Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary materials for:

A novel phosphonic acid functional polythiophene fluorescent sensor for Ca^{2+} and its live cell imaging

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Experimental

Reagents and Apparatus

3,4-dimethoxythiophene, 3-bromo-1-propanol, triethyl phosphite, anhydrous ferric chloride, bromotrimethylsilane were purchased from Energy chemical technology co., Ltd (Shanghai, China).

Hydrazine hydrate (N_2H_4) was purchased from Tianjin Fu Chen Chemical Reagent Factory (Tianjin, China). Sodium bisulfate, anhydrous calcium chloride and disodium ethylenediamine tetraacetate were purchased from Sinopharm group Chemical Reagent co., Ltd (Shanghai, China). Anhydrous magnesium sulfate was purchased from Tianjin Dong Li Big Chemical Reagent Factory (Tianjin, China). Ammonium chloride was purchased from Xilong chemical co., Ltd. Ammonium hydroxide was purchased from Tianjin Fu Yu Chemical Co., Ltd. (Tianjin, China). Dichloromethane, toluene, petroleum ether, methanol, acetic ether, chloroform were purchased from Beijing Chemical Reagent Corporation (Beijing, China). Thin layer chromatography (TLC) was carried out using silica gel 60 F254, and column chromatography was conducted over silica gel (200-300 mesh), both of which were produced by the Qingdao Ocean Chemicals (Qingdao, China). Twice-distilled water was used throughout all experiments. All chemicals were used without further purification. A549 cells were obtained from American Type Culture Collection. Dulbecco's modified Eagle medium (DMEM) was purchased from Thermofisher / gibico. Phosphate buffer saline (PBS) and Trypsin-EDTA solution were purchased from Beijing Solarbio Science & Technology Co.,Ltd. Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies. Fetal bovine serum (FBS) was purchased from Life Technology. DAPI and Lyso-Tracker Red were purchased from Beijing Soledad Symbol Technologies Ltd.

The nuclear magnetic resonance spectroscopy (NMR) of compounds were identified by ^{1}H NMR

(Varian Mercury YH-400 NMR spectrometer), using tetramethylsilane (TMS) as an internal standard. All fluorescence measurements were carried out in a 1 cm path length quartz cuvette with a Hitachi F-2700 spectrometer (Shimadzu Corporation, Japan). Absorption spectra were measured on a UV-3100 UV-VIS-NIR recording spectrophotometer (Shimadzu, Japan). ESI Mass spectra were obtained using a Q-Trap 2000(Applied Biosystems Corporation, American) without using the liquid phase part. MALDI-TOF Mass spectra were obtained using a Bruker Microflex LRF20. FTIR spectra were obtained using a Perkin Elmer-Spectrum 430 FT-IR spectrometer. All pH measurements were tested with a Sartorius PB-10 digital pH meter. Cell viability assay was obtained on a microplate reader (Epoch). Photograph of cell morphology by using a Nikon microscope (Nikon ECLIPSE Ts2) equipped with a high-resolution spot camera (Nikon DS). The fluorescence images were collected directly by confocal fluorescent microscopy (Nikon ECLIPSE Ti).

Synthetic method

Synthesis of 3,4-bis(3-bromopropoxy)thiophene (M1)

3,4-dimethoxythiophene (0.36 mL, 3 mmoL), 3-bromo-1-propanol (1.35 mL, 15 mmoL), sodium bisulfate (0.072 g, 0.6 mmoL) and toluene (20 mL) were added in a 50mL round bottom flask equipped with a Soxhlet extractor with type 4A molecular sieves in degreasing cotton. The mixture was refluxed overnight under argon atmosphere. After the reaction mixture was cooled to RT, washed three times with twice-distilled water and then dried with anhydrous magnesium sulfate. The toluene was removed under vacuum. The crude product was purified by silica-gel column chromatography (petroleum ether / dichloromethane, v/v = 2:1) to get a white solid M1. Yield: 71%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (m, 4H), 3.62 (t, 4H), 4.14 (t, 4H), 6.27 (s, 2H). MS (MALDI-TOF) m/z: $[M+Na]^+$ $C_{10}H_{14}Br_2O_2S$ Na, 379.245; $[M+K]^+$ $C_{10}H_{14}Br_2O_2S$ K, 396.714.

