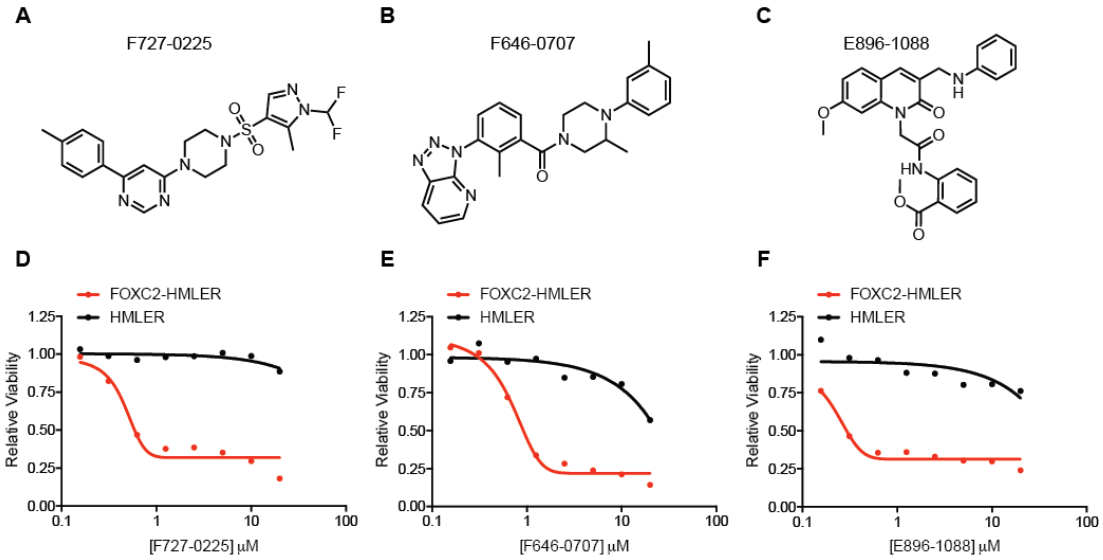
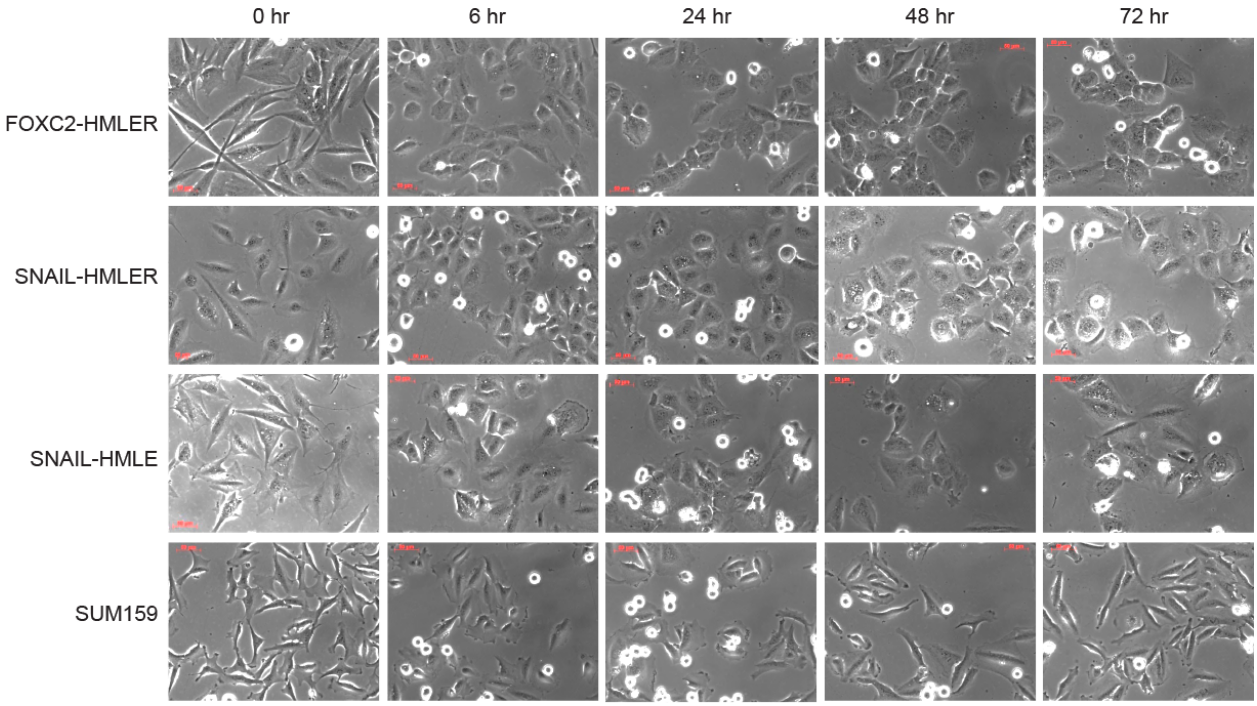
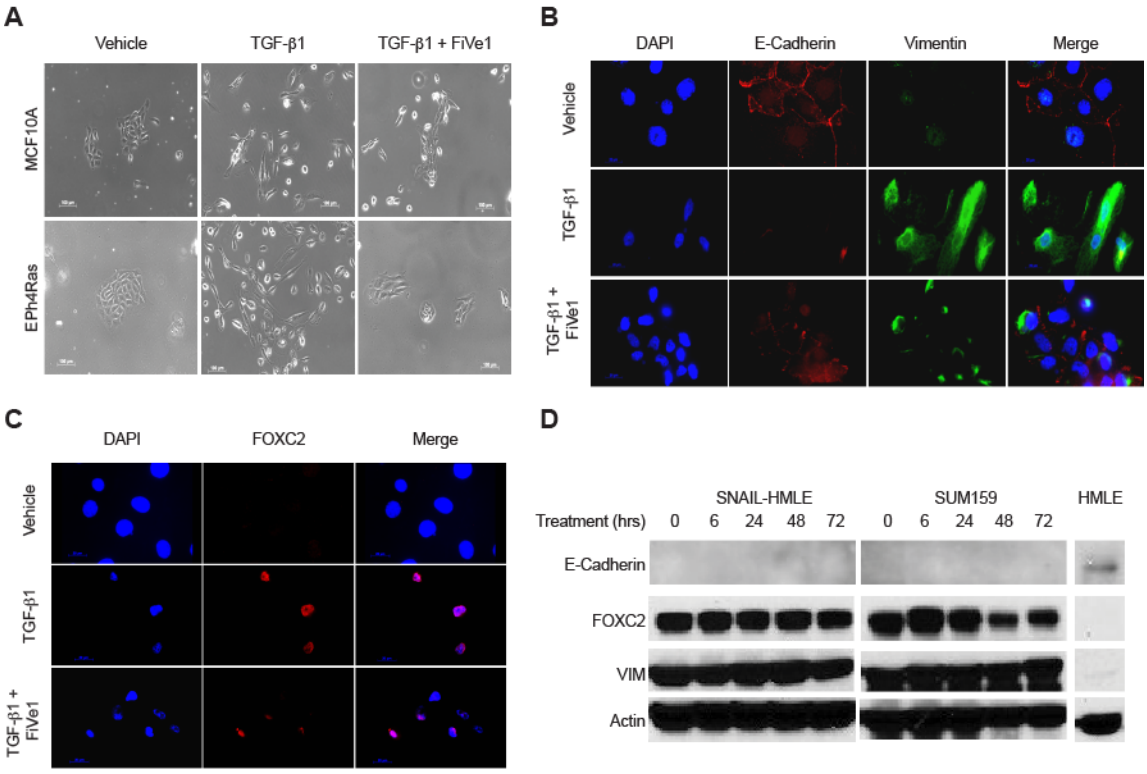


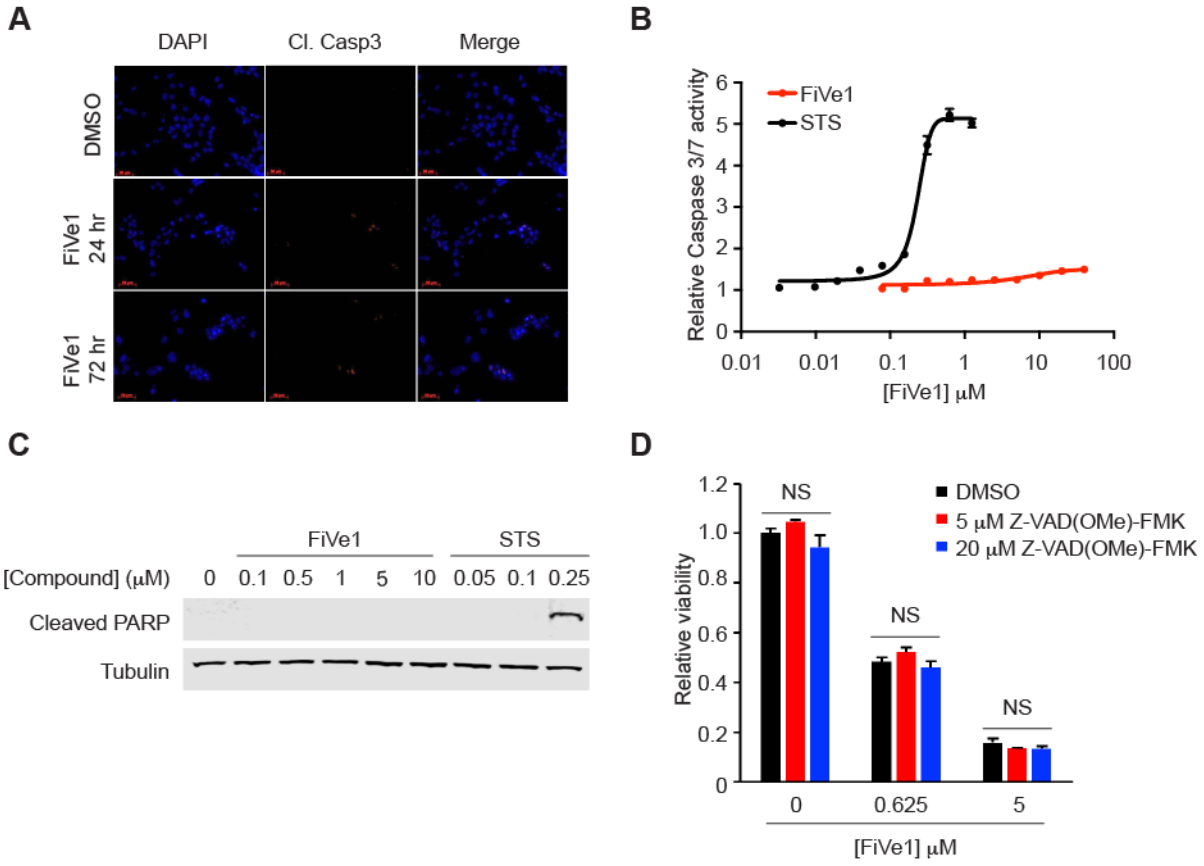

**Supplementary Figure 1. Additional screening hits displaying selective toxicity to FOXC2-expressing HMLER cells.** (**A-C**) Structures of F727-0225, F646-0707, and E896-1088 respectively. (**D-F**) Relative viability measurements of FOXC2-HMLER and control HMLER cells after 72-hour treatment with the indicated doses of compound.



