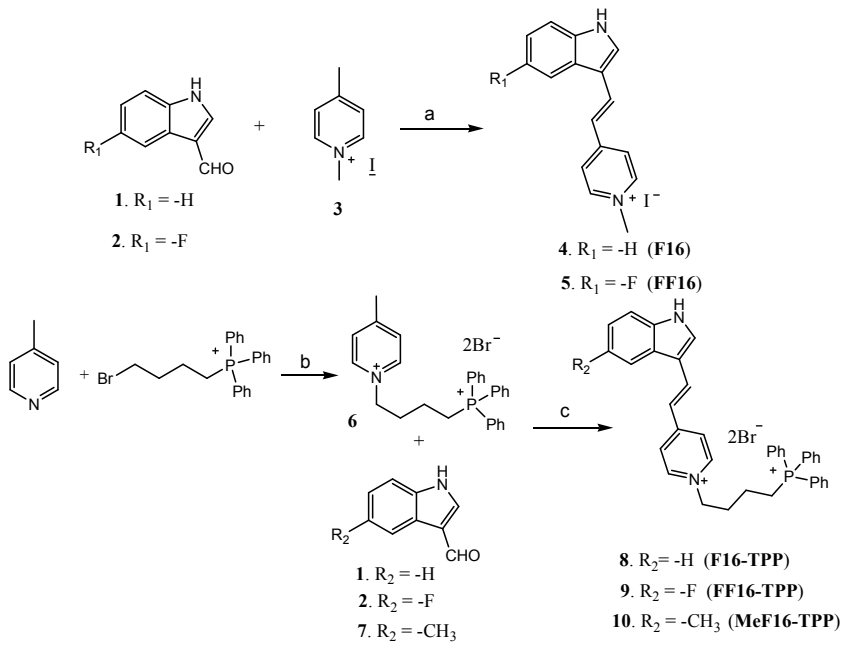
Supplementary Information

MATERIALS AND METHODS

General. All chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) and used without further refinement. Purification and analysis of the F16-TPP analogues were performed with the Dionex Summit high-performance liquid chromatography (HPLC) system (Dionex Corporation, Sunnyvale, CA), equipped with a 340U four-channel UV-Vis absorbance detector. Reverse–phase HPLC column Dionex Acclaim 120 (C18, 4.6 mm × 250 mm) was used for analysis of the products, while reverse-phase semi-preparative HPLC column Zorbax SB (C18, 9.4 mm × 250 mm) was used for purification of the products. The mobile phase was 0.1% trifluoroacetic acid (TFA) and 0.1% TFA in acetonitrile (CH_3CN). The flow were 1 mL/min for analysis and 4 mL/min for separation with the gradient starting from at 5% CH_3CN and ending at 65% CH_3CN at 42 min. UV wavelengths used for detection of all F16 derivatives were 218 nm and 440 nm. Electron spray ionization (ESI) mass spectrometry was performed by Vincent Coats Foundation Mass Spectrometry Laboratory, Stanford University. All NMR spectra were performed on a Varian XL-400 (Varain, Palo Alto, CA).



Reagents and conditions: (a) piperidine, CH_3OH, reflux, 5h; (b) EtOAc, r.t., overnight; (c) piperidine, CH_3OH, reflux, 5h.

Scheme 1: Synthesis of the F16 and TPP Derivatives

*Synthesis of (E)-4-(1H-indol-3-ylvinyl)-N-methylpyridinium iodide (**F16**, compound **4**)*: **F16** was prepared according to the procedure that reported with minor modification.[1] Briefly, equivalent mole of 1, 4-dimethylpyridium iodide (1 mmol) with indole-3-carboxaldehyde (1mmol) in the presence of catalytic amount of piperidine in 10 mL of methanol was refluxed for 5h with continuous stirring. The precipitate was collected, washed with methanol and recrystallized with acetonitrile to give the product as orange powder (yield: 56.0%). ^{1}H NMR(D_2O, 400MHz) : δ(ppm) 8.48 (d, J = 8.0 Hz, 2H), 8.21 (d, J = 16.0 Hz, 1H), 8.07 (m, 1H), 8.00 (d, J = 8.0 Hz, 2H), 7.84 (s, 1H), 7.47 (m, 1H), 7.26 (m, 2H), 7.24 (d, J = 16.0 Hz, 1H), 4.20 (s, 3H).

