Supporting Information

Easily fixed simple small ESIPT molecule with aggregation induced emission for instant and photostable “turn-on” bioimaging

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Experimental section

**Instruments:** \textsuperscript{1}H NMR spectra were obtained on a Bruker Ultra Shield Plus using CDCl\textsubscript{3} as solvent. The UV-vis absorption and fluorescence spectra were measured on a Shimadzu UV-3600 UV-vis spectrophotometer and RF-5301PC spectrofluorophotometer, respectively. Mass spectra was obtained on a Bruker autoflex matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF/TOF) mass spectrometer. Fluorescence quantum yield (\(\Phi_F\)) of powder and the solution sample was measured by an Edinburgh FL 920 instrument with a integrating sephere and by using 9,10-diphenylantrachene (\(\Phi_F = 0.9\) in cyclohexane) or Quinine sulfate dehydrate (\(\Phi_F = 0.55\) in 0.5 mol L\textsuperscript{-1} H\textsubscript{2}SO\textsubscript{4}) or Rhodamine 6G (\(\Phi_F = 0.94\) in ethanol) as standard, respectively. Confocal luminescence images were carried out on an Olympus FV1000 laser scanning confocal microscope.

**Materials:** All materials were commercially available and of reagent grade. PBS buffer (PH 7.4) was prepared using double distilled water. THF (Labscan) was distilled under dry nitrogen from sodium benzophenone ketyl prior to use.
Synthesis of T3BA: 5-Amino-2-(benzo[d]thiazol-2-yl)-phenol (5ABT) was synthesized according to our previously published method.\textsuperscript{1} 5ABT (1.21 g, 5 mmol) was added dropwise to phthaloyl dichloride (1.01 g, 5 mmol) and triethylamine (0.71 mL, 5 mmol) in anhydrous tetrahydrofuran (THF, 40 mL) at 0 °C. The batch was stirred for 3 h, with plenty of white precipitates emerging. The solid was filtered, and the resulting mixture was washed by water and then subjected to silica-gel column chromatography to obtain 0.62 g (1.6 mmol) at 32% yield of pale yellow solid. \textsuperscript{1}H NMR (400MHz, DMSO-$d_6$, 298K): $\delta= 11.85$ (s, 1H, -OH), 8.34-8.36 (d, 1H, Ar-H), 8.16-8.18 (d, 1H, Ar-H), 8.08-8.10 (d, 1H, Ar-H), 7.99-8.01 (m, 2H, Ar-H), 7.92-7.94 (m, 2H, Ar-H), 7.55-7.59 (t, 1H, Ar-H), 7.46-7.49 (t, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 7.15-7.18 (d, 1H, Ar-H). \textsuperscript{13}C-NMR (400 MHz, DMSO-$d_6$, 298K): $\delta= 167.85, 167.36, 165.10, 156.94, 151.53, 143.21, 138.66, 133.98, 131.83, 129.89, 129.62, 129.58, 128.95, 127.83, 126.38, 124.74, 121.91, 121.78, 113.71, 111.26, 106.65$. MALDI-TOF m/z: 391.10 [M+H]$^+$. Anal. calcd for C$_21$H$_{14}$N$_2$O$_4$S: C, 64.60; H, 3.61; N, 7.18; Found: C, 64.76; H, 3.55; N, 7.23.

Cell Culture: HeLa cells were obtained from the Cell Bank (Cell Institute, Sinica Academica Shanghai, Shanghai, China) and cultured in Dulbecco’s modified Eagle’s medium (DMEM) media supplemented with fetal bovine serum (10%), penicillin (100 units/mL) and streptomycin (50 units/mL). Cells were cultured with 5% CO$_2$ at 37°C in a humidified incubator. Prior to the imaging experiments, the cells were cultured until confluence was reached.

MTT cell viability assay: To test the biocompatibility of T3BA for imaging in living cells, cell cytotoxicity assay was conducted on HeLa cells by following standard protocols.\textsuperscript{2} HeLa cells were plated in 96-well microassay culture plates (1 x 10$^4$ cells per well) and maintained for 24 h. Compounds were then added to the wells to achieve final concentrations at 5, 10, 25, 50, 75, 100 µM and negative control wells were prepared by addition 200 µl of complete medium containing 0.2% DMSO. Wells containing complete medium without cells were used as blanks. The plates were incubated at 37 °C in a 5% CO$_2$ incubator for 24 h. Cell proliferation was evaluated
by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, from Sigma-Aldrich) assay. 20 μL MTT solution (5 mg mL⁻¹) was added to each well. After 4 h incubation, the medium was removed and dimethyl sulfoxide (DMSO) was added to solubilize the MTT formazan. Cell growth was determined using the ELISA reader (Sunrise, Tecon) according to the manufacturer’s instructions. The optical density of each well was then measured on a microplate spectrophotometer at a wavelength of 590 nm. Absorbance values of experimental cultures were used to indicate the levels of cell proliferation.