The ^{1}H NMR and MS spectra were shown in Fig. S1 and Fig. S2.

Synthesis of tetraethyl ((thiophene-3,4-diylbis(oxy))bis(propane-3,1-diyl))bis(phosphonate) (M2)

M1 (0.36 g, 1 mmoL) and triethyl phosphite (5 mL) were added in a round bottom flask (50mL). The reaction mixture was refluxed in oil bath under argon protection for 12 h. After the reaction mixture was cooled to RT, excess triethyl phosphite was evaporated by decompression. The crude product was purified TLC on silica gel (dichloromethane / methanol, v/v = 25:1) to get the product M2 (yield: 80%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): 1.29 (t, 12H), 1.87 (m, 4H), 2.08 (m, 4H), 4.00 (t, 4H), 4.08 (m, 8H), 6.17 (s, 2H). MS (ESI) m/z: $[M+Na]^+$ $C_{18}H_{34}O_8P_2S$ Na, 494.90. The ^{1}H NMR and MS spectra were shown in Fig. S3 and Fig. S4.

Synthesis of poly {tetraethyl ((thiophene-3,4-diylbis(oxy))bis(propane-3,1-diyl))bis-(phosph-ornate)} (M3)

Anhydrous ferric chloride (0.97 g, 6 mmoL) was added in a flask with 20 ml dry chloroform and stirred for 0.5 h under argon atmosphere. Then M2 (0.58 g, 1.23 mmoL) was added dropwise to the mixture. The reaction mixture was stirred at RT for 24h under Ar. The solvent was removed after the reaction was completed. Then the solid was dissolved by methanol and added hydrazine hydrate in order to precipitate ferric chloride. This mixture was stirred at RT overnight. The filtrate was evaporated under vacuum after filter. The obtained substance was washed with distilled water repeatedly and dried in vacuum oven to obtain a purple solid (yield: 76%). ^{1}H NMR (400 MHz, $CDCl_3$): 1.27 (s, 12H), 1.96 (s, 4H), 2.13 (s, 4H), 4.10 (s, 12H). GPC: 10.13kDa. The ^{1}H NMR spectra was shown in Fig. S5.

Synthesis of poly {((thiophene-3,4-diylbis(oxy))bis(propane-3,1-diyl))bis(phosphonic acid)}

(PT-PHO)

M3 (0.33 g, 0.7 mmoL) and bromotrimethylsilane (3 mL, 22 mmoL) in Anhydrous dichloromethane (20 mL) was stirred at RT for 24 h. After the reaction complete, remove the excess bromotrimethylsilane and then added 20mL methanol into flask continue to stir at RT overnight. Remove the solvent under vacuum and the crude product was washed with methanol three times to gain a purple solid eventually (yield: 84%). ^{1}H NMR (400 MHz, DMSO): 1.24 (s, 4H), 2.74 (s, 4H), 2.90 (s, 4H). The ^{1}H NMR and FT-IR spectra were shown in Fig. S6 and Fig. S7.

