Supporting Information

Rational design of asymmetric red fluorescent probes for live cell imaging with high AIE effect and large two-photon absorption cross section by tunable terminal group

Zheng-Feng Chang, Ling-Min Jing, Bin Chen, Mengshi Zhang, Xiaolei Cai, Jun-Jie Liu, Yan-Chun Ye, Xiaoqing Lou, Zujin Zhao, Bin Liu, Jun-Liang Wang, and Ben Zhong Tang

a) Beijing Key Laboratory of Photoelectronic/Electrophotonic Conversion Materials, Beijing Institute of Technology, Beijing, 100081, China. E-mail: jinlwang@bit.edu.cn
b) State Key Laboratory of Luminescent Materials and Devices, South China University of Technology, Guangzhou 510640, China. E-mail: mszjzhao@scut.edu.cn
c) School of Chemistry and Chemical Engineering, Huazhong University of Science and Technology, Wuhan 430074, China. E-mail: louxiaoding@hust.edu.cn
d) Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore 117585

1. Experimental Details

1: To a solution of di(thiophen-2-yl)methanone (1.06 g, 5.46 mmol), CBr₄ (3.57 g, 10.93 mmol) and PPh₃ (5.73 g, 21.86 mmol) in toluene (50 mL). After refluxing for 24 h, the mixture was cooled to 25 °C and poured into an aq. NH₄Cl solution. The aqueous layer was extracted with CH₂Cl₂, and then the combined organic phase was washed with brine and dried (MgSO₄). The solvents were then removed under reduced pressure. The residue was purified by silica gel chromatography, eluting with petroleum ether (PE) to give yellow oil (1.23 g, 65%). ¹H NMR (CDCl₃, 400 MHz, ppm): δ 7.39-7.41 (d, J = 5.2 Hz, 2H), 7.10-7.11 (d, J = 3.2 Hz, 2H), 7.00-7.02 (t, J = 4.4 Hz, 2H). ¹³C NMR (CDCl₃, 100 MHz, ppm): δ 142.1, 134.3, 130.0, 127.6, 126.8, 92.2. HR-EIS-MS(m/z): calcd for C₁₀H₆Br₂S₂: 349.8257(100%). Found: 350.8330 ([M+H]+, 100%).

2: To a solution of 1 (0.50 g, 1.73 mmol), (4-(diphenylamino)phenyl)boronic acid (0.25 g, 0.72 mmol), Pd(PPh₃)₄ (0.07 g, 0.06 mmol) in toluene (40 mL) and ethanol(80 mL), aqueous NaOH solution (1.0 M, 5.0 mL) and were added. After refluxing for 12 h under N₂, the mixture was cooled to 25 °C and poured into an aq. NH₄Cl solution. The aqueous layer was extracted with CH₂Cl₂, and then the combined organic phase was washed with brine and dried (MgSO₄). The solvents were then removed under reduced pressure. The residue was purified by silica gel chromatography, eluting with petroleum ether (PE)-CH₂Cl₂ (4:1) to give yellow solid (0.31 g, 64%). ¹H NMR (CDCl₃, 400MHz, ppm) δ: 7.22-7.26 (m, 8H), 7.18-7.20 (d, J = 4.8 Hz, 2H), 7.07-7.09 (d, J = 7.6 Hz, 8H), 6.98-7.04 (m, 8H), 6.87-6.89 (d, J =
8.4 Hz, 4H), 6.83-6.84 (t, J = 4.0 Hz, 2H), 6.80-6.81 (dd, J = 3.6 Hz, 2H). $^{13}$C NMR (CDCl$_3$, 100MHz, ppm) δ: 147.8, 146.8, 146.5, 142.0, 137.2, 1319, 129.6, 129.4, (126.23, 126.18), 125.6, 124.7, 123.1, 122.9. HR-ESI-MS (m/z): calcd for C$_{46}$H$_{34}$N$_{2}$S$_{2}$: 678.2163 (100%). Found: 678.2168 (100%).