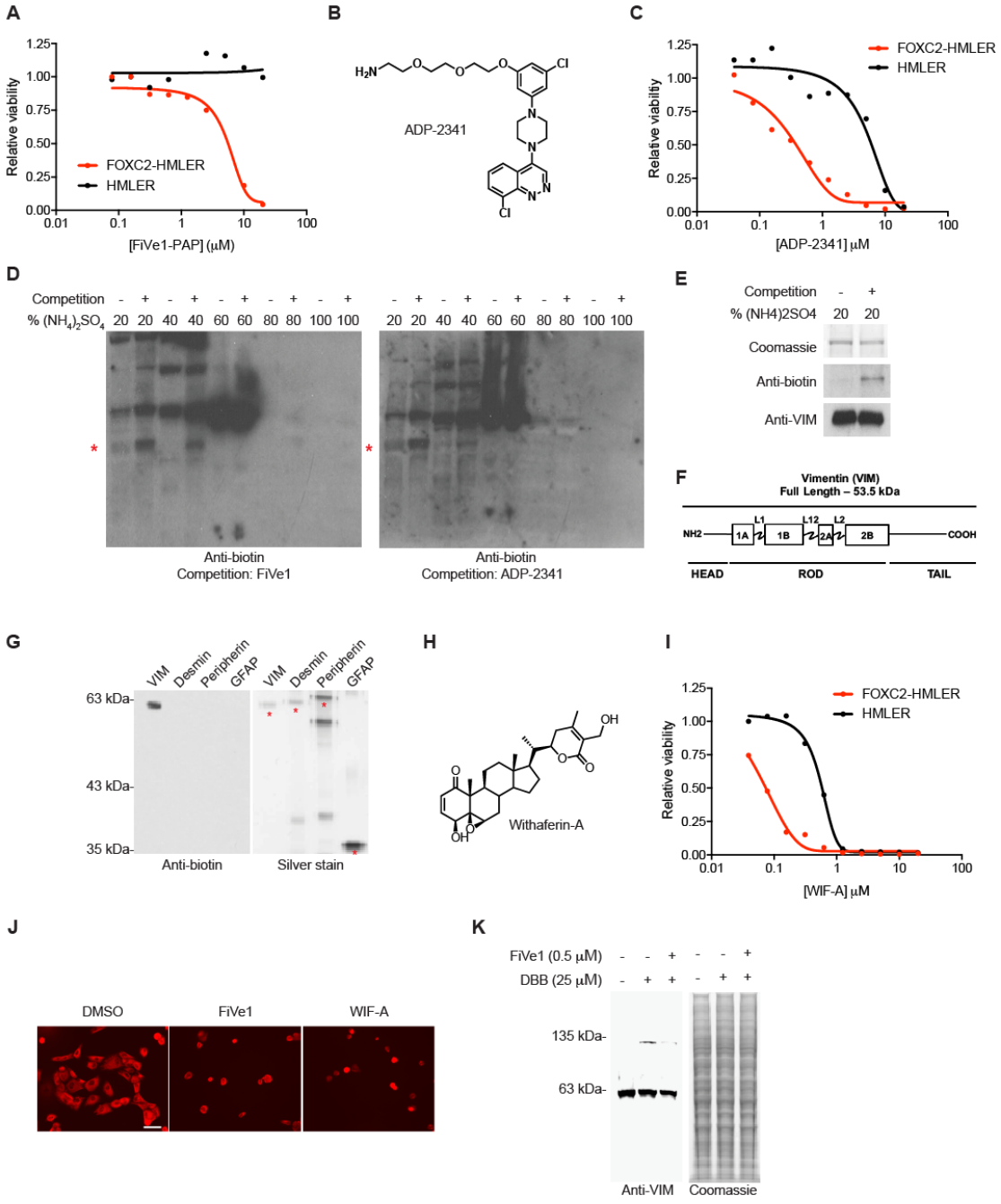

**Supplementary Figure 2. FiVe1 treatment reverts FOXC2-expressing breast cancer cells to a more epithelial morphology.** Representative images of FOXC2-expressing cell types treated with 500 nM FiVe1 for the indicated time intervals. (Scale bars, 50 μm).




**Supplementary Figure 3. FiVe1 treatment inhibits the morphological changes of TGF-β1-induced EMT but does not alter the EMT transcriptional program.** (**A**) Representative images of MCF10A or EPh4Ras cells treated for 72 hours with TGF-β1 in the presence of 500 nM FiVe1 (Scale bars, 100 μm). (**B, C**) Immunofluorescent staining for E-Cadherin, Vimentin, and FOXC2 in MCF10A cells treated for 72 hours with TGF-β1 in the presence of 500 nM FiVe1 (Scale bars, 20 μm). (**D**) Western blot analysis of EMT markers in HMLE-SNAIL and SUM159 cells treated with 500 nM FiVe1 at the indicated time points.

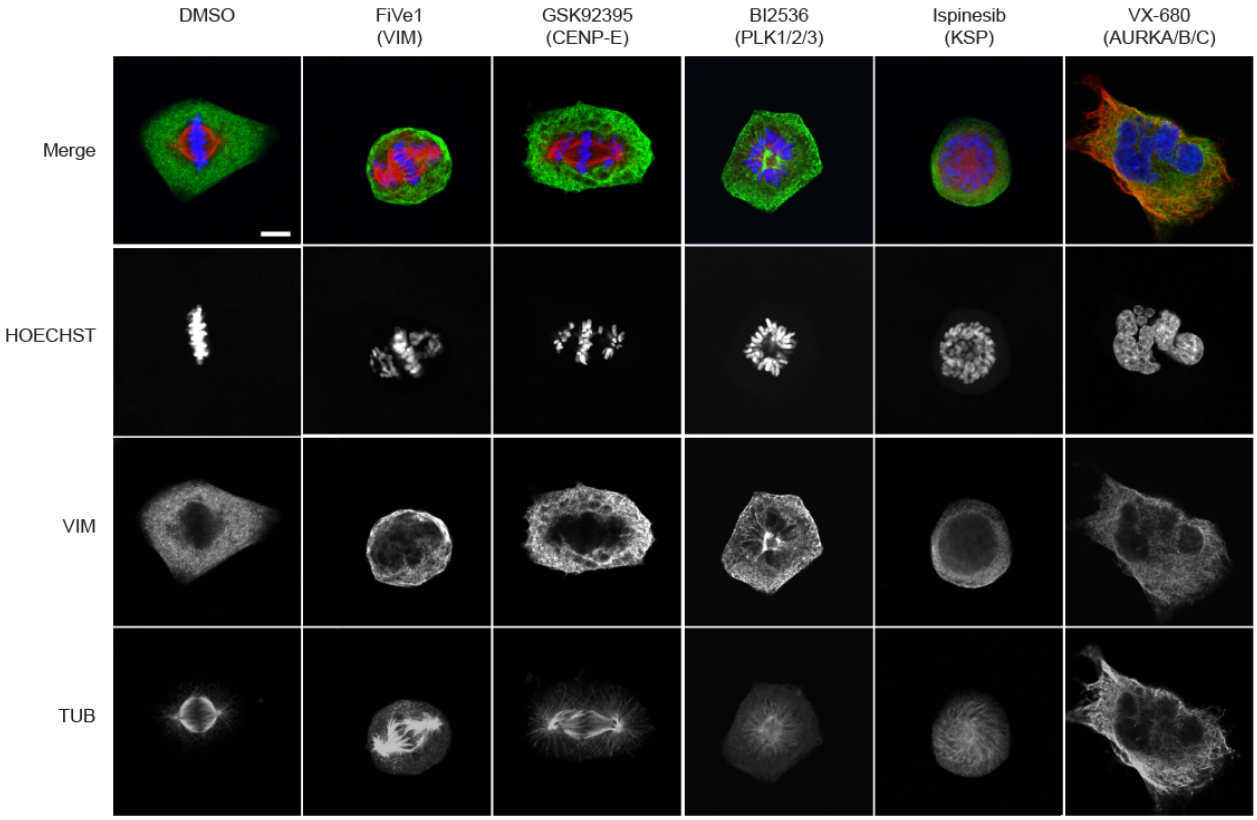



**Supplementary Figure 4. FiVe1 does not inhibit proliferation by inducing apoptosis.** (**A**) Representative images of immunofluorescent staining for cleaved Caspase 3 in FOXC2-HMLER cells treated with 500 nM FiVe1 for the indicated time periods. (**B**) Relative Caspase 3/7 measurements from FOX2-HMLER cells treated for 24 hours with the indicated doses of compound (STS = staurosporine, *n*=3, mean and s.e.m.). (**C**) Western blot analysis of cleaved PARP levels from FOXC2-HMLER cells treated for 24 hours with the indicated doses of FiVe1 or Staurosporine (STS). (**D**) Relative viability measurements from FOXC2-HMLER cells treated for 48 hours with the indicated doses of FiVe1 in the presence of the indicated doses of the pan-Caspase inhibitor Z-VAD(OMe)-FMK (*n*=3, mean and s.d., NS = not significant).

