77 (m, 2H), 3.07-3.16 (m, 2H), 3.87 (t, *J* = 6.4, 2H), 4.53 (bs, 1H), 6.60 (s, 1H), 6.79 (d, *J* = 2.8, 1H), 7.10 (d, *J* = 2.8, 1H), 7.24 (d, *J* = 8.4, 2H), 7.44 (d, *J* = 8.5, 2H), 7.97 (s, 1H). ¹³C NMR (125 MHz, CDCl₃) δ 25.8, 26.6, 28.6, 29.1, 30.1, 40.6, 68.9, 73.6, 79.3, 111.7, 114.7, 119.6, 123.8, 123.8, 128.6, 129.2, 132.1, 135.2, 142.3, 144.0, 154.6, 156.1. HRMS [M+Na]⁺ calculated for C₂₆H₃₀N₂O₆Br₂Na 647.0368, found 647.0372.

6-(3-Aminopropoxy)-8-bromo-2-(4-bromophenyl)-3-nitro-2*H*-chromene hydrochloride (**8b**)

To a solution of 8-bromo-6-(3-*tert*-butoxycarbonylaminopropoxy)-2-(4-bromophenyl)-3-nitro-2*H*-chromene **7b** (270.0 mg, 0.46 mmol) in methanol (5.0 mL) was added freshly distilled acetyl chloride (66.0 μL, 0.92 mmol, 2.0 eq.) at 0 °C. The solution was warmed to room temperature, stirred for 12 h then acetyl chloride (33.0 μL, 0.46 mmol, 1.0 eq.) was again added. After 4 h the reaction mixture was concentrated under reduced pressure and the obtained residue washed with diethyl ether leading to the expected product **8b** which is dried under vacuum (204.0 mg, 85% yield). ¹H NMR (500 MHz, CD₃OD) δ 2.10-2.15 (m, 2H), 3.13 (t, *J* = 7.2, 2H), 4.09 (t, *J* = 5.8, 2H), 6.65 (s, 1H), 7.12 (d, *J* = 2.9, 1H), 7.23 (d, *J* = 2.9, 1H), 7.32 (d, *J* = 8.4, 2H), 7.49 (d, *J* = 8.6, 2H), 8.21 (s, 1H). ¹³C NMR (125 MHz, CD₃OD) δ 28.2, 38.5, 67.2, 74.9, 112.3, 115.9, 121.6, 124.5, 124.6, 130.0, 130.3, 132.9, 136.8, 143.7, 145.4, 155.3. HRMS [M+H]⁺ calculated for C₁₈H₁₇N₂O₄Br₂ 482.9555, found 482.9553.

6-(3-Aminopropoxy)-2-(4-bromophenyl)-3-nitro-2*H*-chromene hydrochloride (**8a**)

Prepared in the same way as 8-bromo-6-(3-aminopropoxy)-2-(4-bromophenyl)-3-nitro-2*H*-chromene hydrochloride **8b** starting from 2-(4-bromophenyl)-6-(3-*tert*-butoxycarbonylaminopropoxy)-3-nitro-2*H*-chromene **8a**. ¹H NMR (300 MHz, CD₃OD) δ 2.08-2.23 (m, 2H), 3.14 (t, *J* = 7.0, 2H), 4.03-4.19 (m, 2H), 6.52 (s, 1H), 6.79 (d, *J* = 8.8, 1H), 6.98 (dd, *J* = 2.8, 8.9, 1H), 7.08 (d, *J* = 3.0, 1H), 7.28 (d, *J* = 8.5, 2H), 7.47 (d, *J* = 8.4, 2H), 8.20 (s, 1H).