*Synthesis of (E)-4-(1H-5-fluro-indole-3-ylvinyl)-N-methylpyridinium iodide (**FF16**, compound **5**):* Preparation of compound **5** was conducted with the procedure

similar to **F16** at a yield of 52.2%. ^{1}H NMR(D_2O, 400MHz) : δ(ppm) 8.51 (d, J = 8.0 Hz, 2H), 8.14 (d, J = 16.0 Hz, 1H), 8.04 (d, J = 8.0 Hz, 2H), 7.89 (s, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.20 (d, J = 16.0 Hz, 1H), 7.04 (dd, J = 8.0 Hz, 1H), 4.22 (s, 3H). MS(ESI+): 253.2(for calculated $C_{16}H_{14}FN^{2+}$ 253.3).

Synthesis of 4-picoline-TPP ***6:*** 4-picoline (1 mL, 10.2 mmol) and (4-bromobutyl)triphenylphosphonium bromide(4.88 g, 10.2 mmol) were dissolved in 50 mL of ethyl acetate. The solution was stirred at room tempreture for 2 days before the solvent was removed under vacuum. The residue was then washed with dichloromethane (3×30 mL) and dried to give the product as pale powder. Yield: 72.4%. ^{1}H NMR(DMSO-d_6, 400MHz) : δ(ppm) 8.87(d, J = 8.0Hz, 2H), 7.95 (d, J = 8.0 Hz, 2H), 7.89 (m, 3H), 7.77 (m, 12H), 4.54 (t, J = 8.0 Hz, 2H), 3.64 (s, 3H), 2.58 (m, 2H), 2.07 (m, 2H), 1.50 (m, 2H). MS(ESI+): 205.8(for calculated $C_{28}H_{30}NP^{2+}$/2 205.8).

General Procedures for Synthesis of F16-TPP Derivatives. Compound **6** and equivalent amount of indole-3-carboxaldehyde analogue was dissolved in methanol and refluxed overnight in the presence of catalytic amount of piperidine (5% molar ratio). The dark red residue obtained by evaporation of the solvent of the mixture was purified by HPLC on a semipreparative C-18 column. The flow rate was set as 3 mL/min, with the mobile phase starting from 95% solvent A and 5% solvent B (0-3 min) to 35% solvent A and 65% solvent B at 33 min, then going to 15% solvent A and 85% solvent B(33-36 min), maintaining this solvent composition for

another 3 min(36-39 min), and going back to the initial composition by 42 min. the desired fractions were collected, concentrated, and lyophilized to give the product as orange to dark red powder.

F16-TPP: ^{1}H NMR(D_2O, 400MHz) : δ(ppm) 8.00 (d, J = 8.0 Hz, 2H), 7.91 (m, 1H), 7.85 (d, J = 16.0 Hz, 1H), 7.71 (s, 1H), 7.64 (m, 2H), 7.54-7.40 (m, 15H), 7.39 (m, 1H), 7.17 (m, 2H), 6.75 (d, J = 16.0 Hz, 1H), 4.14 (t, 2H), 3.12 (m, 2H), 1.94 (m, 2H), 1.35 (m, 2H). MS(ESI+): 269.3 (for calculated $C_{37}H_{35}N_2P^{2+}$/2 269.3).

