Electronic Supplementary Material (ESI) for Polymer Chemistry

Supporting Inforamtion

Synthesis of a New Conjugated Polymer for Cell Membrane Imaging by Using an Intracellular Targeting Strategy

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Experimental Section

Materials and Instruments: All chemicals were purchased from Acros, Aldrich Chemical Company or Alfa-Aesar, and used without any purification. All organic solvents were purchased from Beijing Chemical Works and used as received. 5-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)furan-2-carbaldehyde was purchased from Nanjing CHICO pharmaceutical Co., Ltd. MCF-7, MDA-MB-231 and SK-BR-3 cell lines were obtained from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Xinjingke Biotechnology Co., Ltd (Beijing, China). Fetal bovine serum (FBS) was purchased from Sijiqing Biological Engineering Materials (Hangzhou, China). Dulbecco's modified Eagle medium (DMEM) and Modified RPMI 1640 (RP 1640) were purchased from HyClone/Thermofisher (Beijing, China). The absorbance for MTT analysis was recorded on a microplate reader (BIO-TEK Synergy HT, USA) at a wavelength of 570 nm. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. Mass spectra were recorded on a Bruker Apex IV FTMS for high resolution mass spectra (HRMS), a SHIMADZU LCMS-2010 spectrometer for ESI. Elemental analyses were carried out on a Flash EA1112 instrument. The GPC measurement was performed on a Water-410 system against polystyrene standards with THF as the eluent. UV-Vis absorption spectra were taken on a JASCO V-550 spectrophotometer. Fluorescence spectra were measured on a Hitachi F-4500 fluorometer equipped with a xenon lamp excitation source. Confocal laser scanning microscopy (CLSM) characterization was conducted with a confocal laser scanning biological

microscope (FV1000-IX81, Olympus, Japan). The size of polymers was measured on a Nano ZS (ZEN3600) system.

Cell culture: MCF-7 and MDA-MB-231 cells were cultured in DMEM and SK-BR-3 cells were cultured in RP1640, supplemented with 10% FBS at 37 °C in a humidified atmosphere containing 5% CO_2.

In Vitro Imaging and Localization: MCF-7 and SK-BR-3 cells were seeded in 35 mm culture plates at a density of approximately 20% per plate and cultured for 6 h, and then the culture medium was replaced with medium containing 10 μM **PTL**. After the cells were cultured for 48 h, Dil was added by conforming to the provided protocol. After further culture for 15 min, culture medium was discarded, the plate was washed once by PBS and the cells were fixed with 4% paraformaldehyde at room temperature for 10 min. The cells were washed with PBS and stained with Hoechst 33258 for 5 min. Then the sample was washed with PBS and characterized by using CLSM. The wavelength of stimulating laser of Hoechst 33258, **PTL** and Dil is 405 nm, 488 nm and 559 nm, respectively. The false colors of Hoechst 33258, **PTL** and Dil are blue, green and red, respectively.

In vitro cytotoxicity using MTT assay: The in vitro cytotoxicity of conjugated polymer **PTL** was measured by MTT assay. The stock solution of **PTL** were prepared in DMSO. The final concentration of DMSO in the culture medium is below 0.25%, which scarcely induce cytotoxicity. In all the experiments, cells were seeded in 96-well plates at a density of 4×10^3 cells/well. After 12 h, cells were incubated with various concentrations of **PTL** in fresh medium. After treatment for 5 days, MTT (5 mg mL^{-1} in water, 10 μL/well) was added to the wells by incubation at 37 °C for 4 h. The supernatant was removed and 100 μL DMSO per well was added to dissolve the produced formazan. After shaking the plates for 5 min, absorbance values of the wells were recorded on a microplate reader at 570 nm. The cell viability rate (VR) was calculated according to the following equation:

$$VR = \frac{A}{A_0} \times 100\%$$

where A is the absorbance of the experimental group treated by **PTL** and A_0 is the absorbance of the

control group without any treatment.