6-(6-Aminoheptyloxy)-8-bromo-2-(4-bromophenyl)-3-nitro-2*H*-chromene hydrochloride (**8c**)

Prepared in the same way as 8-bromo-6-(3-aminopropoxy)-2-(4-bromophenyl)-3-nitro-2*H*-chromene hydrochloride **8b** starting from 8-bromo-2-(4-bromophenyl)-6-(6-*tert*-butoxycarbonylaminoheptyloxy)-3-nitro-2*H*-chromene **7c**. ¹H NMR (500 MHz, CD₃OD) δ 1.42-1.49 (m, 2H), 1.50-1.56 (m, 2H), 1.65-1.71 (m, 2H), 1.76-1.81 (m, 2H), 2.93 (d, *J* = 7.7, 2H), 3.95 (t, *J* = 6.3, 2H), 6.63 (s, 1H), 7.06 (d, *J* = 2.8, 1H), 7.16 (d, *J* = 2.8, 1H), 7.32 (d, *J* = 8.4, 2H), 7.49 (d, *J* = 8.5, 2H), 8.20 (s,

1H). ¹³C NMR (125 MHz, CD₃OD) δ 26.6, 27.2, 28.5, 30.0, 40.7, 69.7, 74.9, 112.3, 115.9, 121.6, 124.5, 124.5, 130.0, 130.4, 132.9, 136.9, 143.7, 145.0, 156.0. HRMS [M+H]⁺ calculated for C₂₁H₂₃N₂O₄Br₂ 525.0024, found 525.0021.

DHM25-Biotin

To a solution of (+)-biotin-*N*-hydroxysuccinimide ester **9** (400 mg, 0.12 mmol, 1.2 eq.) in a 1/1 mixture of *N,N*-dimethylformamide and triethylammonium bicarbonate buffer pH 8.5 (2.0 mL) was added 6-(3-aminopropoxy)-8-bromo-2-(4-bromophenyl)-3-nitro-2*H*-chromene hydrochloride **8b** (51.0 mg, 0.10 mmol.) and the reaction mixture was stirred at room temperature for 20 h. Dichloromethane and aqueous 1 M HCl were added and the mixture was extracted with dichloromethane. The combined organic phase was washed with brine, dried over MgSO₄, filtered and the filtrate concentrated under reduced pressure. The DHM25-Biotin was purified by column chromatography on silica gel using dichloromethane/methanol 95/5 to 9/1 as eluent (48.0 mg, 55% yield). ¹H NMR (500 MHz, CDCl₃) δ 1.38-1.44 (m, 2H), 1.60-1.74 (m, 4H), 1.94-1.99 (m, 2H), 2.21 (t, *J* = 7.2, 2H), 2.68 (d, *J* = 12.7, 1H), 2.84-2.88 (m, 1H), 3.09-3.13 (m, 1H), 3.38-3.41 (m, 2H), 3.94 (t, *J* = 5.8, 2H), 4.24-4.27 (m, 1H), 4.41-4.45 (m, 1H), 5.51 (bs, 1H), 6.41 (bs, 1H), 6.53 (bs, 1H), 6.60 (s, 1H), 6.84 (t, *J* = 2.2, 1H), 7.11 (d, *J* = 2.0, 1H), 7.25 (dd, *J* = 2.2, 8.6, 2H), 7.44 (d, *J* = 8.6, 2H), 8.02 (s, 1H). ¹³C NMR (125 MHz, CDCl₃) δ 25.5, 27.9, 28.0, 29.0, 35.8, 36.5, 40.5, 55.5, 60.1, 61.6, 66.7, 73.4, 111.5, 114.5, 114.5, 119.5, 123.6, 123.7, 123.7, 128.4, 129.2, 129.2, 131.9, 135.0, 142.0, 142.0, 144.0, 154.0, 163.9, 173.4. HRMS [M+H]⁺ calculated for C₂₈H₃₁N₄O₆Br₂S 709.0325, found 709.0317.