FF16-TPP: ^{1}H NMR(D_2O, 400MHz) : δ(ppm) 8.01 (m, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.63 (d, J = 16.0 Hz， 1H), 7.59 (s, 1H), 7.46-7.37 (m, 15H), 7.32 (d, J = 8.0 Hz, 2H), 7.22 (m, 1H), 7.01 (m, 1H), 6.80 (m, 1H), 6.58 (d, J = 16.0 Hz, 1H), 4.08 (t, J = 8.0 Hz, 2H), 3.09 (m, 2H), 1.90 (m, 2H), 1.33 (m, 2H). MS(ESI+): 279.0 (for calculated $C_{37}H_{34}FN_2P^{2+}$/2 278.4).

MeF16-TPP: ^{1}H NMR(D_2O, 400MHz) : δ(ppm) 8.47 (d, J = 8.0 Hz, 2H), 8.16 (d, J = 16.0 Hz, 1H), 7.81(m, 4H), 7.74(m, 12H), 7.36 (d, J = 8.0Hz, 1H), 7.22 (d, J = 16.0 Hz, 1H), 7.12 (d, J = 8.0 Hz,1H), 4.43 (t, J = 8.0 Hz, 2H), 3.48 (m, 2H), 2.52 (s, 3H), 2.06 (m, 2H), 1.75 (m, 2H). MS (ESI+):276.4 (for calculated $C_{38}H_{37}N_2P^{2+}$/2 276.3).

Cell Lines. Human breast cancer cell line MDA-MB-231, glioma cell line U87MG, and mouse embryonic fibroblasts cell line NIH 3T3 were obtained from American Type Culture Collection (Manassas, VA). U87MG cells were cultured in minimum essential medium (Eagle) (MEM, Invitrogen, Carlsbad, CA) supplemented

with 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate, 0.01 mg/ mL bovine insulin, 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin. MDA-MB-231 and NIH 3T3 cells were grown in Dulbecco's modified Eagle high glucose medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% FBS and 1% penicilin-streptomycin.

Fluorescence Microscopy Studies. U87MG or MDA-MB-231 cell lines (1×10^5) were incubated in 35 mm MatTek glass-bottom cultures dishes (Ashland, MA). The cells were washed with PBS (pH = 7.4) after 24h's incubation and then incubated with the compounds separately for a further 1h. Thereafter cells were washed three times with ice-cold PBS. The fluorescent signal of the cells was measured with an Axiovert 2000M fluorescence microscope (Carl Zeiss Micro-Imaging, Inc., Thornwood, NY) with the eGFP filter set (excitation 450/490 nm, emission 515/565 nm).An AttoArc HBO 100W microscopic illuminator was used as a light source. Images were recorded with a thermoeletrically cooled charged-coupled device (CCD)(Micromax, model RTE/CCD-576, Princeton Instruments, Inc., Trenton, NJ) and analyzed with *MetaMorph* software version 6.2r4 (Molecular Devices Corporation, Downingtown, PA).

Cell Proliferation Assay. Both U87MG and NIH 3T3 cell lines at a density of 3000 cells/well were incubated in 96-well plates overnight. The culture medium was replaced with 200 μL of culture medium in which the testing compound dispersed at various concentrations (0.5 - 200 μM). After incubation for 4 days, the viable cell

numbers were checked and directly counted under microscopy (10X). A minimum of 1mm×1mm area was counted from each of at least three widely separated regions of cell culture. The cell proliferation rate was calculated by the following formula: cell proliferation rate (%) = (average cell number of sample wells / average cell number of control wells ×100. The intact culture medium was evaluated as a control.

Statistical Methods. All data were presented as mean ± SD. Means were compared using the Student's t-test. A 95% confidence level was chosen to determine the significance between groups, with *P* values of < 0.05 indicating statistically significant differences.

RESULTS

Table 1. Summary of the half inhibitory concentration (IC_{50}) of various compounds against both U87MG cells and NIH 3T3 cells.

