Electronic Supplementary Information (ESI)

A Highly Fluorescent AIE-active Theranostic Agent with Anti-Tumor Activity to Specific Cancer Cells

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Table of Contents

Experimental Section

Scheme S1 Synthetic route to TPE-TMX.

Fig. S1 $^1$H NMR spectrum of TPE-TMX in CDCl$_3$.

Fig. S2 $^{13}$C NMR spectrum of TPE-TMX in CDCl$_3$.

Fig. S3 Mass spectrum of TPE-TMX.

Fig. S4 (A) PL spectra of TPE-TMX in THF/H$_2$O mixtures with different water fractions. (B) Plot of $(I/I_0)$ values versus the compositions of the aqueous mixtures of TPE-TMX. $I_0 = PL$ intensity of TPE-TMX in pure THF solution. [TPE-TMX]: 10 μM; excitation wavelength: 320 nm.

Fig. S5 PL spectra of TPE-TMX (red) and TMX (black) in THF/H$_2$O mixtures with 95% water fraction. [TMX] = [TPE-TMX]: 10 μM; excitation wavelength (nm): 277 nm (TMX) and 320 (TPE-TMX).

Fig. S6 (A–D) Bright-field and (E–H) fluorescent images of different cell lines treated with TPE-TMX (2 μM) for 24 h. Excitation wavelength: 330–385 nm. All images share the same scale bar (30 μm).

Fig. S7 (A–C) Bright-field and (D–F) fluorescent images of MCF-7 cells taken at different times after stained with LTR (50 nM). Excitation wavelength: 520–560 nm. All the images share the same scale bar (30 μm).

Fig. S8 (A–D and I–L) Bright-field and (E–H and M–P) fluorescent images of MCF-7 cells taken at different times (from 24 to 196 h) after treated with TPE-TMX (2 μM). Excitation wavelength: 330–385 nm. All images share the same scale bar (30 μm).
**Experimental section**

**Materials**

Benzophenone, 4-hydroxybenzophenone, zinc powder, titanium tetrachloride (TiCl₄), potassium carbonate (K₂CO₃), anhydrous dimethylsulfoxide (DMSO), acetone and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide (MTT) and other chemicals were all purchased from Sigma-Aldrich and used as received. 2-Bromo-N,N-dimethylethanamine hydrobromide was purchased from Annker Organics Co., Ltd. Dulbecco’s Modified Eagle Medium (DMEM), modified essential medium (MEM) and LysoTracker red DND-99 (LTR) were the commercial products of Invitrogen. Fetal bovine serum (FBS) and trypsin-EDTA solution were purchased from Life Technologies. Tetrahydrofuran (THF) and dichloromethane (DCM) were distilled from sodium benzophenone ketyl and calcium hydride under nitrogen, respectively. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, United states).

**Instruments**

¹H NMR and ¹³C NMR spectra were measured on a Bruker AV 400 NMR spectrometer using CDCl₃ as a solvent and tetramethylsilane (TMS; δ = 0) as an internal reference. Absorption spectra were measured on a Milton Roy Spectronic 3000 Array spectrophotometer. Photoluminescence (PL) spectra were recorded on a Perkin-Elmer LS 55 spectrofluorometer with a xenon discharge lamp excitation. High-resolution mass spectra (HRMS) were recorded on a GCT Premier CAB 048 mass spectrometer system operated in MALDI-TOF mode.

**Synthesis of TPE-OH**

Into a two-necked round bottom flask equipped a condenser were added a dry THF
solution (40 mL) of benzophenone (1.82 g, 10 mmol), 4-hydroxybenzophenone (1.90 g, 10 mmol), zinc powder (1.60 g, 24 mmol) under nitrogen. The mixture was cooled to -78 °C and TiCl$_4$ (1.3 mL, 12 mmol) was injected dropwise. The mixture was slowly warmed to room temperature, stirred for 0.5 h, and then heated to reflux overnight. After the mixture was cooled to room temperature, dilute hydrochloric acid was added to quench the reaction. The mixture was extracted with dichloromethane (DCM) three times and the combined organic layer was washed with brine and dried over anhydrous sodium sulfate. After solvent evaporation, the crude product was purified on a silica-gel column using petroleum ether/DCM as eluent. A white solid was obtained. $^1$H NMR (400 MHz, CDCl$_3$), $\delta$ (ppm): 7.12–7.07 (m, 9H), 7.07–7.01 (m, 6H), 6.90–6.88 (d, 2H, $J$ = 6.57 Hz), 7.14–7.10 (m, 9H), 7.07–7.03 (m, 6H), 6.96–6.94 (d, 2H, $J$ = 8.4 Hz), 4.06–4.03 (d, 2H, $J$ = 4.5 Hz), 2.78–2.76 (d, 2H, $J$ = 4.5 Hz), 2.39 (m, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$ (TMS, ppm): 157.2, 144.0, 143.8, 140.5, 140.2, 136.3, 132.6, 131.4, 131.4, 131.3, 127.7, 127.6, 126.4, 126.3, 113.7, 77.4, 77.1, 76.8, 65.5, 58.2, 45.8. MS (MALDI-TOF): $m/z$ 418.2 (M$^+$, calcd. 419.2).