Synthesis of tert-butyl (2-mercaptoethyl) carbamate (2): To dichloromethane (150 ml) were added 2-mercaptoethanaminium chloride (**1**) (5.7 g, 50 mmol) and di-tert-butyl dicarbonate (12.0 g, 55 mmol) in sequence under argon at room temperature. Triethylamine (5.9 g, 60 mmol) was then dropwise added slowly. The mixture was stirred for 2 h at 25 °C, and then filtered. The residue was washed with CH_2Cl_2, and the filtrate was evaporated to dryness under vacuum. The crude product was purified by silica gel column chromatography using petroleum ether/ethyl acetate (5:1) as the eluent to afford a colorless liquid (7.8 g, 87%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 4.70−5.10 (br, 1H), 3.28 (t, 2H), 2.62 (m, 2H), 1.43 (s, 9H), 1.34(t, 1H).

Synthesis of compound 3: To anhydrous acetone (120 ml) was added potassium carbonate (5.5 g, 40 mmol) and compound **2** (4.2 g, 24 mmol). The mixture was stirred under argon and 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (6.9 g, 20mmol) was added. The mixture was stirred at 60 °C for 24 h. Then the solvent was removed under reduced pressure, and the residue was dissolved in 80 ml water. The resulting solution was extracted with $CHCl_3$ for three times, and the organic layer was combined and dried over Na_2SO_4. The solvent was removed and the crude product was purified by silica gel column chromatography using petroleum ether/ethyl acetate/methanol/dimethoxyethylene (5:15:1:2) as the eluent to afford a colorless liquid (6.6 g, 94 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 5.21 (br, 1H), 3.59 (t, 2H), 3.40-3.60 (m, 12H), 3.10−3.20 (m, 2H), 3.06 (br, 1H), 2.50−2.65 (m, 4H), 1.32 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 155.75, 79.04, 72.41, 70.95, 70.40, 70.34, 70.23, 70.14, 70.08, 61.39, 39.82, 32.47, 31.08, 28.26. ESI−MS *m/z*: $[M + Cl]^-$ Calcd. 388.2, found 388.1.

Synthesis of compound 4: To a solution of compound **3** (4.0 g, 11 mmol) in methanol (12 ml) was slowly added H_2O_2 (12 g, 30% in aqueous solution, 110 mmol) at 0 °C. Next, two droplets of acetic acid were added as catalyst. The mixture was refluxed at 70 °C for 2 days, and evaporated to dryness under vacuum carefully. The residue was purified by silica gel column chromatography using chloroform/methanol/ dimethoxyethylene (30:1:2) as the eluent to afford a colorless liquid (2.4 g, 55 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 5.47 (br, 1H), 3.90 (t, 2H), 3.71 (t, 2H),

3.55−3.68 (m, 12H), 3.35 (t, 2H), 3.23 (t, 2H), 2.80 (br, 1H), 1.42 (s, 9H). ^{13}C NMR (DMSO, 100 MHz) δ (ppm): 155.76, 78.48, 72.70, 70.06, 69.89, 64.41, 60.57, 53.69, 53.17, 33.90, 28.53. ESI−MS *m/z*: $[M + Na]^+$ Calcd. 408.2, found 408.2.

Synthesis of compound 5: To dichloromethane (30 mL), compound **4** (2.3 g, 6 mmol) and pyridine (0.8 g, 10 mmol) were added and the mixture was kept at 0 °C. 4-methylbenzene-1-sulfonyl chloride (2.1 g, 11 mmol) in dichloromethane (10 mL) was then added slowly. The mixture was stirred for 24 h under room temperature. After that, the mixture was washed with excess HCl (1 M) solution, and then dried over $MgSO_4$. The solvent was removed and the residue was purified by silica gel column chromatography using petroleum ether/ethyl acetate (1:2) as the eluent to afford a colorless liquid (3.4 g, 53 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.79 (d, 2H), 7.34(d, 2H), 5.40 (br, 1H), 4.15 (t, 2H), 3.91 (t, 2H), 3.70 (t, 2H), 3.55−3.65 (m, 10H), 3.31 (t, 2H), 3.23 (t, 2H), 2.45 (s, 3H), 1.43 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 155.78, 145.00, 133.06, 129.99, 128.08, 79.78, 70.91, 70.63, 70.50, 70.40, 69.36, 68.84, 64.85, 54.98, 54.38, 34.42, 28.48, 21.77. ESI−MS *m/z*: $[M + Na]^+$ Calcd. 562.2, found 562.2.