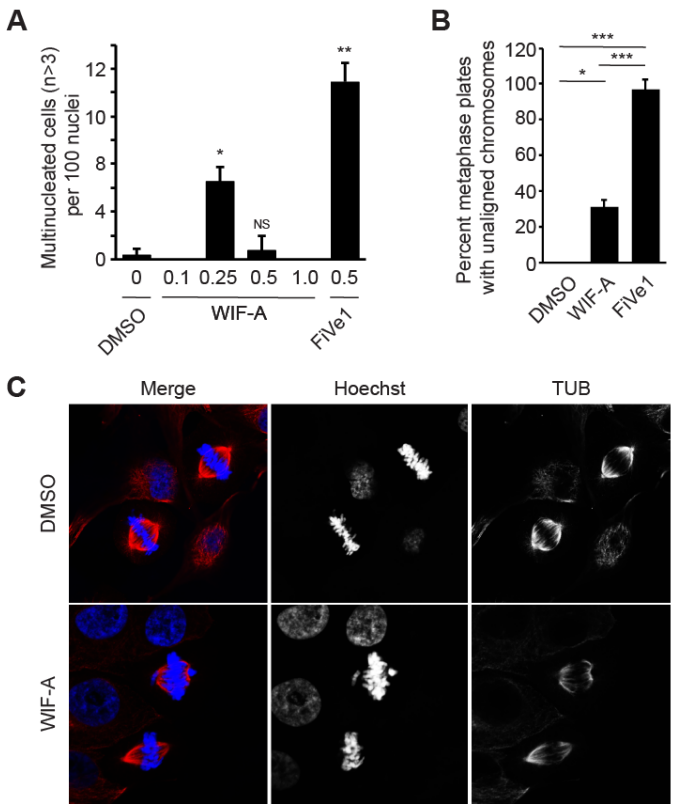



**Supplementary Figure 5. Target identification experiments reveal VIM as the relevant cellular target of FiVe1.** (**A**) Relative viability measurements of FOXC2-HMLER and control HMLER cells treated with the indicated doses of FiVe1-PAP. (**B**) Structure of the soluble FiVe1 analog ADP-2341. (**C**) Relative viability measurements of FOXC2-HMLER and control HMLER cells treated with the indicated doses of ADP-2341 for 72 hours. (**D**) FOXC2-HMLER cells were treated with 2.5 μM FiVe1-PAP in the presence of DMSO or 50 μM competition for 15 minutes and crosslinked to cellular targets by UV irradiation. Shown are anti-biotin Western blots of ammonium sulfate fractions when competed with either FiVe1 (left) or ADP-2341 (right). Red

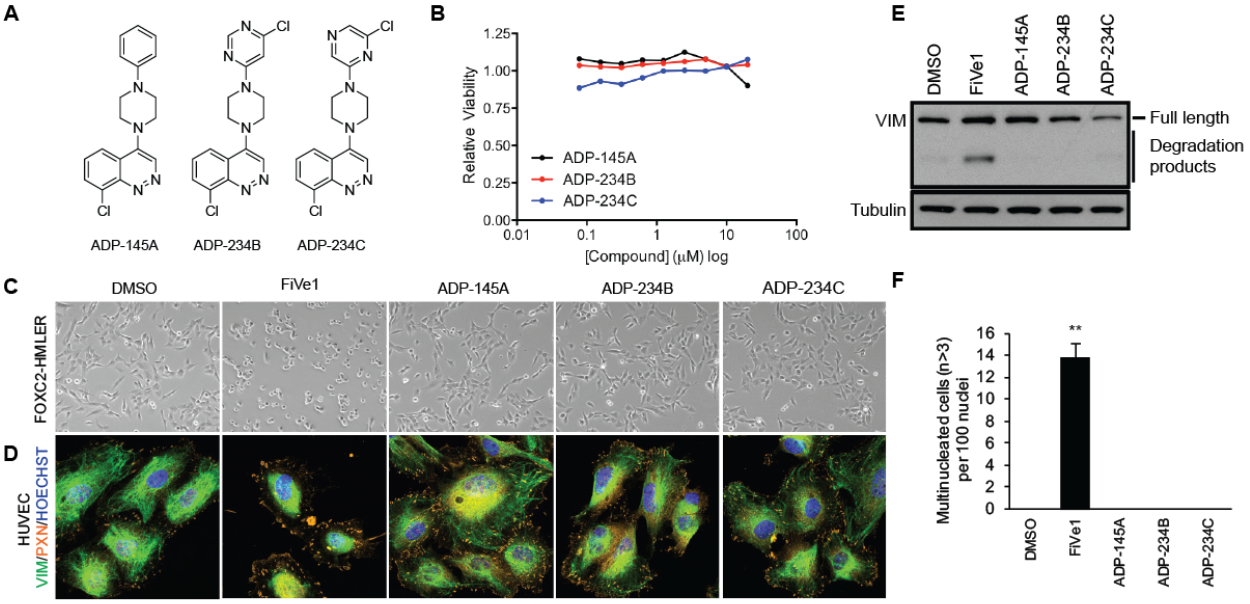
asterisks indicate probe labeled VIM content confirmed by LC-MS/MS. (**E**) The 20% ammonium sulfate fraction from FOXC2-HMLER cells UV crosslinked with FiVe1-PAP (2.5 μM) and 50 μM ADP-2341 competition was stained with Coomassie blue or visualized by anti-biotin or anti-VIM Western blotting. (**F**) Schematic depicting the domains of the intermediate filament Vimentin. Boxes 1A, 1B, 2A, and 2B indicate helical portions of the rod domain. Head, rod, and tail refer to previously identified domains expressed as GST fusion proteins. (**G**) Anti-biotin Western blotting analysis of the indicated type 3 intermediate filament proteins exposed to *in vitro* crosslinking conditions (FiVe1-PAP, 20 nM). Red asterisks indicate the full length protein product of the recombinant protein preparations. (**H**) Structure of Withaferin A (WIF-A). (**I**) Relative viability measurements of FOXC2-HMLER and control HMLER cells treated with the indicated doses of Withaferin A for 72 hours. (**J**) Representative images of FOXC2-HMLER cells treated for 30 minutes with FiVe1 (500 nM) or WIF-A (500 nM) and then immunostained for VIM protein (red). (**K**) Western blotting analysis of dimerized VIM protein content from cells treated for 30 minutes with 500 nM FiVe1 and exposed to the crosslinking agent dibromobimane (DBB, 25 μM) for 15 minutes.



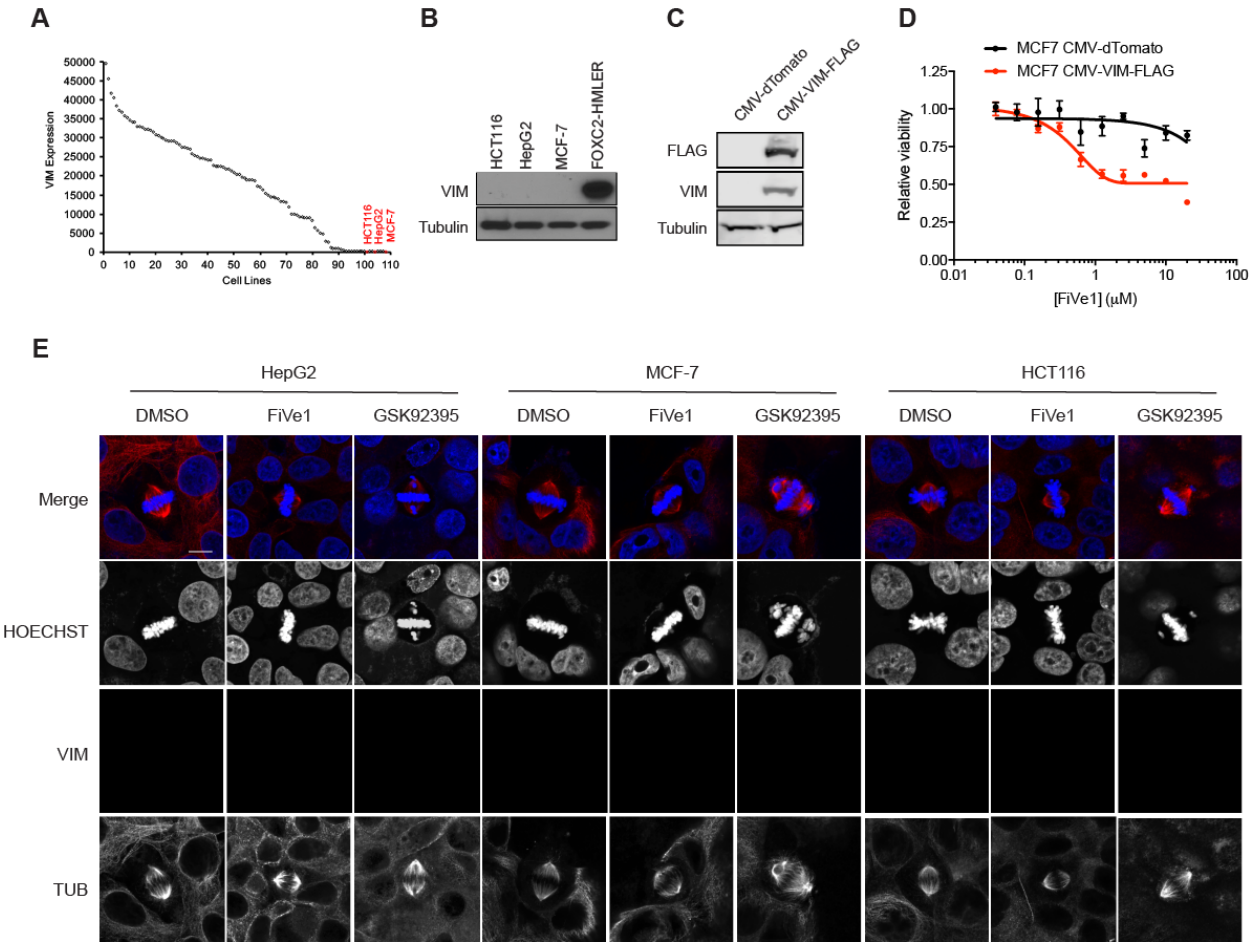

**Supplementary Figure 6. FiVe1 induces a metaphase phenotype distinct from other mitosis-modulating inhibitors.** Confocal images of thymidine synced FOXC2-HMLER cells treated with the indicated chemical inhibitors with their respective targets in parenthesis (scale bar, 10 μm; FiVe1, 250 nM; GSK92395, 25 nM; BI2536, 10 nM; Ispinesib, 100 nM; VX-680 100 nM).