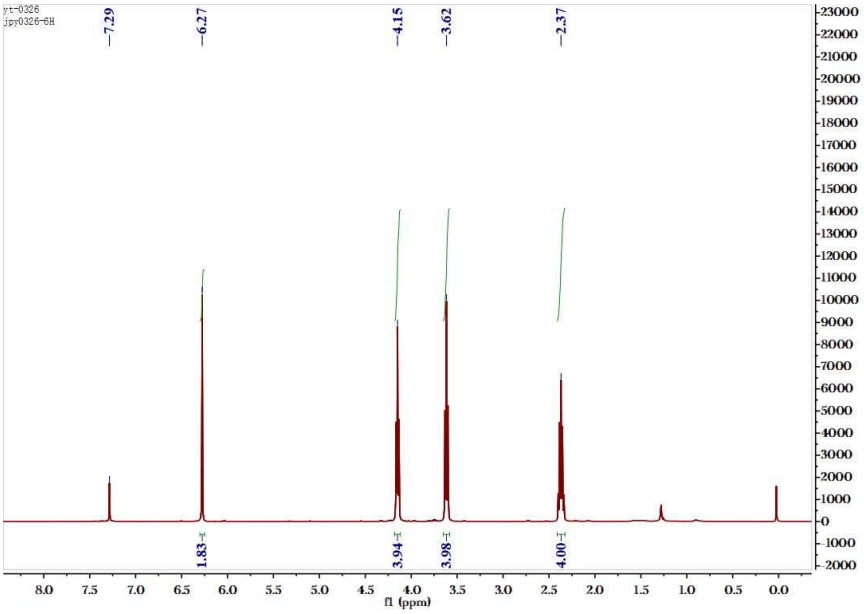

Fig. S1 The ^{1}H NMR spectrum of M1.

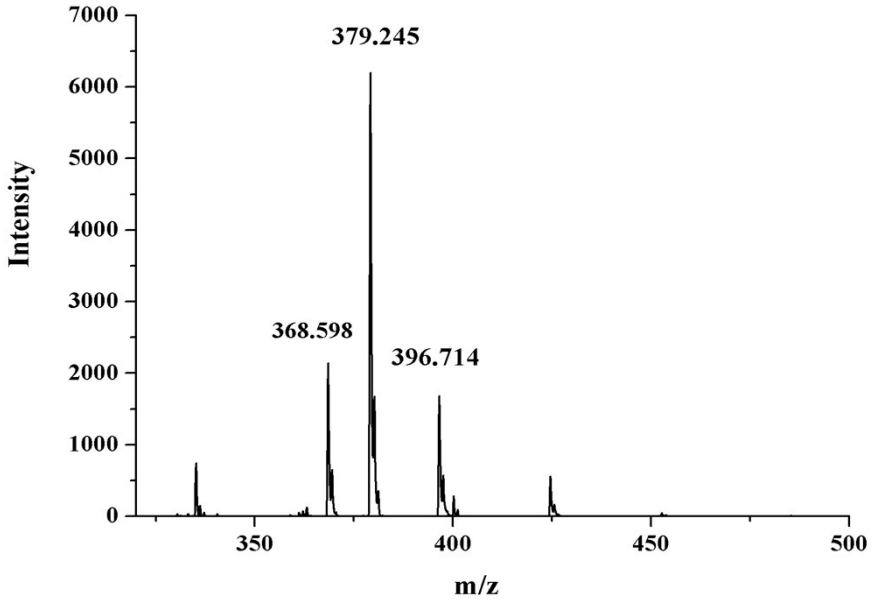

Fig. S2 The mass spectrum of M1.