DHM9-Biotin

Prepared in the same way as DHM25-Biotin starting from 6-(3-aminopropoxy)-2-(4-bromophenyl)-3-nitro-2*H*-chromene hydrochloride **8a**. ¹H NMR (500 MHz, CDCl₃) δ 1.39-1.47 (m, 2H), 1.62-1.74 (m, 4H), 1.93-2.02 (m, 2H), 2.21 (t, *J* = 7.4, 2H), 2.68 (d, *J* = 12.8, 1H), 2.84-2.88 (dd, *J* = 4.7, 12.8, 1H), 3.08-3.14 (m, 1H), 3.38-3.45 (m, 2H), 3.96 (t, *J* = 6.0, 2H), 4.24-4.27 (m, 1H), 4.41-4.45 (m, 1H), 5.57 (bs, 1H), 6.44 (t, *J* = 5.6, 1H), 6.47 (s, 1H), 6.53 (bs, 1H), 6.77 (d, *J* = 8.6, 1H), 6.85 (t, *J* = 2.7, 1H), 6.88 (dd, *J* = 2.9, 8.6, 1H), 7.21 (d, *J* = 8.4, 2H), 7.43 (d, *J* = 8.4, 2H), 8.02 (s, 1H). ¹³C NMR (125 MHz, CDCl₃) δ 25.6, 28.0, 28.1, 29.1, 35.8, 36.9, 40.5, 55.6, 60.3, 61.8, 66.7, 73.3, 114.6, 114.6, 118.1, 118.4, 121.4, 121.4, 123.5, 128.7, 129.7, 131.9, 135.6, 141.2, 147.2, 154.0, 163.9, 173.6. HRMS [M+H]⁺ calculated for C₂₈H₃₁N₄O₆BrNaS 653.1045, found: 653.1042.

DHM25-FITC

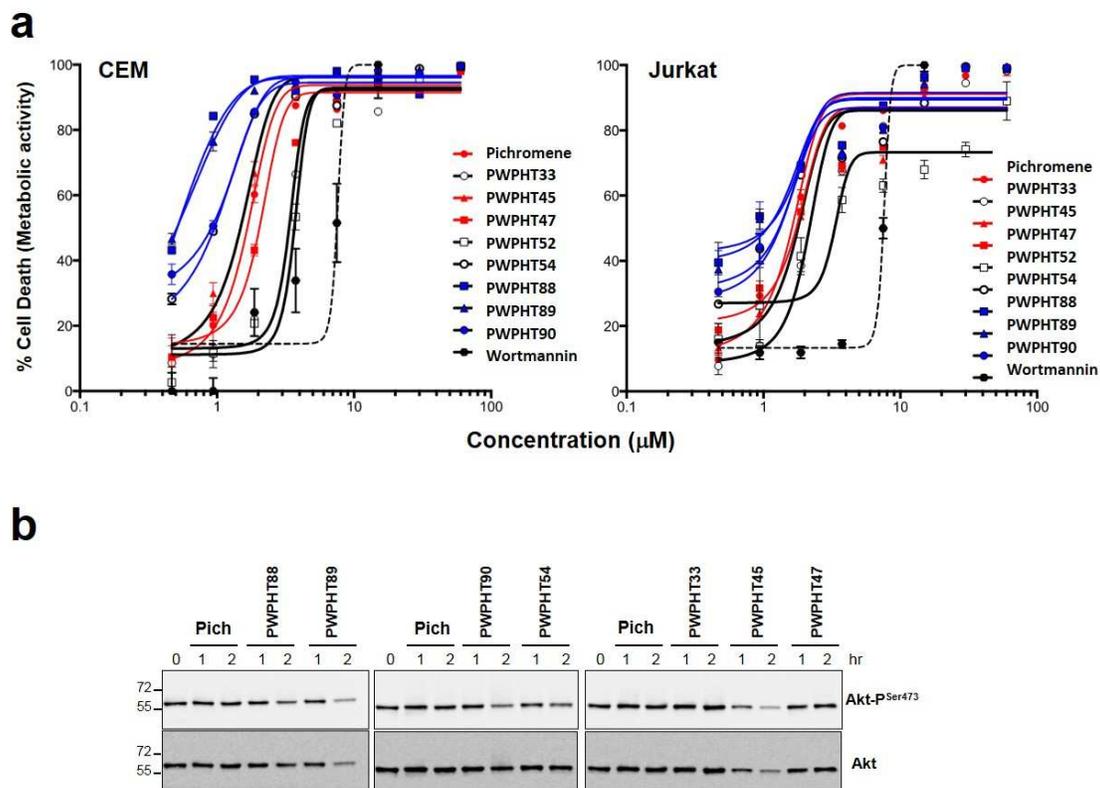
