Supporting information for

**Red-emissive D-A-D type fluorescent probe for lysosomal pH imaging**

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1. Materials

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was purchased from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Nigericin was obtained from Aladdin Industrial Corporation (Shanghai, China). LysoTracker Red, MitoTracker Red, fetal bovine serum (FBS), and RPMI 1640 media were purchased from Thermo Fisher Scientific Co., Ltd (Shanghai, China). All inorganic salt was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All the reagents were of analytical grade and used as received.

2. Instruments

UV-vis spectra and fluorescence spectra were recorded with a Cary 60 spectrophotometer and a Cary Eclipse Spectrofluorophotometer, respectively (Agilent Technologies, Palo. Alto, CA, USA). MTT assays and pH fluorescence cell imaging were acquired with a Cytation 3 Cell Imaging Multi-Mode Reader equipped with Acridine Orange (ACR OR) Filter cube (BioTek Instruments, Inc., VT, USA). Co-localization and autophagy experiments were acquired with a Leica TCS-SP8 confocal scanning microscope (Leica Microsystems Inc., Wetzlar, Germany).

3. Theoretical calculations

Geometry optimizations of DBTD and H+-DBTD-H+ were obtained at the B3LYP level with standard 6-31g (d, p) basis set optimized with density functional theory (DFT). The structures and levels of HOMO and LUMO of two compounds had been calculated at the same way (B3LYP/6-31g (d, p)). All the calculations were procured through Gaussian program.
4. Cell culture and cytotoxicity experiments

Gastric cancer SGC-7901 cells were grown in RPMI 1640 medium containing 10% FBS, 1% Penicillin-Streptomycin solution at the environment of 37 °C, 5% CO$_2$. The cells were used for imaging after incubating for at least 12 h. The cytotoxicity of DBTD has been evaluated with SGC-7901 cells using MTT assay. The SGC-7901 cells were seeded in a 96-well plate at a density of $10^4$ cells per well and incubated at the environment of 37 °C, 5% CO$_2$ for 24 h. Then, the cells were treated with different concentrations (1-10 μM) of DBTD for 12 h. After that, cells were incubated with new culture medium containing MTT (0.5 mg/mL) for another 4 h. 100 μL DMSO was added to each well after removing the MTT. When shaking for 15 min, the optical density values were measured at 490 nm.
Fig. S1. $^1$H NMR spectrum of 4,5-dimethoxybenzene-1,2-diamine (1) in CDCl$_3$.

Fig. S2. $^1$H NMR spectrum of 5,6-dimethoxybenzo[c][1,2,5]thiadiazole (2) in CDCl$_3$. 
Fig. S3. $^1$H NMR spectrum of 4,7-dibromo-5,6-dimethoxybenzo[c] [1,2,5]thiadiazole (3) in CDCl$_3$.

Fig. S4. $^1$H NMR spectrum of DBTD in CDCl$_3$. 
Fig. S5. $^{13}$C NMR spectrum of DBTD in CDCl$_3$.

Fig. S6. HRMS spectrum of DBTD.
Fig. S7. The absorbance spectra of DBTD in different solvents.

Fig. S8. Plot of fluorescence emission maximum of DBTD (20 μM) in various solvents vs $E_T(30)$. 

$\lambda_{\text{em}} = 7.27 \times E_T(30) + 335.92$

$R^2 = 0.9719$
Fig. S9. The absorbance spectra of DBTD (20 μM) in BR buffer solution (with 10% DMAC) with pH change from 3.0 to 7.4.

Fig. S10. Fluorescence changes ($F_{614\text{nm}}$) of DBTD (20 μM) treated with metal ions (2 mM K$^+$, Na$^+$ and 100 μM Zn$^{2+}$), reactive oxygen species (500 μM ONOO$^-$, 100 μM ·OH), active biomolecules (500 μM Cys, 1 mM Hcy and GSH), Reaction time: 20 min, $\lambda_{\text{ex}} = 465$ nm.
Fig. S11. Photostability investigation of DBTD (20 μM) in BR buffer solution (with 10% DMAC) of pH 4.0 and 7.4. All solutions were continuously irradiated under a 365 nm UV lamp (8 W), $\lambda_{ex} = 465$ nm.

Fig. S12. Fluorescence reversibility of DBTD (20 μM) in BR buffer solution (with 10% DMAC) between pH 4.0 and 7.4, $\lambda_{ex} = 465$ nm.
Fig. S13. Plot of the fluorescence intensity ($F_{659\ nm}$) vs H$^+$ equivalent in CH$_3$CN.

Fig. S14. Cell viability of SGC-7901 cells with different concentrations of DBTD evaluated by MTT assay.
Table S1. Quantum yield (QY) of DBTD in different solvents.*

<table>
<thead>
<tr>
<th>Solvent</th>
<th>QY (%)</th>
<th>Solvent</th>
<th>QY (%)</th>
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<tbody>
<tr>
<td>Hexane</td>
<td>52.3</td>
<td>Toluene</td>
<td>33.0</td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>31.0</td>
<td>THF</td>
<td>28.8</td>
</tr>
<tr>
<td>EA</td>
<td>20.2</td>
<td>Acetone</td>
<td>10.5</td>
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<tr>
<td>DMAC</td>
<td>8.0</td>
<td>DMSO</td>
<td>3.7</td>
</tr>
</tbody>
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* Carboxyl fluorescein (QY = 79%, in 0.1 M NaOH) was used as the standard.