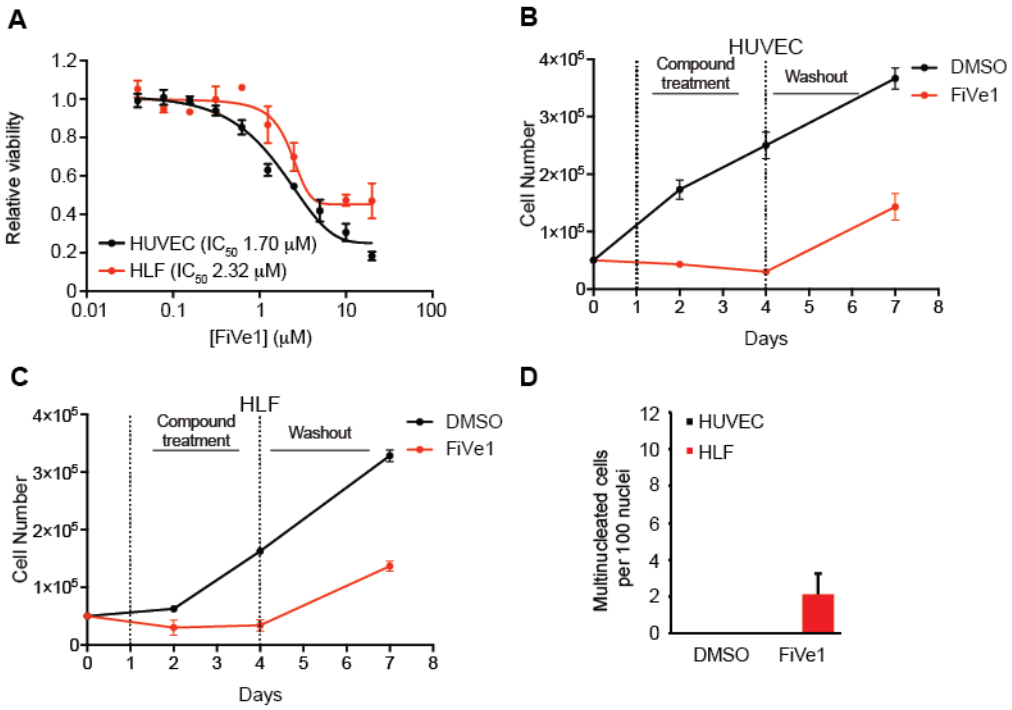

**Supplementary Figure 7. WIF-A induces multinucleation and chromosomal misalignment within a limited concentration range.** (**A**) Quantification of multinucleation (n>3 per cell) from FOXC2-HMLER cells treated with the indicated doses (μM) of WIF-A or FiVe1 (*n*=3, mean and s.d.). (**B, C**) Quantification and representative images of metaphase plates with misaligned chromosomes from thymidine synched FOXC2-HMLER cells treated with WIF-A (0.25 μM) or FiVe1 (0.5 μM) (*n*=3, mean and s.d.; **P*<0.05, ***P*<0.005, ****P*<0.0005, NS= not significant; t-test).




**Supplementary Figure 8. FiVe1 analogs without FOXC2-HMLER cytotoxicity do not degrade VIM or induce multinucleation.** (**A**) Chemical structures of ADP-145A, ADP-234B, and ADP-234C. (**B**) Relative viability measurements of FOXC2-HMLER cells exposed to the indicated doses of compound for 72 hours. (**C**) Bright field images of FOXC2-HMLER cells exposed to the indicated compounds for 30 minutes. (FiVe1, 500 nM; ADP-145A, ADP-234B, and ADP-234C, 5 μM). (**D**) Maximum intensity projections of Vimentin (VIM) and Paxillin (PXN) stained HUVECs after one hour treatment with 5 μM of the indicated compounds. (**E**) Western blot analysis for VIM degradation from FOXC2-HMLER cells 4 hours after exposure to 5 μM of the indicated compounds (**F**) Results of multinucleation image analysis of FOXC2-HMLER cells exposed to the indicated compounds for 24 hours. (FiVe1, 500 nM; ADP-145A, ADP-234B, and ADP-234C, 5 μM). ($n$=3, mean and s.d., $^{**}P$<0.005, t-test).




**Supplementary Figure 9. FiVe1-induced effects on growth arrest and mitotic disruption are restricted to VIM-expressing cells.** (**A**) VIM transcript levels across the NCI-60 dataset as retrieved from BioGPS. (**B**) Western blot analysis of VIM protein levels from HCT116, HepG2, and MCF-7 cells relative to FOXC2-HMLER cells. (**C**) Western blot analyses of FLAG and VIM protein levels from MCF-7 cell lines stably expressing dTomato or VIM-FLAG. (**D**) Relative viability measurements of MCF-7 cell lines stably expressing lentivirally delivered dTomato or VIM-FLAG proteins. (**E**) Representative confocal images of the indicated thymidine synced, VIM negative cell types at metaphase treated with FiVe1 or GSK92395 (FiVe1, 500 nM; GSK92395, 25 nM; scale bar, 10 μm).




**Supplementary Figure 10. Evaluation of the anti-proliferative effects of FiVe1 on normal mesenchymal cell lines.** (**A**) Relative viability measurements from HUVEC and HLF cells treated with the indicated concentration response of FiVe1 (*n*=3, mean and s.e.m.). Growth curves of HUVEC (**B**) or HLF (**C**) cells treated with 10 μM FiVe1 during days 1-4 as noted by dashed vertical lines. (*n*=3, mean and s.d.). (**D**) Multinucleation analysis of HUVEC and HLFs treated for 24 hours with 500 nM FiVe1 (*n*=3, mean and s.d.).

| Kinase | Conc. Tested (μM) | % Inhibition |
|---|---|---|
| ABL1 | 1 | 8 |
| ABL1 | 10 | 8 |
| AKT1 | 1 | 7 |
| AKT1 | 10 | 2 |
| ALK | 1 | 1 |
| ALK | 10 | -2 |
| AURORA-A | 1 | 7 |
| AURORA-A | 10 | 4 |
| CDK2 | 1 | 4 |
| CDK2 | 10 | 4 |
| CHEK1 | 1 | 4 |
| CHEK1 | 10 | 6 |
| CLK1 | 1 | 7 |
| CLK1 | 10 | 5 |
| CLK2 | 1 | 9 |
| CLK2 | 10 | 3 |
| CLK3 | 1 | 11 |
| CLK3 | 10 | -13 |
| CSK | 1 | 8 |
| CSK | 10 | 0 |
| DAPK1 | 1 | 4 |
| DAPK1 | 10 | 2 |
| DYRK1A | 1 | 6 |
| DYRK1A | 10 | 15 |
| DYRK1B | 1 | -7 |
| DYRK1B | 10 | 4 |
| EGFR-002 | 1 | 5 |
| EGFR-002 | 10 | 2 |
| EPH-A2 | 1 | 3 |
| EPH-A2 | 10 | 10 |
| EPH-B4 | 1 | -1 |
| EPH-B4 | 10 | -2 |
| FGFR1 | 1 | 4 |
| FGFR1 | 10 | 2 |

| Kinase | Conc. Tested (μM) | % Inhibition |
|---|---|---|
| FLT-3 | 1 | 5 |
| FLT-3 | 10 | 2 |
| GSK-3-BETA | 1 | 6 |
| GSK-3-BETA | 10 | 10 |
| IGF1R | 1 | 3 |
| IGF1R | 10 | 4 |
| IKK-ALPHA | 1 | -9 |
| IKK-ALPHA | 10 | -11 |
| IKK-BETA | 1 | -15 |
| IKK-BETA | 10 | 5 |
| IRAK4 | 1 | 4 |
| IRAK4 | 10 | 1 |
| ITK | 1 | 1 |
| ITK | 10 | 4 |
| JAK3 | 1 | 4 |
| JAK3 | 10 | 2 |
| JNK1 | 1 | -22 |
| JNK1 | 10 | 1 |
| JNK2 | 1 | -17 |
| JNK2 | 10 | -2 |
| JNK3 | 1 | -15 |
| JNK3 | 10 | 2 |
| KDR | 1 | 6 |
| KDR | 10 | 7 |
| LCK | 1 | 1 |
| LCK | 10 | 0 |
| MAP4K4 | 1 | 10 |
| MAP4K4 | 10 | 11 |
| MAPK1 | 1 | -7 |
| MAPK1 | 10 | 6 |
| MEK1 | 1 | -8 |
| MEK1 | 10 | 2 |
| MEK2 | 1 | -12 |
| MEK2 | 10 | 18 |