**Confocal luminescence imaging:** The HeLa cells were cultured in cell plates specially for observation under CLSM at 37 °C. The T3BA was dispersed in DMSO buffer and were added to the plate at a final concentration of 20 μM. After incubation for 10 min, the cells were washed three times with 1 × PBS buffer and imaged by CLSM (Olympus FV1000, Tokyo, Japan) using the Olympus Fluoview FV1000 imaging software. The fluorescent signals from the T3BA were collected at 480-580 nm upon excitation at 405 nm (1.25 mW). To better understand the intracellular distribution of T3BA in the cell, luminescence intensity profile corresponding to the extracellular region, nuclear region and cytoplasm was investigated, respectively.

**Photostability and pH effect analysis:** To further explore the application of T3BA complexes as cellular dyes, the photostability of the T3BA in living HeLa cells were examined under continuous laser scanning upon excitation at 405 nm via photobleaching experiments. The fluorescence intensity for each image was analyzed using the Image Pro Plus software. The photostability of T3BA was expressed by the ratio of the fluorescence intensity after excitation for a designated time interval to its initial value as a function of the exposure time. The images were taken under successive irradiation. The fluorescence stability of T3BA was also investigated by continuous observation of staining cell under CLSM for 24 h and images of the staining cells were taken after addition of T3BA for 0 s, 20 s, 3 min, 3 h, 12 h, and 24 h, respectively. The pH effect on the imaging response of T3BA was investigated³ by the pH titration curve reflecting luminescence intensity with the pH varied from 4-10.
**Mechanisms of Cellular Uptake:** To investigate the mechanism of cellular uptake of the T3BA, confocal microscopy and flow cytometry were used to study the cellular entry pathway. To determine whether T3BA entered the cell *via* an energy-dependent or energy-independent transport pathway, HeLa cells were either incubated with T3BA under conditions of different temperatures or in the presence of the metabolic inhibitors 2-deoxy-D-glucose and oligomycin. Cells cultured in plates were incubated with 20 μM T3BA for 10 min at 4 °C or at 37 °C with or without the cells preincubated with metabolic inhibitor 2-deoxy-D-glucose (50 mM) and oligomycin (5 μM) for 1 h. To quantitatively analyze the uptake of the T3BA to the tumor cells, HeLa cells were plated in 96-well microassay culture plates (1 × 10^4 cells per well) and maintained for 24 h. The above-mentioned procedure of sample preparation was repeated and a fluorescent microplate reader was used to quantify the fluorescent intensity (Beckman Coulter, USA). Such prepared cells were then analyzed under CLSM upon excitation at 405 nm.
Fig. S1  $^1$H-NMR spectrum of T3BA in DMSO-$d_6$.

Fig. S2  $^{13}$C-NMR spectrum of T3BA in DMSO-$d_6$. 
Fig. S3 (a) Absorption spectra of $10^{-5}$ M T3BA in the THF/H$_2$O mixture at different water fractions; (b) excitation spectra detected at different wavelengths of T3BA nanoparticles dispersed in H$_2$O.
**Fig. S4** Cell viability values (%) assessed by MTT proliferation test *vs.* incubation concentrations of 5-100 µM T3BA in PBS buffer at 37 °C for 24 h.

**Fig. S5** Luminescence intensity profile and luminescence image (across the line) of HeLa cells incubated with the T3BA.
Fig. S6 Confocal luminescence and bright-field images (a) and fluorescence intensity histogram profile from fluorescent microplate reader (b) of living HeLa cells incubated with 20 μM T3BA under different conditions. Cells were incubated with 20 μM T3BA for 10 min at 4 °C or at 37 °C with or without the cells preincubated with 50 mM 2-deoxy-D-glucose and 5 μM oligomycin in PBS for 1 h. PI staining is included for each to indicate cell viability.
**Fig. S7** Photostability of T3BA in HeLa cells upon continuous laser excitation at 405 nm. $I_0$ is the initial fluorescence intensity and $I$ is the fluorescence intensity of the sample at various time points after illumination.

**Fig. S8** The normalized emission intensity of 20 μM T3BA at 405 nm under different pH in Britton-Robinson buffer.
**Fig. S9** The signal to noise (S/N) ratio of T3BA and several commonly commercialized fluorescent dyes. Confocal luminescence images of living HeLa cells incubated with 20 μM of T3BA, MitoTracker Red, LysoTracker Red, CellView Blue and Acridine Orange, respectively, for 10 min at 37 °C. All experiments were conducted under the same condition (pinhole diameter was 100 μm, bin was 1, sampling speed was 8 μs/pixel and laser transmisivity was 10.0%).
**Fig. S10** The chemical structures of several commonly commercialized fluorescent dyes. (The quantum efficiencies of Mito tracker Red, CellView Blue, Lyso Tracker Red and Acridine Orange in water are 7.9%, 3.3%, 1.5% and 12%, respectively)

**References**


2 Mueller, H., Kassack, M. U., Wiese, M. Comparison of the usefulness of the MTT, ATP, and calcein assays to predict the potency of cytotoxic agents in various human cancer cell lines. J. Biomol. Screen. 2004, 9, 506-515