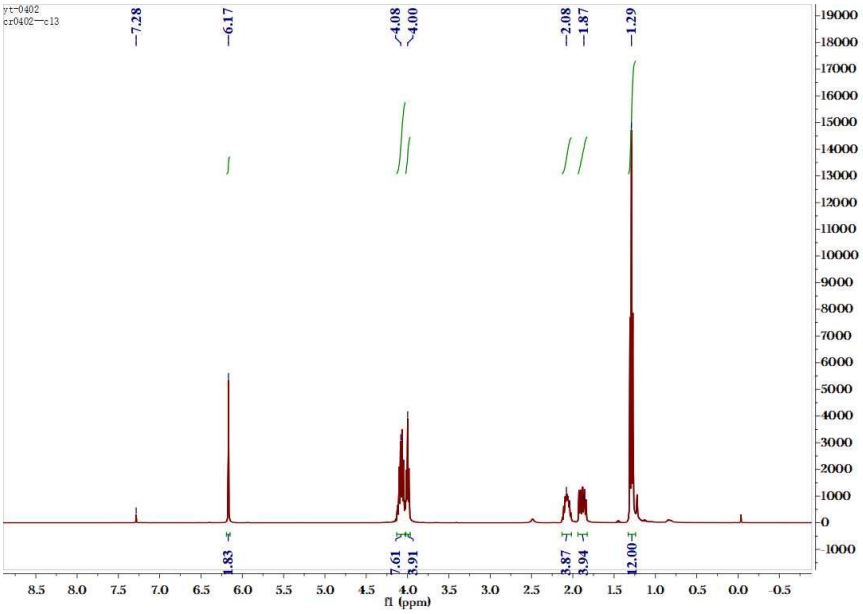

Fig. S3 The ^{1}H NMR spectrum of M2.

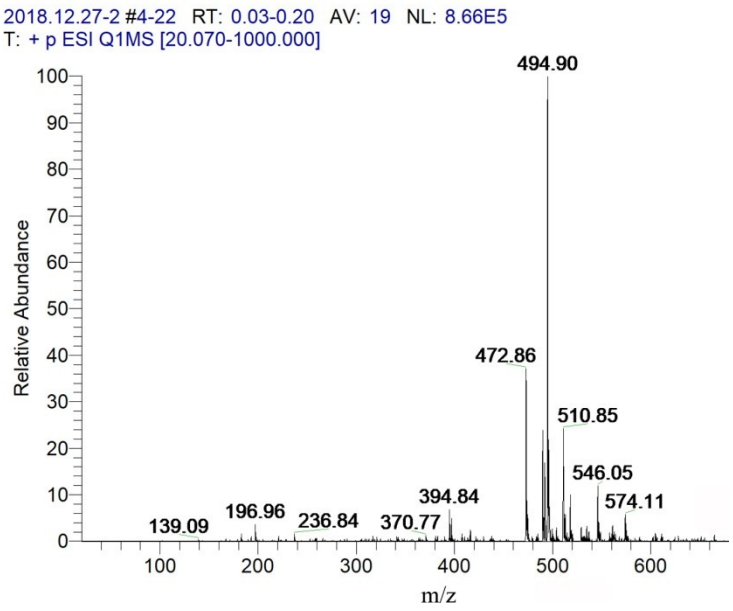

Fig. S4 The mass spectrum of M2.

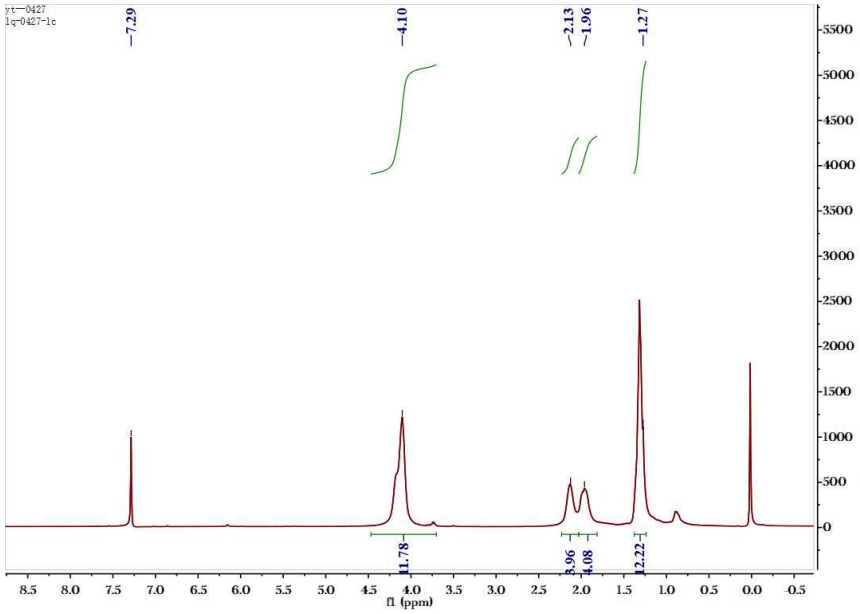

Fig. S5 The ^{1}H NMR spectrum of M3.