| Kinase | Conc. Tested (μM) | % Inhibition |
|---|---|---|
| MET | 1 | 2 |
| MET | 10 | 0 |
| MNK2 | 1 | 4 |
| MNK2 | 10 | -1 |
| MST1 | 1 | 6 |
| MST1 | 10 | 5 |
| NEK2 | 1 | 5 |
| NEK2 | 10 | 1 |
| P38-ALPHA | 1 | 8 |
| P38-ALPHA | 10 | 8 |
| P38-BETA | 1 | 7 |
| P38-BETA | 10 | 10 |
| P38-DELTA | 1 | 5 |
| P38-DELTA | 10 | 3 |
| P38-GAMMA | 1 | 7 |
| P38-GAMMA | 10 | 0 |
| PAK2 | 1 | 0 |
| PAK2 | 10 | 1 |
| PDGFR-ALPHA | 1 | 3 |
| PDGFR-ALPHA | 10 | 3 |
| PDK1 | 1 | 4 |
| PDK1 | 10 | 4 |
| PIM-1-KINASE | 1 | 3 |
| PIM-1-KINASE | 10 | 2 |
| PKC-ALPHA | 1 | 2 |
| PKC-ALPHA | 10 | 2 |
| PLK1 | 1 | 13 |
| PLK1 | 10 | 0 |
| PYK2 | 1 | 4 |
| PYK2 | 10 | 0 |
| ROCK1 | 1 | 6 |
| ROCK1 | 10 | -1 |
| ROCK2 | 1 | 7 |
| ROCK2 | 10 | 1 |
| SGK1 | 1 | 10 |
| SGK1 | 10 | 2 |

| Kinase | Conc. Tested (μM) | % Inhibition |
|---|---|---|
| SRC | 1 | -1 |
| SRC | 10 | 2 |
| SYK | 1 | 3 |
| SYK | 10 | 2 |
| TBK1 | 1 | 3 |
| TBK1 | 10 | 6 |
| TIE2 | 1 | 7 |
| TIE2 | 10 | -2 |
| TNIK | 1 | 4 |
| TNIK | 10 | 12 |
| TRKA | 1 | 5 |
| TRKA | 10 | 4 |
| TSSK1 | 1 | 7 |
| TSSK1 | 10 | -1 |
| TTK | 1 | 4 |
| TTK | 10 | 4 |
| AXL | 1 | 4 |
| AXL | 10 | 0 |
| CK1-EPSILON | 1 | 7 |
| CK1-EPSILON | 10 | 11 |
| MAPKAPK-2 | 1 | 3 |
| MAPKAPK-2 | 10 | 0 |
| MER | 1 | 0 |
| MER | 10 | 3 |
| TYRO3 | 1 | 2 |
| TYRO3 | 10 | 6 |
| AMPA1B1G1 | 1 | -2 |
| AMPA1B1G1 | 10 | 8 |
| CAMK4 | 1 | 8 |
| CAMK4 | 10 | 1 |
| P70S6K1 | 1 | 3 |
| P70S6K1 | 10 | 2 |
| PRKACA | 1 | 1 |
| PRKACA | 10 | 2 |
| FAK | 1 | -2 |
| FAK | 10 | -1 |

| % Inhibition |
|---|
| -20 |
| -10 |
| 0 |
| 10 |
| 20 |
| 30 |
| 40 |
| 50 |
| 60 |
| 70 |
| 80 |
| 90 |
| 100 |

**Table 1. Kinase profiling results for FiVe1.** Shown are percent inhibition values for the indicated kinase at 1 and 10 μM of FiVe1.

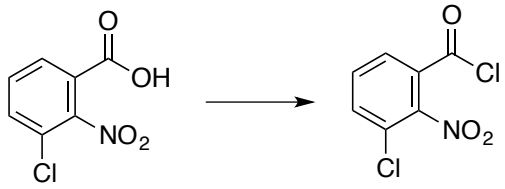
| Antigen | Species | Supplier | Catalog Number | WB Dilution | IF Dilution |
|---|---|---|---|---|---|
| VIM (V9) | Mouse | Abcam | ab8069 | 1:1000 | 1:500 |
| VIM | Rabbit | Abcam | ab16700 | 1:1000 | 1:500 |
| PS39-VIM | Rabbit | Cell Signaling Technologies | 13614 | 1:1000 | N/A |
| PS56-VIM | Rabbit | Cell Signaling Technologies | 7391 | 1:1000 | 1:200 |
| PS83-VIM | Rabbit | Cell Signaling Technologies | 3878 | 1:1000 | N/A |
| Tubulin (TUBB) | Mouse | Sigma-Aldrich | T8328 | N/A | 1:500 |
| FLAG | Mouse | Sigma-Aldrich | F1804 | 1:2000 | N/A |
| HA | Rabbit | Cell Signaling Technologies | 3724 | 1:1000 | N/A |
| Biotin | Rabbit | Abcam | ab1227 | 1:500 | N/A |
| Tubulin (TUBG1) | Mouse | Sigma-Aldrich | T6557 | 1:1000 | N/A |
| PXN | Rabbit | Abcam | ab32084 | N/A | 1:200 |

**Table 2. Primary antibodies used in this study.**

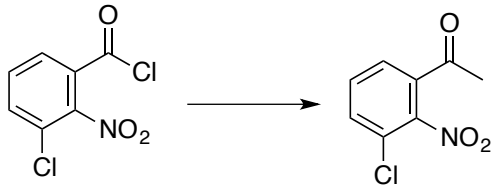
**Supplemental Methods.**

**General synthetic procedures.**

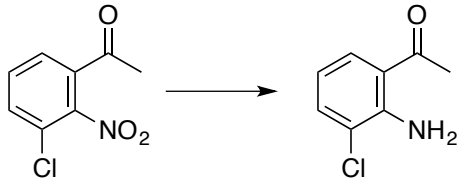
All non-aqueous reactions were carried out in oven-dried glassware under an atmosphere of nitrogen. All solvents, starting materials and reagents were purchased from commercial vendors and used without further purification. All reagent grade solvents used for chromatography were purchased from Fisher Scientific. A Biotage FLASH column chromatography system was used to purify mixtures and the flash column chromatography silica cartridges were obtained from Biotage. All NMR spectra were recorded on a Varian INOVA-400 spectrometer. Chemical shifts (δ) are reported in parts per million relative to the residual solvent peak, and coupling constants (J) are reported in hertz (Hz). HPLC Gradient conditions: solvent A (0.05% TFA in water) and solvent B (0.05% TFA in Acetonitrile): 0-2 min 95% A, 2-12 min 5-95% B (linear gradient), 12-15 min 100% B. Detection by UV-Vis (220-400nm).




**3-chloro-2-nitrobenzoyl chloride.** To a solution of 2-nitro-3-chlorobenzoic acid (10.08 g, 50 mmol) in dichloromethane (67 mL) was added thionyl chloride (18.2 mL, 250 mmol) followed by DMF (3 drops). The reaction was refluxed 2 hours and evaporated in vacuo to give the acid chloride (10.34 g, 94%). 1H-NMR (400 MHz; DMSO-d6): δ 8.01-7.97 (m, 2H), 7.71 (t, *J* = 8.0 Hz, 1H).

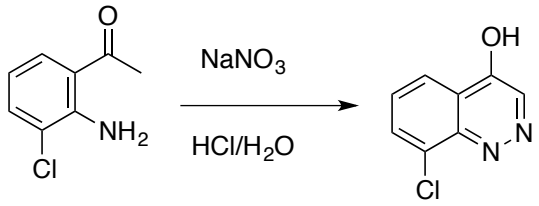



**1-(3-chloro-2-nitrophenyl)ethan-1-one.** To a suspension of magnesium (10g, 0.41 mol) in dry THF (750 mL) at 50°C was added ethanol (40.1 mL) and carbon tetrachloride (1 mL). After 30 min. a solution of diethyl malonate (62.5 mL, 0.41 mol) in ethanol (28 mL, 0.69 mol) was added and the reaction was allowed to stir at 60°C for 2 hrs. Quench with 10% sulfuric acid (250 mL). Separate and evaporate the organic layer to give a yellow oil. To the oil was added acetic acid (365 mL), sulfuric acid (49mL) and water (248 mL). This mixture was refluxed 10 hours and the product was extracted with ethyl acetate. The combined extracts were washed with water followed by brine and dried over sodium sulfate and evaporated in vacuo to give the product 65 g, 79%). 1H-NMR (400 MHz; CDCl3): δ 7.76 (d, *J* = 7.8 Hz, 1H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 2.61 (s, 3H).

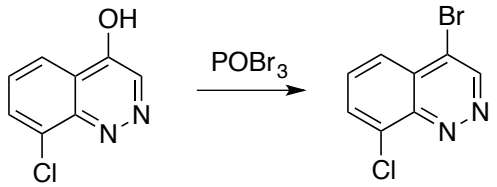



**1-(2-amino-3-chlorophenyl)ethan-1-one.** To a solution of 1-(3-chloro-2-nitrophenyl)ethan-1-one (65 g, 0.33 mol) in glacial acetic acid (500 mL) was added iron powder (55 g, 0.98 mol). Shake the slurry until it thick. Use cooling to prevent a reflux. Let the mixture sit for 1 hr at 80°C shaking ever hour to make sure it is mixed. Cool and quench with 10% sodium hydroxide. Extract product by shaking with ethyl acetate and then separating the emulation by centrifugation followed by decanting the organic layer. Repeat 3

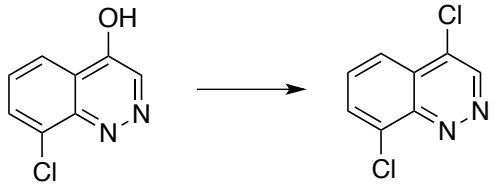
times. Filter through celite, dry over sodium sulfate and evaporate in vacuo to give the aniline (44.1 g, 80%). 1H-NMR (400 MHz; CDCl3): δ 7.76 (d, *J* = 7.8 Hz, 1H), 7.70 (d, *J* = 8.1 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 2.61 (s, 3H).