Samples	IC50 (μM)*	
	U87MG	NIH 3T3
F16	36.5 ± 1.1	~100
FF16	28.0 ± 1.2	~491
F16-TPP	>200	N.O.T. †
FF16-TPP	28.9 ± 1.1	~111.1
MeF16-TPP	64.0 ± 1.3	~109.6

*Both cell lines at a density of 3000 cells/well were incubated in 96-well plates for 4 days. The viable cell numbers were checked and directly counted under microscopy (10X). A minimum of 1mm×1mm area was counted from each of at least three widely separated regions of cell culture. †N.O.T.: Not obvious toxicity.

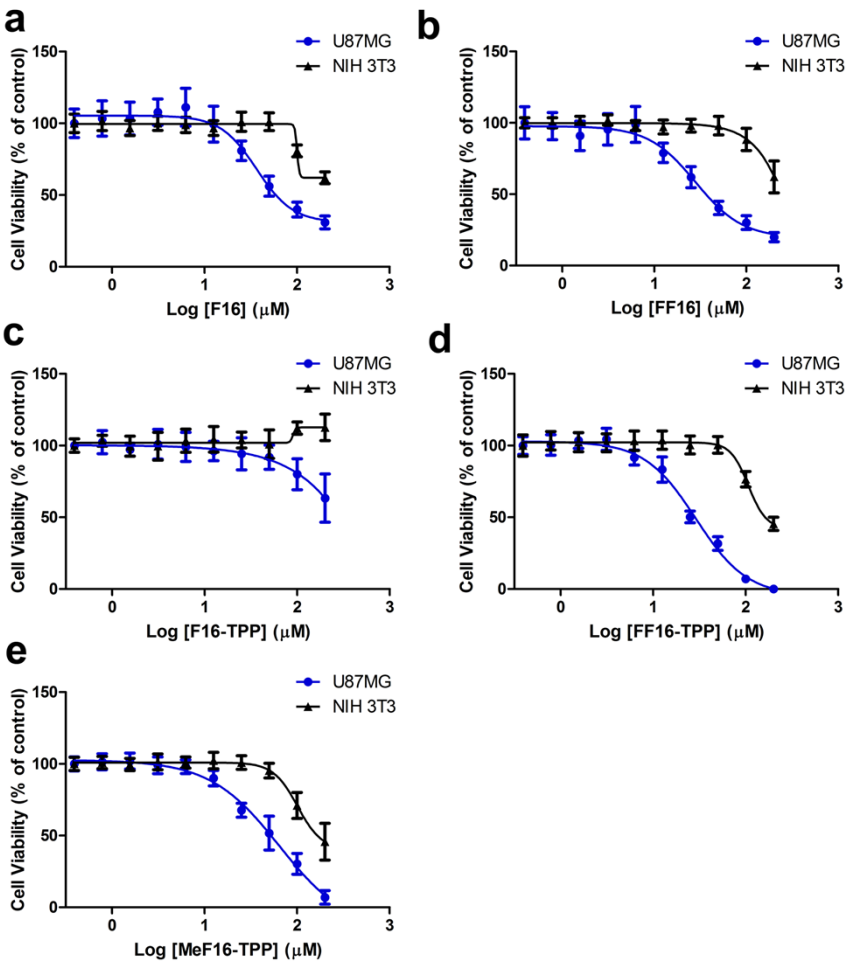


Figure S1. Antiproliferative effect on the F16 and F16-TPP analogues. Data are expressed in cell proliferative ratio with exposure of the compounds for 4 days to the negative control in PBS buffer. The proliferation status of treated cultures was determined by direct counting of cells. Data are presented as mean ± SD. (n = 4)

