Materials and methods

Cell Culture

AML12 and THLE-2 were provided by Procell Life Science&Technology Co.,Ltd. (Wuhan, China) and maintained in State Key Laboratory of Antiviral Drugs. AML12 cells were maintained in DMEM/F-12 with 10%(v/v) fetal bovine serum, 1% Insulin & Transferrin & Selenium (ITS) liquid media supplement and 40 ng/mL dexamethasone. THLE-2 was cultured in a dedicated medium provided by Procell.All cell lines were cultured in a humidified cell incubator (37 °C, 5% CO2).

Cytotoxicity assay

The cytotoxic effect of TPE-Q on cells was evaluated using the Cell Counting Kit-8 (CA1210, Solarbio, China) in accordance with the manufacturer's guidelines. In brief, 100 μL of the medium containing 1×10^4 cells per well was dispensed into 96-well plates, after which varying concentrations of TPE-Q were added. Absorbance readings were taken at 450 nm using a Microplate reader (BioTek, USA) after 24 hours.

Cell Imaging

TPE-Q is stored in DMSO, and then a certain amount of TPE-Q is added to the culture medium or PBS to obtain the corresponding concentration of working solution. Cells were seeded in a small confocal laser dish or cell slide at 2×104 cells/well. After lipid droplet induction, TPE-Q solution was added to the well before or after fixation with 4% paraformaldehyde (PFA). Incubate cells with TPE-Q at 37℃ for 30min-2 hours. Images were visualized with Nikon Ni-E (Tokyo, Japan), Zeiss LSM880 confocal microscope (Oberkotchen, Germany) and Olympus FV3000 confocal microscope (Tokyo, Japan). Process the images using ImageJ, NIS Elements viewer, and Zen3.8. Images were analyzed using the NIS-Elements Viewer5.22, Zen3.8 and Fiji (ImageJ, V2.0.0). The excitation wavelengths of TPE-Q and Nile Red are 330-405nm and 532-561nm, respectively.

Oil red O staining

Cells were grown in a medium containing 250 μ M oleic acid for 24 hours. The lipid droplets were validated using Oil Red O reagent(IO1720, Solarbio, China). In short, fix the cells with 4% PFA for 15-20 minutes, wash three times with PBS, and then rinse the cells with 60% isopropanol for 20-30 seconds. Next, immerse the cells in Oil Red O working solution for 20-30 minutes. Finally, remove the working solution and rinse the cells with 60% isopropanol. Hematoxylin is used to stain cell nuclei.

Photostability Test

The fluorescent images were recorded by continuous laser scanning by Zeiss LSM880 confocal microscope. The images were captured every 60 seconds, and the measurement was performed three times in parallel. The fluorescence intensity was assessed by Fiji (ImageJ, V2.0.0) software.