**Synthesis of TPE-TMX**

Into a two-necked round bottom flask equipped a condenser were added an acetone solution (15 mL) of 2-bromo-$N,N$-dimethylethanamine hydrobromide (1.74 g, 7.5 mmol), TPE-OH (1.74 g, 5 mmol), potassium carbonate (3.45 g, 25 mmol). Afterwards, the mixture was heated to 85 °C overnight. After the mixture cooled to room temperature, the mixture was extracted with DCM three times and the combined organic layer was washed with brine and dried over anhydrous sodium sulfate. After solvent evaporation, the crude product was purified on an aluminum oxide-gel column to afford the desirable white solid. $^1$H NMR (400 MHz, CDCl$_3$), $\delta$ (ppm): 7.14–7.10 (m, 9H), 7.07–7.03 (m, 6H), 6.96–6.94 (d, 2H, $J$ = 8.4 Hz), 4.06–4.03 (d, 2H, $J$ = 4.5 Hz), 2.78–2.76 (d, 2H, $J$ = 4.5 Hz), 2.39 (m, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$ (TMS, ppm): 157.2, 144.0, 143.8, 140.5, 140.2, 136.3, 132.6, 131.4, 131.4, 131.3, 127.7, 127.6, 126.4, 126.3, 113.7, 77.4, 77.1, 76.8, 65.5, 58.2, 45.8. MS (MALDI-TOF): $m/z$ 418.2 (M$^+$, calcd. 419.2).
Cell culture

MCF-7, MDA-MB-231, HeLa and COS-7 cell lines were provided by American Type Culture Collection. The HeLa cells were cultured in MEM while MCF-7, MDA-MB-231 and COS-7 cells were cultured in DMEM at 37 °C in a humidified incubator with 5% CO₂. Both culture mediums contained 10% heat-inactivated FBS 100 U mL⁻¹ penicillin and 100 μg mL⁻¹ streptomycin. Before experiment, the cells were pre-cultured until confluence was reached.

Cell imaging

Cells were grown overnight on a 35 mm petri dish with a cover slip. The live cells were incubated with the aqueous solution of TPE-TMX (2 μM) for 24 h followed by LTR (50 nM) for 15 min. The dye-labelled cells were then mounted in standard mounting media and imaged by fluorescence microscopy (BX 41 Microscope). Conditions: for TPE-TMX, excitation wavelength: 330–385 nm, dichroic mirror: 400 nm and emission long pass filter: 420 nm; for LTR, excitation filter: 540–580 nm, dichroic mirror: 600 nm and emission long pass filter: 610 nm.

Photostability test

The dye-labelled MCF-7 cells were imaged by confocal microscope (Zeiss laser scanning confocal microscope LSM7 DUO). Conditions: excitation wavelength: 405 nm and emission filter: 500–750 nm (TPE-TMX); excitation wavelength: 561 nm and emission filter: 580–750 nm (LTR). Laser power was unified as 0.1 mW.

Cytotoxicity study

MTT assays were used to evaluate the cytotoxicity of TPE-TMX to different cell lines. For each cell line, the cells were seeded in 96-well plates (Costar, IL, USA) at a density of 5 × 10³ cells/well. After 24 h incubation, the cells were exposed to a series
of doses of TPE-TMX (0–10 μM) in culture medium at 37 °C. After 24 h incubation, 10 μL of freshly prepared MTT solution (5 mg/mL in PBS) was added into each well. After 4 h incubation, 100 μL of solubilizing solution containing 10% SDS and 0.01 M HCl was added to dissolve the purple crystals. After 8 h incubation, the absorbance of MTT at 595 nm was monitored using a Perkin-Elmer Victor plate reader. Cell viability was expressed by the ratio of absorbance of the cells incubated with TPE-TMX to that of cells incubated with culture medium only. Each of the experiments was performed at least three times.

Scheme S1 Synthetic route of TPE-TMX.
**Fig. S1** $^1$H NMR spectrum of TPE-TMX in CDCl$_3$.

![Fig. S1 $^1$H NMR spectrum of TPE-TMX in CDCl$_3$.](image1)

**Fig. S2** $^{13}$C NMR spectrum of TPE-TMX in CDCl$_3$.

![Fig. S2 $^{13}$C NMR spectrum of TPE-TMX in CDCl$_3$.](image2)

Chemical Formula: C$_{30}$H$_{29}$NO

$m/z$: 419.2
Fig. S3 Mass spectrum of TPE-TMX.

Fig. S4 (A) PL spectra of TPE-TMX in THF/H$_2$O mixtures with different water fractions ($f_w$). (B) Plot of ($I/I_0$) values *versus* the compositions of the aqueous mixtures of TPE-TMX. $I_0$ = PL intensity of TPE-TMX in pure THF solution. [TPE-TMX]: 10 $\mu$M; excitation wavelength: 320 nm.

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