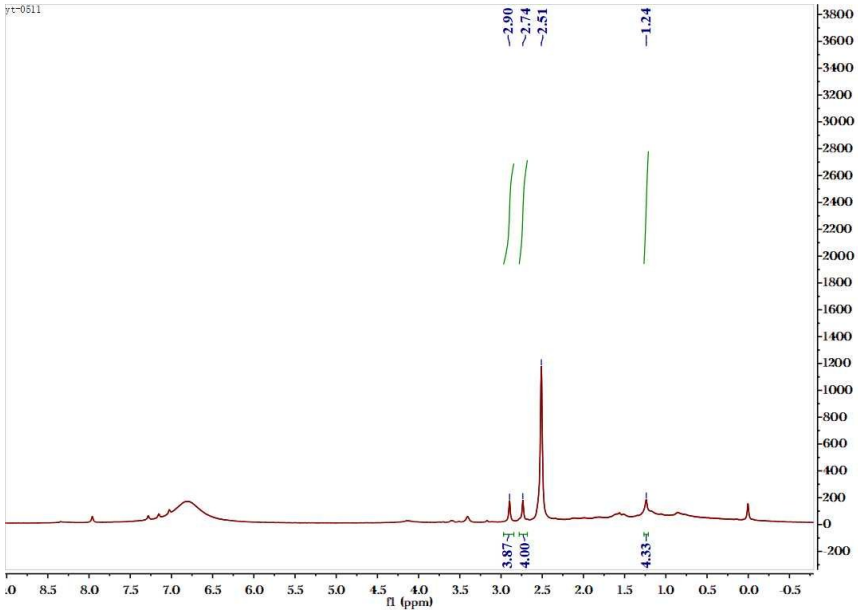

Fig. S6 The ^{1}H NMR spectrum of PT-PHO.

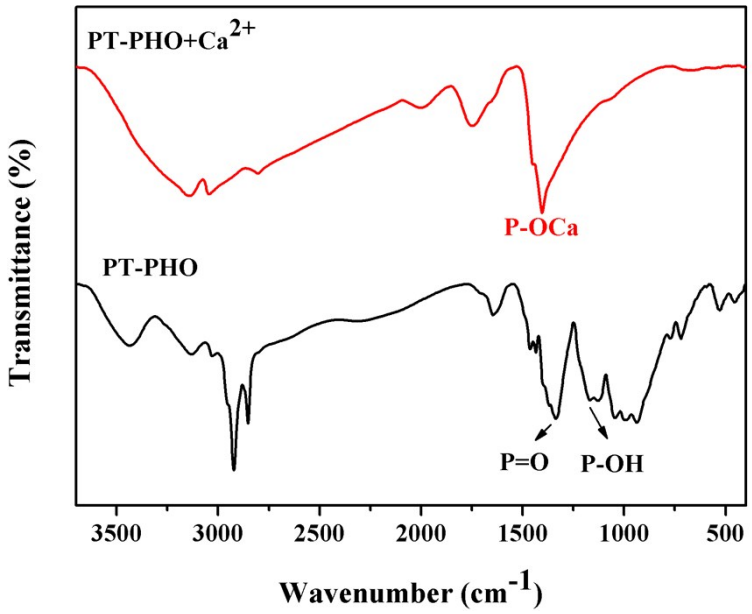

Fig. S7 FT-IR spectra of PT-PHO and PT-PHO-Ca^{2+}.