Synthesis of compound 6: To anhydrous acetone (30 ml), potassium carbonate (1.4 g, 10 mmol) and hydroquinone (2.2 g, 20 mmol) were added. The mixture was stirred under argon and compound **5** (1.1 g, 2 mmol) was added. The mixture was stirred at 70 °C for 24 h. Then the solvent was removed under reduced pressure, and the residue was dissolved in 30 ml CH_2Cl_2. The solution was filtered and washed with CH_2Cl_2. The filtrate was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography using petroleum ether/ethyl acetate (1:2) as the eluent to afford a colorless liquid (480 mg, 49 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 6.75 (s, 4H), 5.46 (br, 1H), 4.04 (t. 2H), 3.87 (t, 2H), 3.80 (t, 2H), 3.50−3.75 (m, 10H), 3.32 (t, 2H), 3.20 (t, 2H), 1.44 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 155.84, 152.32, 150.38, 116.00, 115.70, 79.83, 70.63, 70.42, 70.38, 70.13, 69.83, 68.00, 64.58, 54.67, 54.11, 34.25, 28.32. HRMS (ESI) *m/z*: $[M + Na]^+$ Calcd. 500.1935, found 500.1930.

Synthesis of compound 8: Compound **6** (270 mg, 0.57 mmol) was dissolved in dichloromethane (10 ml), and the solution was bubbled with hydrochloride for 5 h to remove the protective

t-butoxycarbonyl group. The solvent was removed and triethylamine (3 eq.) in methanol (5ml) was added to neutralize the ammonium. The solution was evaporated under reduced pressure and dried under vacuum. The residue was dissolved in anhydrous THF (8 ml) under nitrogen, and 5-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)furan-2-carbaldehyde (**7**) (243 mg 0.5 mmol) was added subsequently. After 30 min, three droplets of AcOH and $NaBH(OAc)_3$ (206 mg, 0.9 mmol) was added slowly in sequence. The solution was stirred for 18 h at room temperature and quenched with two droplets of $NaHCO_3$ aqueous solution. The solution was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography using ethyl acetate/methanol (100:3) as the eluent to afford a yellow powder (260 mg, 50 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.61 (s, 1H), 8.57 (d, 1H), 8.46 (s, 1H), 7.71−7.81 (m, 3H), 7.62 (dd, 1H), 7.33 (m,1H), 7.21 (t, 2H), 7.00 (t, 1H), 6.91 (d, 1H), 6.65 (m, 4H), 6.26 (d, 1H), 5.09 (s,2H), 3.96 (t, 2H), 3.86 (s, 2H), 3.76 (m, 4H), 3.66 (t, 2H), 3.59 (t, 2H), 3.50 (t, 4H), 3.40 (t, 2H), 3.10−3.30 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 164.34, 161.89, 158.15, 154.62, 152.94, 152.48, 152.30, 151.04, 150.82, 148.78, 139.31, 139.23, 132.62, 130.35, 130.27, 129.00, 128.88, 128.45, 125.23, 123.21, 122.58, 116.45, 115.81, 115.59, 115.12, 114.91, 114.27, 114.20, 113.98, 110.10, 107.35, 70.86, 70.58, 70.44, 70.26, 70.09, 68.10, 64.74, 54.68, 54.40, 45.46, 41.77. HRMS (ESI) *m/z:* $[M + H]^+$ Calcd. 835.2636, found 835.2580.

Synthesis of PT: A suspension of anhydrous $FeCl_3$ (130 mg, 0.8 mmol) in $CHCl_3$ (15 mL) was stirred for 30 min at room temperature under nitrogen. To this suspension was added a solution of monomer **9** (30 mg, 0.1 mmol) and monomer **10** (48 mg, 0.1 mmol) in $CHCl_3$ (10 mL), and the resulting solution was stirred for 2 days at room temperature. The solvent was removed gently and the residue was dissolved with methanol. Droplets of hydrazine hydrate were added and the mixture was filtered and the precipitate was washed with $CHCl_3$. The organic solution was collected and was removed under vacuum. then the residue was dissolved in water and dialyzed through a membrane with a molecular weight cutoff of 3500 for 3 days to yield a red sticky substance (29 mg, 33%).^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.8−7.2 (br), 3.5−3.8 (br), 3.07 (br), 2.84 (br).

