Supporting information for

A two-photon endoplasmic reticulum-targeting fluorescent probe for the imaging of pH in living cells and zebrafishes

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1. Materials and instruments

All reagents were mentioned in this work of analytical grade. $^1$H NMR and $^{13}$C NMR spectra were collected on an AVANCE III 400 MHz Digital NMR Spectrometer. High resolution mass spectrometric (HRMS) analyses were measured using a Finnigan MAT 95 XP spectrometer. UV-Vis and Fluorescence spectra were acquired on a Shimadzu UV-2700 spectrometer and HITACHI F-4600 fluorescence spectrophotometer, respectively. Fluorescence imaging of living cells and cells was performed on a Nikon A1MP confocal microscopy.

2. Cytotoxicity experiments

Cytotoxicity of Na-pH was evaluated using the standard MTT assay. HeLa cells were seeded into the 96 well-plates with the density of around 7000 cells/well. After 24 h, various concentrations of Na-pH were added into the wells and the cells were further cultured for 24 h. Then, 10 $\mu$L of 5 mg/mL MTT was mixed into cells and the cells were incubated for another 4 h. Subsequently, 100 $\mu$L DMSO were used to resolve the formazan and the plate was shaken for 20 min, and finally the absorbance was determined by a Thermo Fisher Scientific microplate reader. Cell viability was expressed as the percentage of the control culture value.

![Graph](image)

**Fig. S1.** Photostability of compound Na-Ph at different pH values (4.0 and 7.4, at 531nm).
Fig. S2. MTT assay of HeLa cells in the presence of various concentrations of Na-pH for 24 h.

Fig. S3. $^1$H-NMR spectrum of Na-pH in CDCl$_3$. 

**Fig. S4.** $^{13}$C-NMR spectrum of Na-pH in CDCl$_3$.

**Fig. S5.** $^1$H-NMR spectrum of Compound 1 in CDCl$_3$. 
Fig. S6. $^{13}$C-NMR spectrum of Compound 1 in CDCl$_3$.

Fig. S7. LC-MS data of compound Na-pH
Fig. S8. HR-MS data of t Na-pH