To a solution of 6-(6-aminohexyloxy)-8-bromo-2-(4-bromophenyl)-3-nitro-2*H*-chromene hydrochloride **8c** (85.0 mg, 0.15 mmol) and fluorescein isothiocyanate isomer I **10** (70.0 mg, 0.18 mmol, 1.2 eq.) in dry *N,N*-dimethylformamide (2.0 mL) was added dry trimethylamine (21.0 μL, 0.15 mmol, 1.0 eq.). The reaction mixture was stirred at room temperature for 20 h then ice was added. The precipitate was filtered, washed with water and dried under reduced pressure. The crude material was purified by column chromatography on silica gel using dichloromethane/methanol 95/5 to 9/1 as eluent leading to DHM25-FITC (75.0 mg, 63% yield). ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.37-1.47 (m, 4H), 1.57-1.63 (m, 2H), 1.69-1.74 (m, 2H), 3.50-3.53 (m, 2H), 3.95 (t, *J* = 6.2, 2H), 6.56 (dd, *J* = 2.2, 8.6, 2H), 6.60 (d, *J* = 8.6, 2H), 6.67 (d, *J* = 2.2, 2H), 6.72 (s, 1H), 7.17 (d, *J* = 8.2, 1H), 7.26 (d, *J* = 2.7, 1H), 7.28 (d, *J* = 2.7, 1H), 7.34 (d, *J* = 8.5, 2H), 7.57 (d, *J* = 8.5, 2H), 7.71-7.74 (m, 1H), 8.07 (bs, 1H),