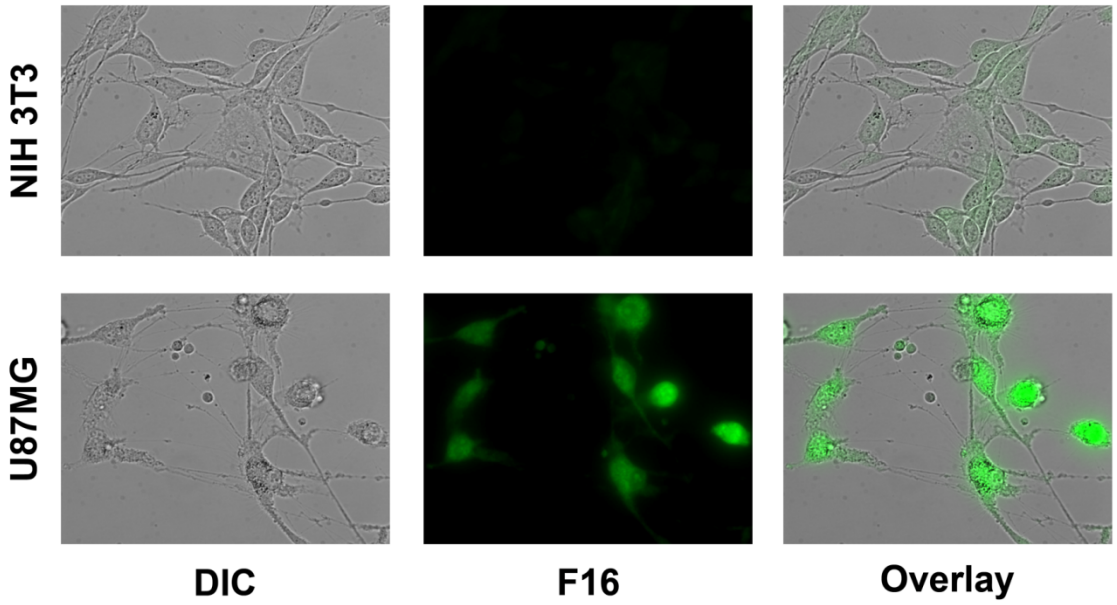


Figure S2. Fluorescence studies of both U87MG and NIH 3T3 cells treated with F16. From left to right columns are differential interference contrast (DIC) images, probes, and overlay channels in each group, respectively.

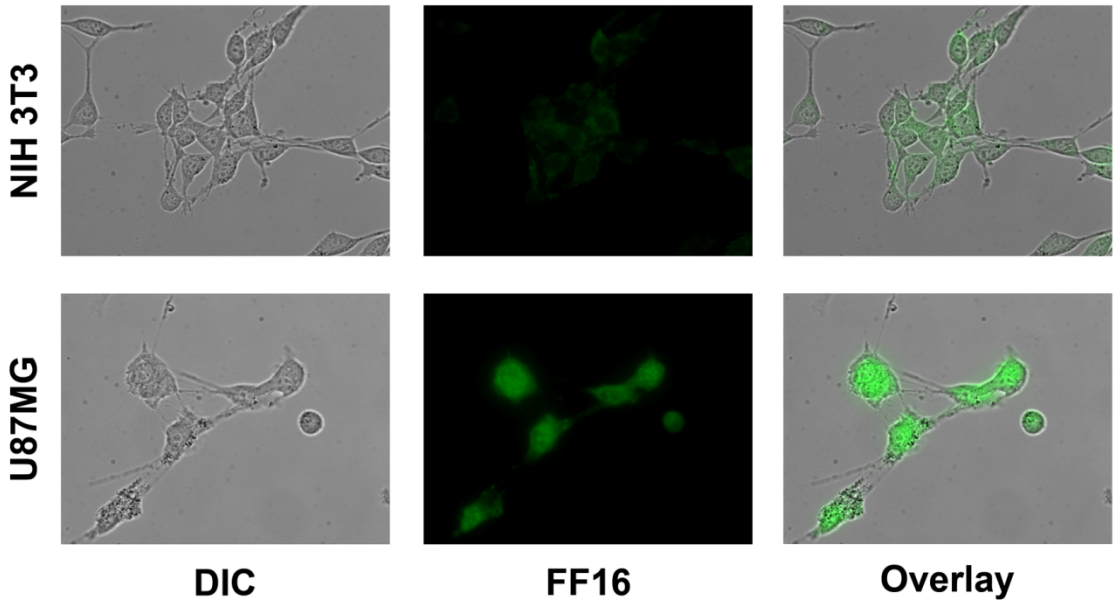


Figure S3. Fluorescence studies of both U87MG and NIH 3T3 cells treated with FF16. From left to right column are DIC images, probes, and overlay channels in each group, respectively.