Synthesis of polythiophene PTI: A suspension of anhydrous $FeCl_3$ (200 mg, 1.2 mmol) in $CHCl_3$ (15 mL) was stirred for 30 min at room temperature under nitrogen. To this suspension were added

a solution of monomer **9** (39.5 mg, 0.13 mmol) and monomer **11** (54 mg, 0.13 mmol) in $CHCl_3$ (10 mL), and the resulting solution was stirred for 2 days at room temperature. The solvent was removed gently and the residue was dissolved in methanol. The aqueous solution of phosphorus pentoxide was added. After stirring for a while, the supernatant was collected and the precipitate was washed repeatedly by methanol. The combined solution was dried and the residue was dissolved in DMSO/H_2O (1:10). Then the solution was dialyzed through a membrane with a molecular weight cutoff of 3500 for 3 days to yield a red sticky substance (36 mg, 38 %). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.8–7.2 (br), 3.25–4.0 (br), 3.10 (br), 2.80–3.00 (br).

Synthesis of PTL: To a solution of **PTI** (10 mg, 0.028 mmol in RUs) and potassium carbonate (10 mg, 0.073 mmol) in DMF, compound **8** (30 mg, 0.036 mmol) was added. The mixture was stirred at 70 °C under nitrogen for 2 days. The solution was dried and the residue was dissolved in DMSO/H_2O (1:10). Then the solution was dialyzed through a membrane with a molecular weight cutoff of 3500 for 3 days. After filtration, the filtrate was collected and the solvent was removed to yield a orange sticky substance (15 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.63 (s), 8.31 (s), 8.00 (br), 7.60−7.90 (br), 7.10−7.50 (m, br), 6. 90 (d), 6.60−6.80 (br), 5.27 (s), 4.53 (s), 3.91 (br), 3.25−3.75 (m, br), 2.70−3.10 (m, br).

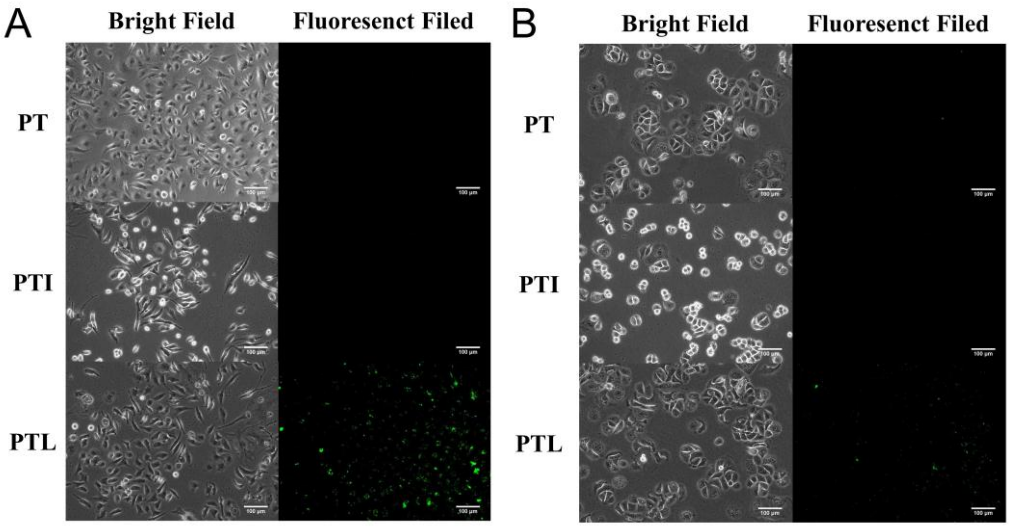

Figure S1. The cellular location of **PT**, **PTI** and **PTL** after 48h incubation using a fluorescence microscope: (A) MDA-MB-231 cell line; (B) SK-BR-3 cell line.