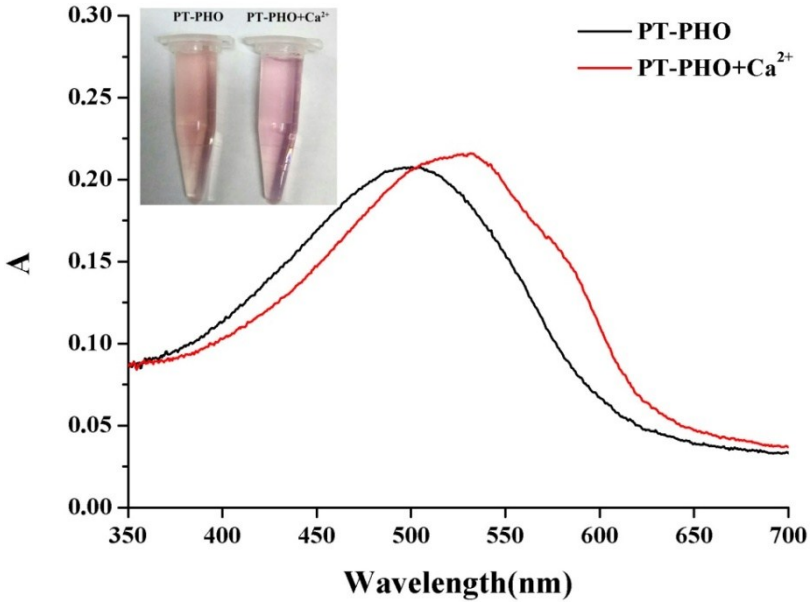

Fig.S8 The absorption spectra of PT-PHO and PT-PHO-Ca^{2+}.

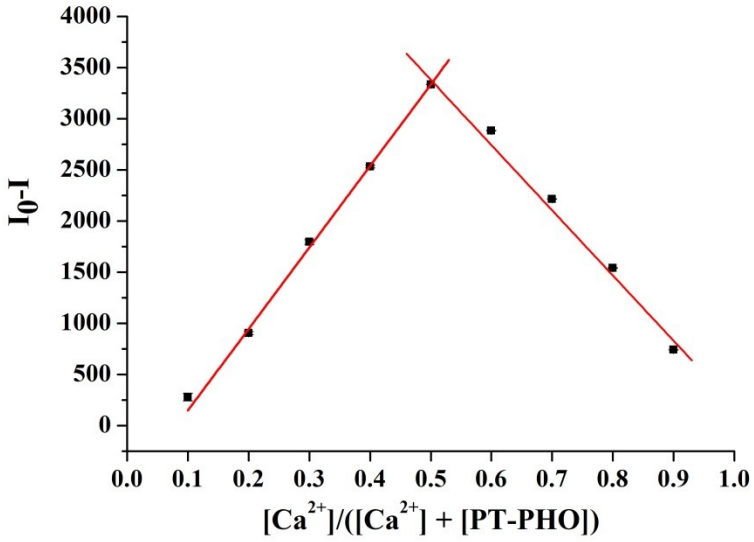

Fig. S9 Job's plot for PT-PHO in NH_3-NH_4Cl buffer solution (0.1 mol L^{-1}, pH = 9). [Ca^{2+}] + [PT-PHO] = 0.1 mmol L^{-1}. I_0 and I are the fluorescence intensity in the absence and presence of Ca^{2+}, respectively.

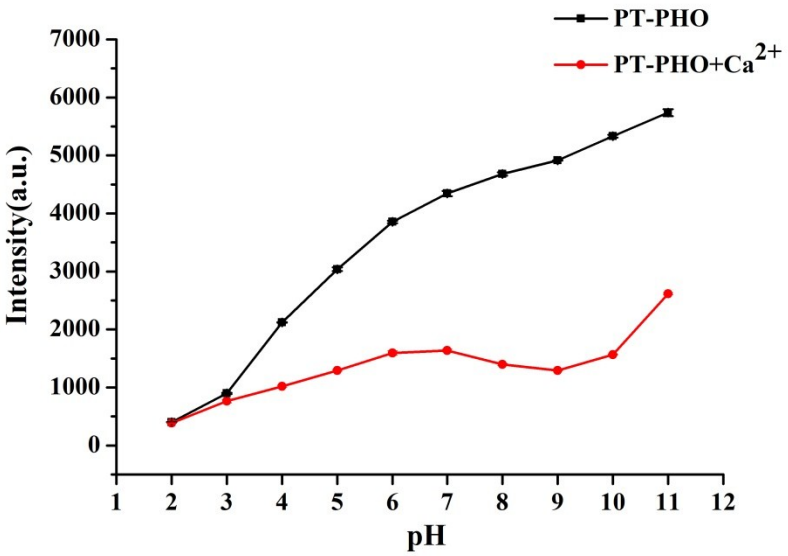

Fig. S10 The effect of different pH value on fluorescence intensity of PT-PHO (50 μmol L^{-1}) and PT-PHO added with Ca^{2+} (50 μmol L^{-1}).