8.22 (s, 1H), 8.38 (s, 1H), 9.85 (bs, 1H), 10.11 (s, 2H). ^{13}C NMR (125 MHz, DMSO_{d6}) δ 25.1, 26.0, 28.2, 28.4, 30.7, 35.7, 68.3, 72.8, 82.9, 102.1, 109.6, 110.2, 112.4, 115.8, 119.9, 122.7, 122.8, 128.9, 129.0, 129.7, 131.7, 135.3, 141.6, 142.8, 151.7, 153.8, 159.3, 162.2, 168.4. HRMS $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{42}\text{H}_{34}\text{N}_3\text{O}_9\text{Br}_2\text{S}$ 914.0377, found 914.0375.

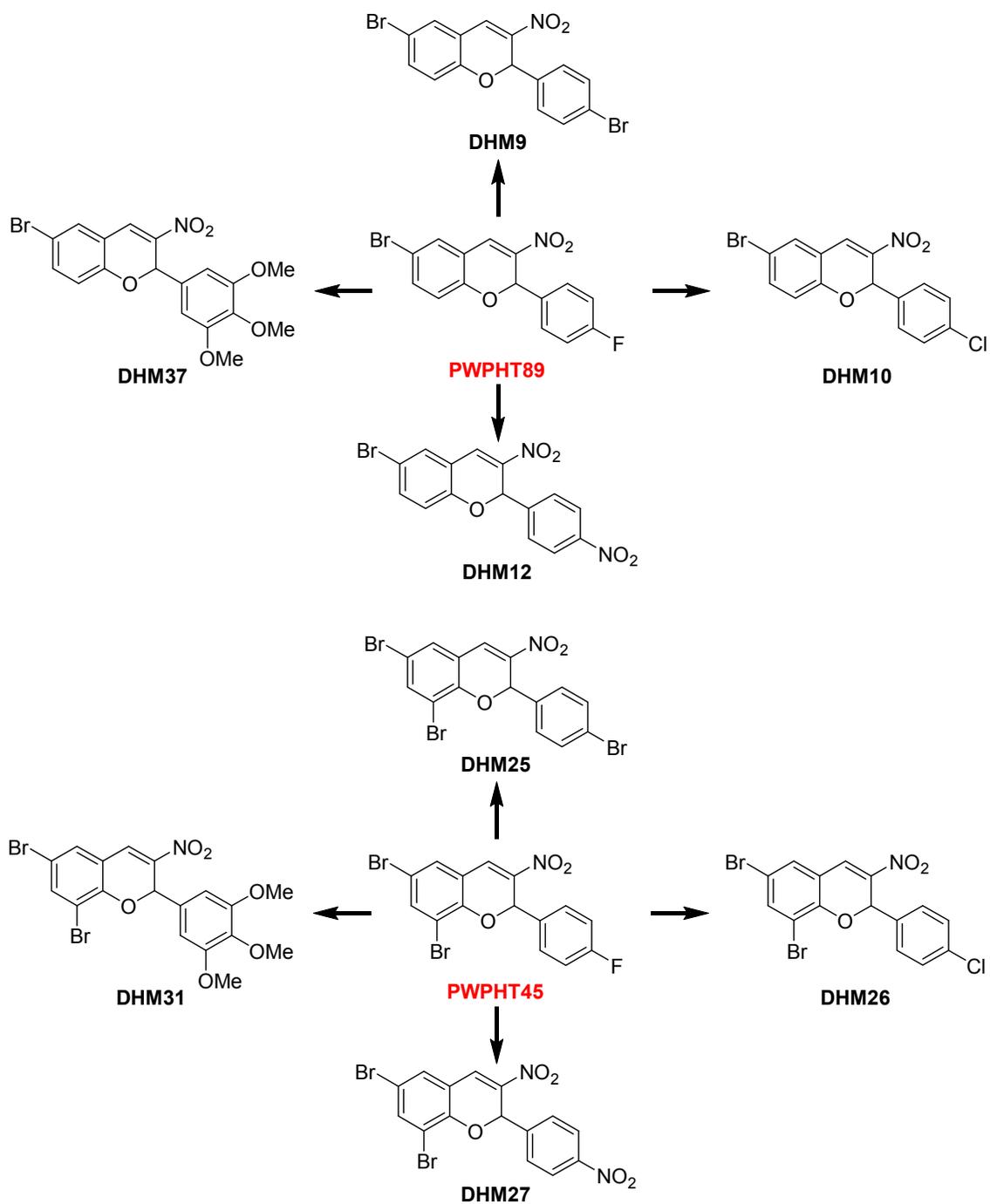
6,8-Dibromo-2-(4-bromophenyl)-3-nitrochromane (DHM25-H2)