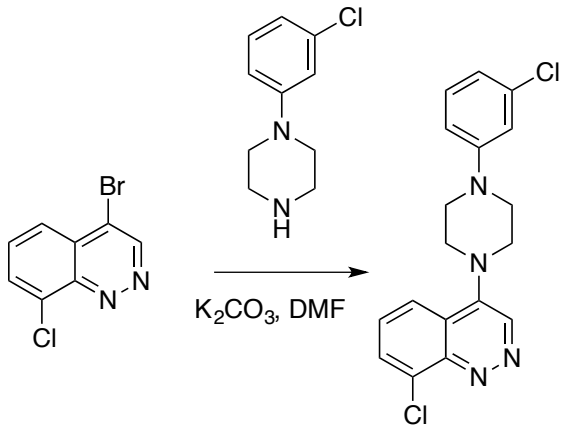

**8-chlorocinnolin-4-ol.** To a suspension of 1-(2-amino-3-chlorophenyl)ethan-1-one (5 g, 29.5 mmol) in water (29 mL) at 0°C was added conc. HCl (205 mL). A solution of sodium nitrite (2.05g, 29.7 mmol) in water (7.5 mL) was added dropwise. The reaction was stirred for 1 hr. at 0°C then heated at 65°C for 4 hr. After cooling, the product was collected by filtration. The product was triturated with acetone and recrystallized from boiling 6M HCl to give the product as pink needles (2.81 g, 15.7 mmol). 1H-NMR (400 MHz; CDCl3): δ 10.32 (s, 1H), 8.19 (dd, *J* = 8.2, 0.6 Hz, 1H), 7.89 (s, 1H), 7.77 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.35 (t, *J* = 7.9 Hz, 1H).



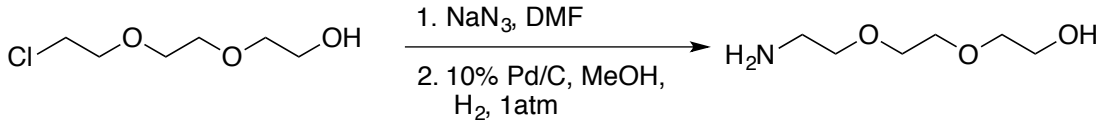

**4-bromo-8-chlorocinnoline.** A bottle of phosphorus oxybromide (25g, 87.2 mmol) was melted with a heat gun and poured into chloroform (150 mL). To this solution was added 8-chlorocinnolin-4-ol (2.8 g, 15.5 mmol) and was stirred until a thick slurry was obtained (~20 min). The reaction was refluxed 2 hr. and then basified with 10% sodium carbonate. The mixture was filtered through celite and extracted with chloroform. The combined organic layers were dried over sodium sulfate and evaporated to give the product (3.5 g, 92%) as a green-brown solid. 1H-NMR (400 MHz; DMSO-d6): δ 9.81 (s, 1H), 8.23 (d, *J* = 7.5 Hz, 1H), 8.11 (d, *J* = 8.5 Hz, 1H), 7.98 (t, *J* = 8.0 Hz, 1H).



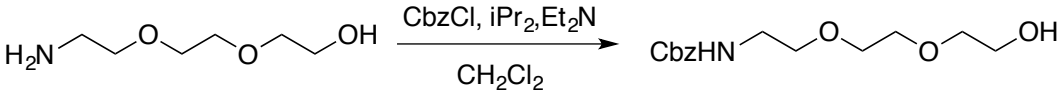

**4-chloro-8-chlorocinnoline.** To a flask containing 8-chlorocinnolin-4-ol (4.67 g, 25.9 mmol) was added thionyl chloride (100 mL) the mixture was refluxed for 2 hr. and evaporated in vacuo to give the product (5.15 g, 100%). 1H-NMR (400 MHz; CDCl3): δ 9.47 (s, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 8.03 (d, *J* = 7.2 Hz, 1H), 7.81 (t, *J* = 8.0 Hz, 1H).



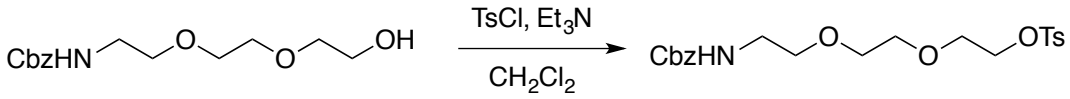

**8-chloro-4-(4-(3-chlorophenyl)piperazin-1-yl)cinnoline.** To a solution of 4-bromo-8-chlorocinnoline (25 mg, 0.10 mmol) and 1-(3-chlorophenyl)piperazine HCl (23.9 g, 0.10 mmol) in dimethylformamide (1 mL) was added potassium carbonate (42.6 mg, 0.31 mmol). The reaction was stirred at 60°C overnight. Water was added to the mixture to precipitate the product, which was collected by filtration or centrifugation. The product was washed with methanol and dried in vacuo to give the product (28 mg, 75%). Mass calculated for $C_{18}H_{16}Cl_2N_4$ 358.0751; Mass observed by HR-MS (ESI+) 359.0825 (M+H). 1H-NMR (400 MHz; CDCl3): δ 9.07 (s, 1H), 7.93 (dd, *J* = 8.5, 1.0 Hz, 1H), 7.89 (dd, *J* = 7.4, 1.0 Hz, 1H), 7.60 (dd, *J* = 8.4, 7.5 Hz, 1H), 7.23 (d, *J* = 8.1 Hz, 1H), 6.97 (t, *J* = 2.1 Hz, 1H), 6.92-6.86 (m, 2H), 3.54 (dd, *J* = 6.8, 3.1 Hz, 4H), 3.49 (dd, *J* = 6.8, 2.9 Hz, 4H).



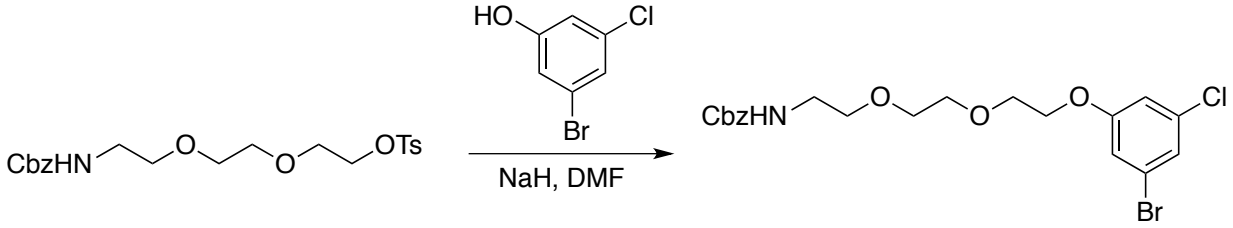

**2-(2-(2-aminoethoxy)ethoxy)ethan-1-ol.** A solution of 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol (12.5 g, 74.1 mmol) and sodium azide (7.25 g, 111.5 mmol) in DMF (125 mL) was allowed to stir at 100°C overnight. The mixture was filtered and evaporated to give 2-(2-(2-azidoethoxy)ethoxy)ethan-1-ol. A solution of the crude material and 10% Pd/C (1.12 g) in methanol (100 mL) was hydrogenated (1 atm) overnight. The mixture was filtered, evaporated and purified via flash chromatography (0-20% MeOH/DCM) to give the amine (3.53 g, 32% over 2 steps) 1H-NMR (400 MHz; CDCl3): δ 3.75-3.71 (dd, 2H), 3.69-3.64 (m, 2H), 3.64-3.61 (m, 2H), 3.59-3.53 (m, 2H), 3.48-3.45 (m, 2H), 2.88 (t, *J* = 5.1 Hz, 2H).



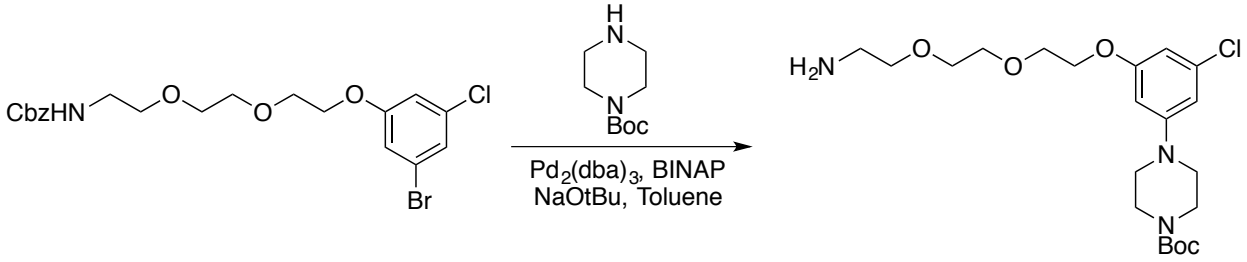

**benzyl (2-(2-(2-hydroxyethoxy)ethoxy)ethyl)carbamate.** To a solution of 2-(2-(2-aminoethoxy)ethoxy)ethan-1-ol (3.53 g, 23.7 mmol) and diisopropylethylamine (8.2 mL, 47.3 mmol) in dichloromethane (75 mL) at 0°C was added benzyl chloroformate (5 mL, 35.5). The solution was allowed warm to room temperature and stirred for 2 hours. The mixture was evaporated and purified by flash chromatography (20-100% EtOAc/hexanes) to give the benzyl carbamate (3 g, 45%). 1H-NMR (400 MHz; CDCl3): δ 7.37-7.31 (m, 5H), 5.37 (s, 1H), 5.14-5.09 (s, 2H), 3.72-3.71 (m, 2H), 3.64-3.59 (m, 8H), 3.40 (q, *J* = 5.2 Hz, 2H).