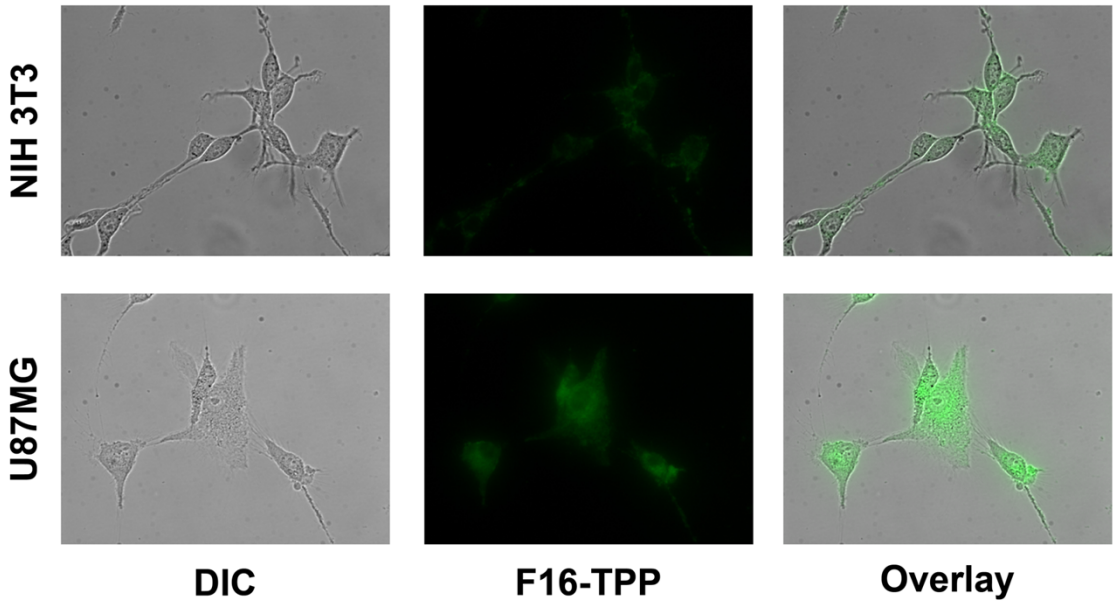


Figure S4. Fluorescence studies of both U87MG and NIH 3T3 cells treated with F16-TPP. From left to right column are DIC images, probes, and overlay channels in each group, respectively.