To a solution of DHM25 in a mixture of THF and methanol (9/1) was added sodium borohydride by portion at 0 °C. After 10 minutes water was added and the solution was extracted with EtOAc. The combined organic phases were washed with brine, dried over MgSO_4 , filtered and the filtrate concentrated under reduced pressure. HRMS M^{++} calculated for $\text{C}_{15}\text{H}_{10}\text{NO}_3\text{Br}_3$ 488.8211, found 488.8231.

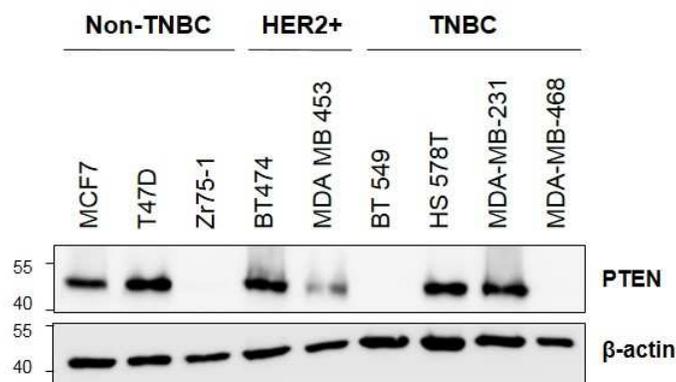
SUPPORTING FIGURES



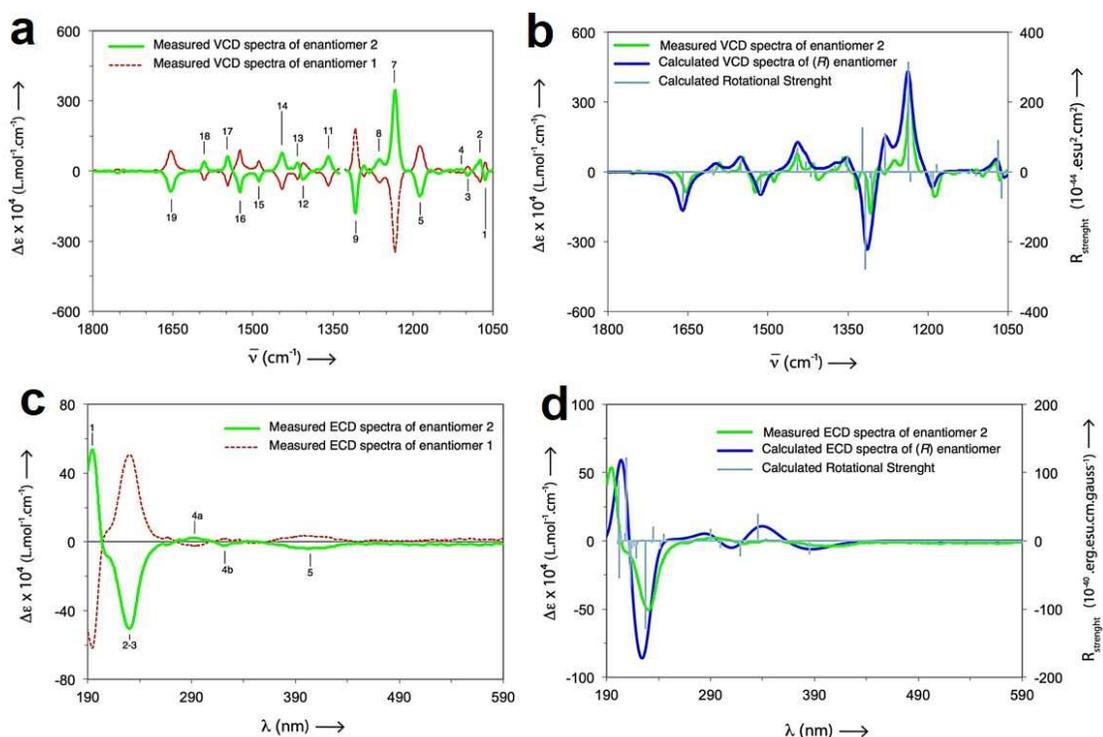
Supporting Figure 1. Novel chromologs with cytotoxic activity against PI3K-addicted cancer cells. A. The leukemic T cell lines CEM or Jurkat were incubated with the indicated chemicals for 24 hours, and cell death was measured by MTT viability assay. **B.** Jurkat cells were incubated with the indicated chromologs (10 μM) for the indicated times. Cells were lysed, and immunoblots were performed. Data are representative of three different experiments.



Supporting Figure 2. Synthesis of a second library of chromologs. From PWPHT89 and PWPHT45, a new collection of derivatives was designed and synthesized.