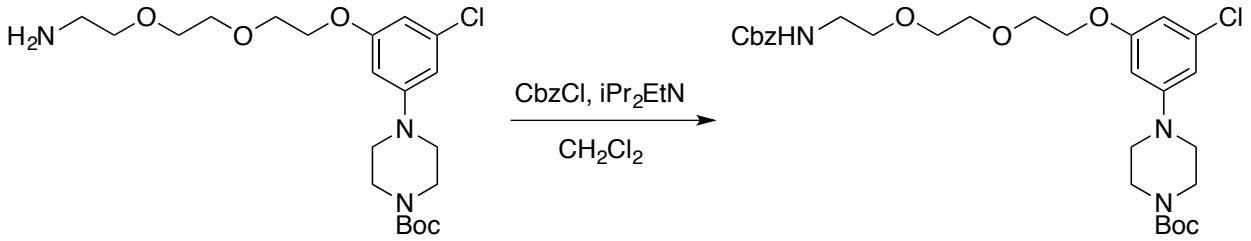

**3-oxo-1-phenyl-2,7,10-trioxa-4-azadodecan-12-yl 4-methylbenzenesulfonate.** To a solution of benzyl (2-(2-(2-hydroxyethoxy)ethoxy)ethyl)carbamate (3 g, 10.6 mmol) and triethylamine (3 mL, 2 eq.) in dichloromethane (50 mL) was added tosyl chloride (2.4 g, 12.7 mmol). The solution was allowed to stir overnight, was evaporated and purified by flash chromatography (20-100% EtOAc/hexanes) to give the tosylate (2.85 g, 62%). 1H-NMR (400 MHz; CDCl3): δ 7.80 (s, 1H), 7.78 (s, 1H), 7.36 (m, 5H), 7.33 (s, 1H), 7.31 (s, 1H), 5.20 (s, 1H), 5.09 (s, 2H), 4.15 (t, *J* = 4.7 Hz, 2H), 3.67 (t, *J* = 4.8 Hz, 2H), 3.56 (d, *J* = 2.3 Hz, 2H), 3.52 (t, *J* = 5.0 Hz, 2H), 3.37 (q, *J* = 5.3 Hz, 2H), 2.43 (s, 3H).



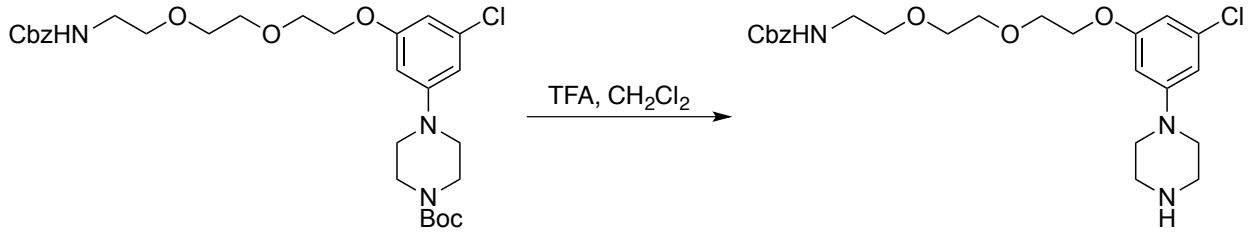

**benzyl (2-(2-(2-(3-bromo-5-chlorophenoxy)ethoxy)ethoxy)ethyl)carbamate.** To a solution of 3-bromo-5-chlorophenol (2.85 g, 6.5 mmol) in DMF (30 mL) at 0°C was added sodium hydride (348 mg, 13 mmol). After stirring for 10 minutes 3-oxo-1-phenyl-2,7,10-trioxa-4-azadodecan-12-yl 4-methylbenzenesulfonate (3.01 g, 6.5 mmol) was added. The mixture was allowed to stir overnight at room temperature. The mixture was evaporated in vacuo and purified by flash chromatography to give the product (1.67 g, 84% brsm) 1H-NMR (400 MHz; CDCl3): δ 7.35-7.30 (m, 4H), 7.10 (s, 1H), 6.96 (s, 1H), 6.84 (s, 1H), 5.25 (s, 1H), 5.09 (s, 2H), 4.07 (t, *J* = 4.4 Hz, 2H), 3.81 (t, *J* = 4.7 Hz, 2H), 3.69-3.66 (m, 2H), 3.64-3.62 (m, 2H), 3.57 (t, *J* = 5.1 Hz, 2H), 3.40 (q, *J* = 5.0 Hz, 2H).



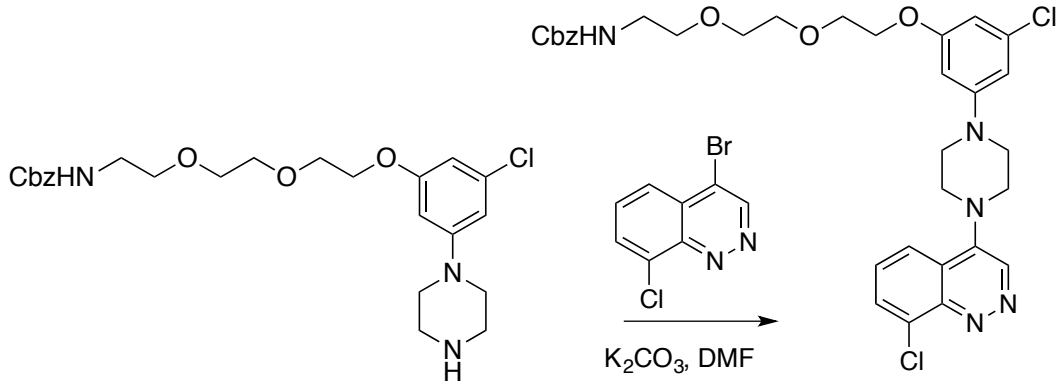

**tert-butyl 4-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)-5-chlorophenyl)piperazine-1-carboxylate.** A solution of benzyl (2-(2-(2-(3-bromo-5-chlorophenoxy)ethoxy)ethoxy)ethyl)carbamate (1.67 g, 3.5 mmol), tert-butyl piperazine-1-carboxylate (658 mg, 1 eq.), Tris(dibenzylideneacetone)dipalladium (97 mg, 3mol%), Bis(diphenylphosphino)-1,1'-binaphthalene (198 mg, 9mol%), sodium tert-butoxide (679 mg, 7.1 mmol) in toluene (30 mL) was allowed to stir at 90°C overnight. The mixture was purified by flash chromatography (40% EtOAc/hexanes then 10% MeOH/DCM) to give the product (1.31 g, 84%). 1H-NMR (400 MHz; CDCl3): δ 6.48 (s, 1H), 6.40 (s, 1H), 6.34 (s, 1H), 4.08 (t, *J* = 4.7 Hz, 2H), 3.81 (t, *J* = 4.8 Hz, 2H), 3.70-3.68 (m, 2H), 3.65-3.62 (m, 2H), 3.56 (t, *J* = 5.1 Hz, 2H), 3.52 (t, *J* = 5.2 Hz, 2H), 3.42 (t, *J* = 5.1 Hz, 2H), 3.10 (t, *J* = 5.0 Hz, 2H), 2.92 (t, *J* = 5.3 Hz, 2H), 2.83 (t, *J* = 5.0 Hz, 2H), 2.14 (s, 2H), 1.44 (s, 9H).




**benzyl tert-butyl 4-(3-chloro-5-((3-oxo-1-phenyl-2,7,10-trioxa-4-azadodecan-12-yl)oxy)phenyl)piperazine-1-carboxylate.** To a solution of tert-butyl 4-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)-5-chlorophenyl)piperazine-1-carboxylate (1.31 g crude, 3 mmol) and diisopropylethylamine (1 mL, 5.9 mmol) in dichloromethane (29 mL) at 0°C was added benzyl chloroformate (0.62 mL, 4.4 mmol). The solution was allowed warm to room temperature and stirred for 2 hours. The mixture was evaporated and purified by flash chromatography to give the benzyl carbamate (1.0 g, 59%). 1H-NMR (400 MHz; CDCl3): δ 7.37-7.32 (m, 5H), 6.50 (s, 1H), 6.40 (s, 1H), 6.33 (s, 1H), 5.26 (s, 1H), 5.09 (s, 2H), 4.07 (t, *J* = 4.3 Hz, 2H), 3.81 (t, *J* = 4.8 Hz, 2H), 3.70-3.67 (m, 2H), 3.65-3.62 (m, 2H), 3.59-3.51 (m, 4H), 3.39 (q, *J* = 5.5 Hz, 2H), 3.11 (t, *J* = 5.0 Hz, 3H), 1.48 (s, 9H).

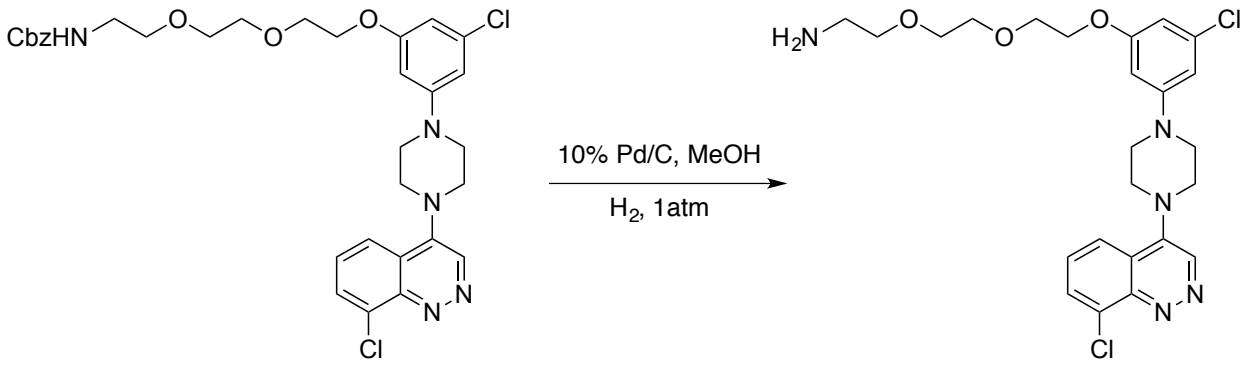



**benzyl (2-(2-(2-(3-chloro-5-(piperazin-1-yl)phenoxy)ethoxy)ethoxy)ethyl)carbamate.** To a solution of tert-butyl 4-(3-chloro-5-((3-oxo-1-phenyl-2,7,10-trioxa-4-azadodecan-12-yl)oxy)phenyl)piperazine-1-carboxylate (1 g, 1.7 mmol) at 0°C in dichloromethane (25 mL) was added trifluoroacetic acid (5 mL). The mixture was stirred for an hour and was evaporated to give the amine (1.39 g) as the TFA salt and was taken on crude.