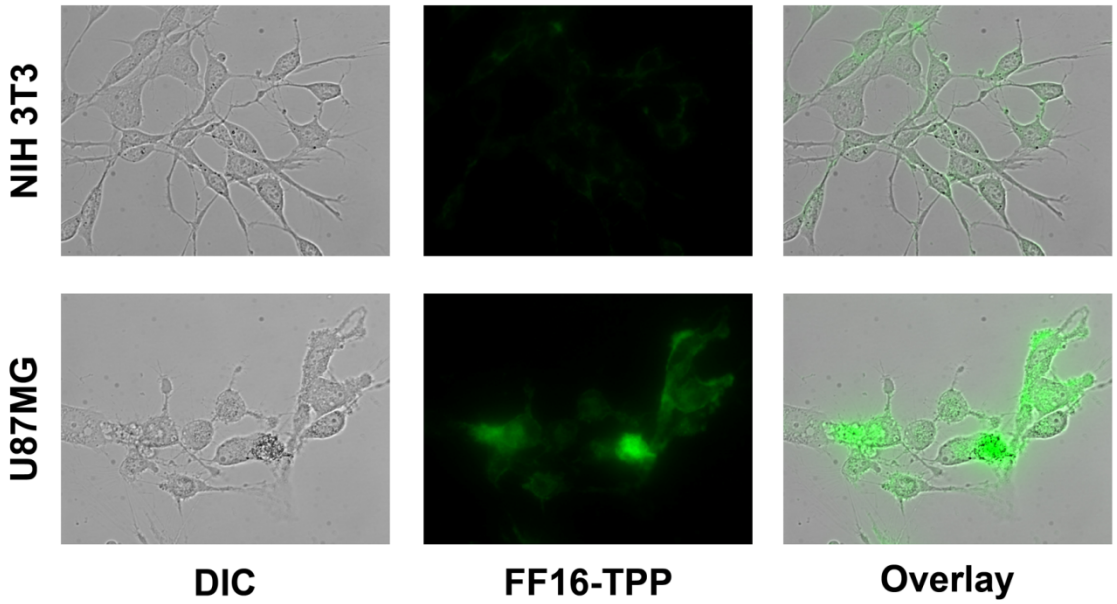


Figure S5. Fluorescence studies of both U87MG and NIH 3T3 cells treated with FF16-TPP. From left to right column are DIC images, probes, and overlay channels in each group, respectively.

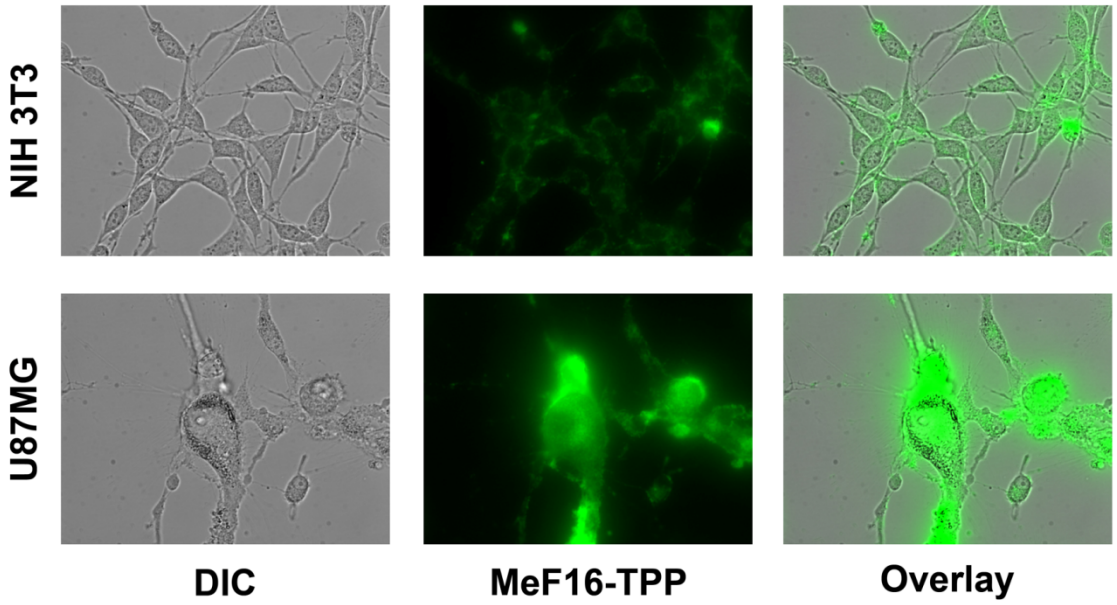


Figure S6. Fluorescence studies of both U87MG and NIH 3T3 cells treated with MeF16-TPP. From left to right column are DIC images, probes, and overlay channels in each group, respectively.

References:

(1) Z. Wang, V. N. Nesterov, O. Y. Borbulevych, R. D. Clark, M. Y. Antipin, T. V. Timofeeva, *Acta. Cryst. Section C.* 2001**, C57**, 1343.