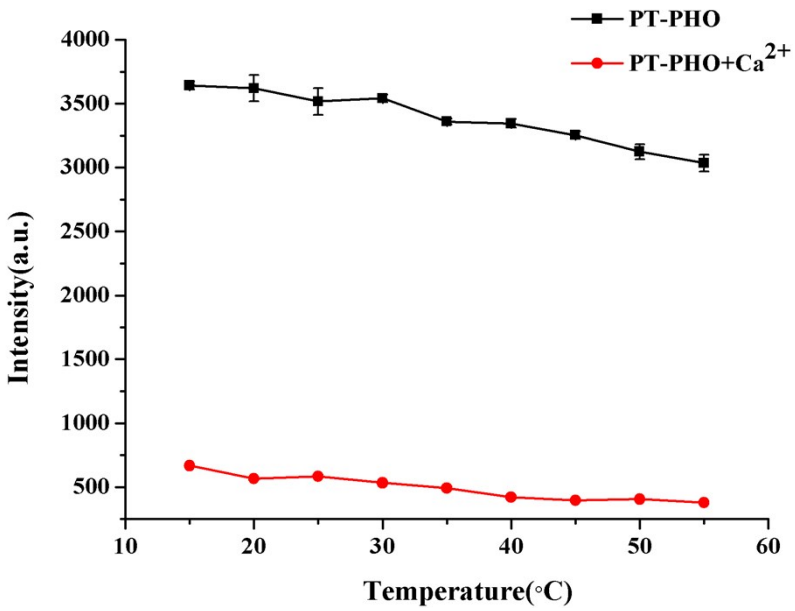

Fig. S11 The effect of different temperature on fluorescence intensity of the PT-PHO (50 μmol L^{-1}) and PT-PHO added with Ca^{2+} (50 μmol L^{-1}) in NH_3-NH_4Cl buffer solution (0.1 mol L^{-1}, pH = 9).

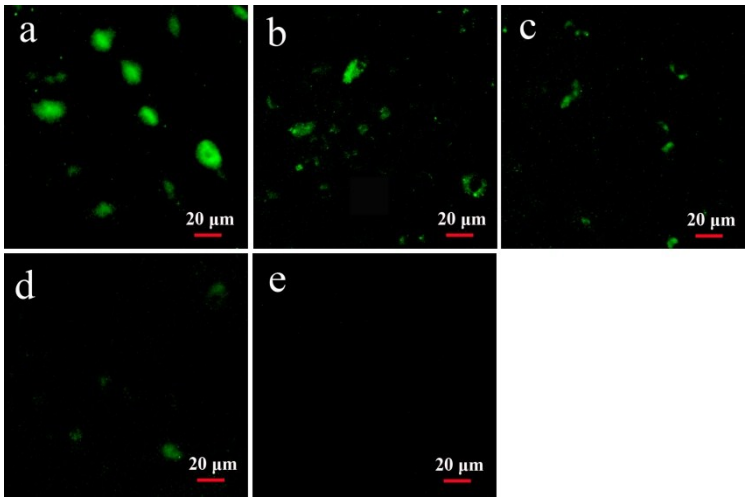

Fig. S12 Fluorescence images of A549 cells stained with 25 μmol L^{-1} PT-PHO for 1 h, then incubated with different concentrations of Ca^{2+} for 1h. a-e: 0 μmol L^{-1}, 25 μmol L^{-1}, 50 μmol L^{-1}, 100 μmol L^{-1}, 200 μmol L^{-1}. Scale bar: 20 μm.