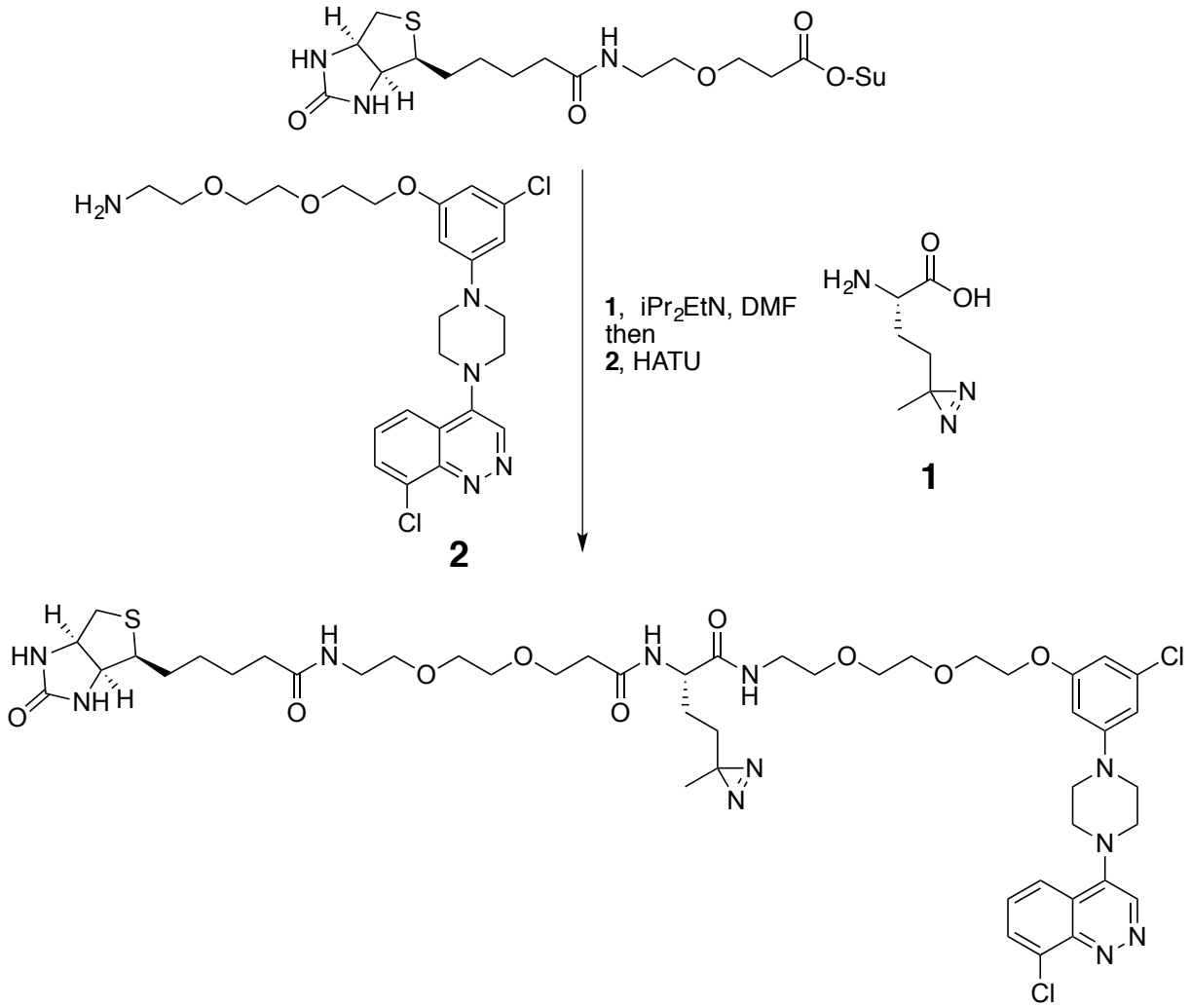



**benzyl (2-(2-(2-(3-chloro-5-(4-(8-chlorocinnolin-4-yl)piperazin-1-yl)phenoxy)ethoxy)ethoxy)ethyl)carbamate.** To a solution of crude (2-(2-(2-(3-chloro-5-(piperazin-1-yl)phenoxy)ethoxy)ethoxy)ethyl)carbamate (1.08 g, 2.3 mmol) in DMF (20 mL) was added potassium carbonate (1.26 g, 6.8 mmol) and 4-bromo-8-chlorocinnoline (666 mg, 2.7 mmol). The mixture was allowed to stir at 60°C overnight and was then filtered and evaporated in vacuo at 60°C. The crude material was purified via flash chromatography (50-100% EtOAc/Hexanes) to give the product (561 mg, 38%) as a white solid. 1H-NMR (400 MHz; CDCl3): δ 9.02 (s, 1H), 7.88 (dd, *J* = 12.1, 8.0 Hz, 2H), 7.57 (t, *J* = 7.9

Hz, 1H), 7.32-7.27 (m, 5H), 6.57 (s, 1H), 6.45 (s, 1H), 6.41 (s, 1H), 5.27 (s, 1H), 5.07 (s, 2H), 4.08 (t, *J* = 4.8 Hz, 2H), 3.81 (t, *J* = 4.7 Hz, 2H), 3.68 (dd, *J* = 6.0, 3.1 Hz, 2H), 3.63 (dd, *J* = 5.7, 3.1 Hz, 2H), 3.56 (t, *J* = 5.0 Hz, 2H), 3.50-3.46 (m, 4H), 3.44-3.41 (m, 4H), 3.39-3.36 (m, 2H).




**2-(2-(2-(3-chloro-5-(4-(8-chlorocinnolin-4-yl)piperazin-1-yl)phenoxy)ethoxy)ethoxy)ethan-1-amine.** To a solution of benzyl (2-(2-(2-(3-chloro-5-(4-(8-chlorocinnolin-4-yl)piperazin-1-yl)phenoxy)ethoxy)ethoxy)ethyl)carbamate (80 mg, 0.12 mmol) in MeOH (10 mL) was added 10% Pd/C (40 mg). The mixture was allowed to stir under hydrogen at 1 atm until complete by TLC (~30 min). The mixture was filtered an then evaporated and purified by HPLC to give the product (58 mg, 92%). Mass calculated for $C_{24}H_{29}Cl_2N_5O_3$ 505.1647; Mass observed by HR-MS (ESI+) 506.1721 (M+H). 1H-NMR (400 MHz; CD3OD): δ 9.03 (s, 1H), 8.08 (d, *J* = 8.5 Hz, 1H), 7.96 (d, *J* = 7.5 Hz, 1H), 7.68 (t, *J* = 8.0 Hz, 1H), 6.64 (s, 1H), 6.51 (s, 1H), 6.47 (s, 1H), 4.11 (t, *J* = 4.6 Hz, 2H), 3.82 (t, *J* = 4.5 Hz, 2H), 3.70 (dd, *J* = 5.6, 3.3 Hz, 2H), 3.65-3.63 (m, 2H), 3.62-3.60 (m, 4H), 3.48-3.46 (m, 4H), 2.80 (t, *J* = 5.3 Hz, 2H).




**N-((S)-1-(3-chloro-5-(4-(8-chlorocinnolin-4-yl)piperazin-1-yl)phenoxy)-11-(2-(3-methyl-3H-diazirin-3-yl)ethyl)-10,13-dioxo-3,6,16,19-tetraoxa-9,12-diazahenicosan-21-yl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide.** To a solution of biotin-PEG2-NHS (19.8 mg, 0.043 mmol) in DMF (2 mL) was added (S)-2-amino-4-(3-methyl-3H-diazirin-3-yl)butanoic acid (**1** (photo-methionine ), 6.2 mg, 0.039). The mixture was allowed to stir for 1 hour in a dark foil wrapped vial (or until complete by HPLC) and 2-(2-(2-(3-chloro-5-(4-(8-chlorocinnolin-4-yl)piperazin-1-yl)phenoxy)ethoxy)ethoxy)ethan-1-amine (**2**, 20 mg, 0.039 mmol) and HATU (22.5 mg, 0.059 mmol) were added. The mixture was allowed to stir overnight and was purified by HPLC to give the product (5 mg, 12%). Mass calculated for $C_{47}H_{65}Cl_2N_{11}O_9S$ 1029.4064; Mass observed by HR-MS (ESI+) 1030.4139 (M+H). 1H-NMR (400 MHz; DMSO-d6): δ 9.11 (s, 1H), 8.04 (d, *J* = 8.8 Hz, 1H), 8.02 (d, *J* = 7.4 Hz, 1H), 7.94 (d, *J* = 8.3 Hz, 1H), 7.90 (t, *J* = 5.8 Hz, 1H), 7.79 (t, *J* = 5.7 Hz, 1H), 7.70 (t, *J* = 8.1 Hz, 1H), 6.63 (s, 1H), 6.49 (s, 1H), 6.45 (s, 1H), 6.38 (s, 1H), 6.32 (s, 1H), 4.28 (t, *J* = 3.6 Hz, 1H), 4.19-4.14 (m, 1H), 4.11-4.06 (m, 3H), 3.70 (t, *J* = 4.3 Hz, 2H), 3.61-3.11 (m, 27H), 3.08-3.02 (m, 1H), 2.79 (dd, *J* = 12.6, 5.0 Hz, 1H), 2.03 (t, *J* = 7.2 Hz, 2H), 1.50-1.40 (m, 2H), 1.25-1.21 (m, 6H), 0.93 (s, 3H), 0.83 (t, *J* = 6.1 Hz, 2H).