Figure S1. Comparison of HOMO and LUMO orbital surfaces of DTPABT and DTPEBT using DFT B3LYP/6-31G(d) method.

Table S1. Summary of calculated parameters for these AIE molecules using DFT B3LYP/6-31G(d) method.

<table>
<thead>
<tr>
<th>Compd</th>
<th>HOMO (eV)</th>
<th>LUMO (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPABT</td>
<td>-4.61</td>
<td>-2.40</td>
</tr>
<tr>
<td>TPEBT</td>
<td>-4.65</td>
<td>-2.44</td>
</tr>
<tr>
<td>DTPABT</td>
<td>-4.54</td>
<td>-2.42</td>
</tr>
<tr>
<td>DTPEBT</td>
<td>-4.60</td>
<td>-2.47</td>
</tr>
</tbody>
</table>
Figure S2: (A) Emission spectra of DTPABT in THF-water mixtures with different water fraction ($f_w$). Inset: photos of DTPABT with different water fraction under UV lamp illumination. (B) Emission spectra of DTPEBT in THF-water mixtures with different water fraction ($f_w$). Inset: photos of DTPEBT with different water fraction under UV lamp illumination.
Figure S3. Fluorescence images of MCF-7 by TPEBT (A-C) and DTPABT (D-F). (A) and (D) are bright field image cells, (B) and (E) are fluorescent image, (C) and (F) are merged image, incubated for 3 hours with 20 µM.

Figure S4. Fluorescence images of Hela by TPEBT (A-C) and DTPABT (D-F). (A) and (D) are bright field image cells, (B) and (E) are fluorescent image, (C) and (F) are merged image, incubated for 3 hours with 20 µM.
Figure S5. Metabolic viability of MCF-7 breast cancer cells and Hela cells after incubation with red emission AIE molecules (A) DTPABT and (B) DTPEBT for 12, 24 and 36 h at different concentrations, respectively.

Figure S6. One-photon excited fluorescence (OPEF) and two-photon-induced fluorescence (TPEF) spectra of A) TPABT; B) DTPABT; C) TPEBT and D) DTPEBT in THF solution (10^{-5} M).
Figure S7. Two-photon excited fluorescence imaging of human breast cancer cells (MCF-7 cells) after 3 h incubation with TPEBT at 37 °C. The images were recorded upon 980 nm excitation with a 560–660 nm band pass filter. (A) brightfield field image cells, (B) two-photon excited fluorescence, (C) two-photon excited fluorescence/brightfield overlay. The scale bar is 50 µm.

Figure S8. Two-photon fluorescence images of ear blood vessels stained with TPEBT NPs including 3D reconstructed images of blood vessels when TPEBT was excited at 980 nm, images at different vertical depths of mouse ear. All the images share the same scale bar of 100 µm.
$^1$H NMR spectrum of TPABT in CDCl$_3$. 
The expended $^1$H NMR spectrum of TPABT in CDCl$_3$. 
$^{13}$C NMR spectrum of **TPABT** in CDCl$_3$. 
The expended $^{13}$C NMR spectrum of TPABT in CDCl$_3$. 
$^1$H NMR spectrum of DTPABT in CDCl$_3$. 
The expended $^1$H NMR spectrum of DTPABT in CDCl$_3$. 
$^{13}$C NMR spectrum of DTPABT in CDCl$_3$. 
The expended $^{13}\text{C}$ NMR spectrum of DTPABT in CDCl$_3$. 
$^1$H NMR spectrum of TPEBT in CDCl$_3$. 
The expended $^1$H NMR spectrum of TPEBT in CDCl$_3$
$^{13}$C NMR spectrum of TPEBT in CDCl$_3$. 
The expanded $^{13}$C NMR spectrum of TPEBT in CDCl$_3$. 
$^1$H NMR spectrum of DTPEBT in CDCl$_3$. 
The expended $^1$H NMR spectrum of DTPEBT in CDCl$_3$. 
$^{13}$C NMR spectrum of \textbf{DTPEBT} in CDCl$_3$. 
The expended $^{13}$C NMR spectrum of DTPEBT in CDCl$_3$. 