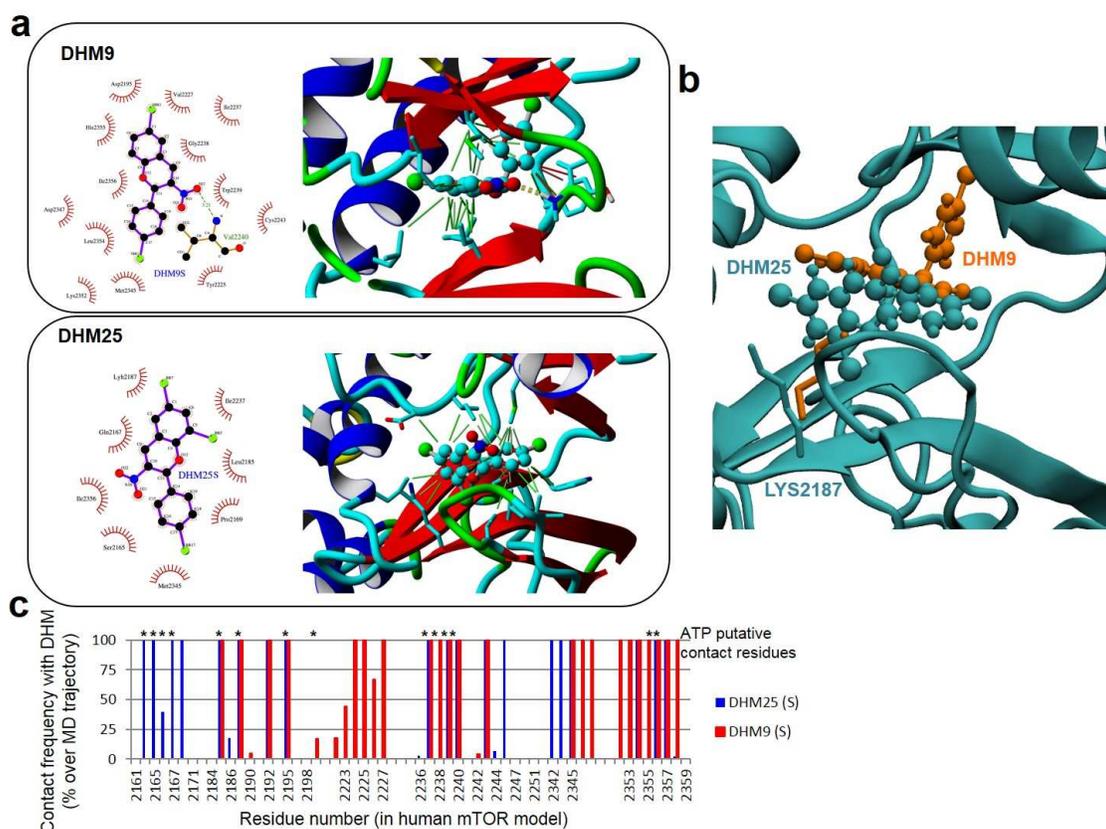


Supporting Figure 3. PTEN expression in breast cancer cell lines. The indicated breast cancer cells were lysed, and 100 μg of protein was loaded per lane and resolved by 12% SDS-PAGE. Immunoblotting was carried out using the indicated antibodies. Data are representative of three independent experiments.

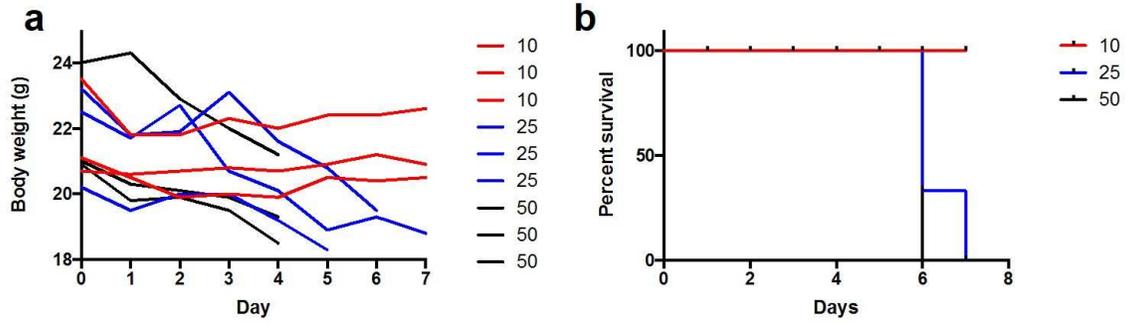


Supporting Figure 4. Determination of the absolute configuration of the DHM25 enantiomers. **A.** Measured vibrational circular dichroism (VCD) spectra of each DHM25 enantiomer. **B.** Assignment of the (*R*) absolute configuration to enantiomer 2 by comparison

of its measured VCD spectra to the predicted spectra. **C.** Measured electronic circular dichroism (ECD) spectra of each DHM25 enantiomer. **D.** Assignment of the (*R*) absolute configuration to enantiomer 2 by comparison of its measured ECD spectra to the predicted spectra.



Supporting Figure 5. Computer modeling of (*S*)-DHM25 and (*S*)-DHM9 insertions inside the ATP pocket of mTOR. **A. Contact residues for both DHM9 and DHM25 in models of the DHM-mTOR complex determined by the LigPlot+ program (left panel) and the Yasara program (right panel). In the tridimensional view, the green sticks indicate hydrophobic contacts. **B.** Superimposition of the DHM9 and DHM25 positioning in the catalytic pocket of human mTOR. DHM molecules are shown in ball-and-stick form, and Lys2187 is shown in stick form; for clarity, only the backbone trace of mTOR is shown. **C.** Contact frequencies measured during the molecular dynamics simulations. ATP putative contact residues are**



Supporting Figure 7. Evaluation of DHM25 toxicity in mice. A. Measure of body weight loss following repeated injections of different DHM25 doses (10, 25, or 50 mg/kg). **B.** Viability of mice treated in A.